Enzyme-Assisted Extraction of Alginate from Beach Wrack *Fucus vesiculosus*

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Dedicated to Prof. Dr. Christian Wandrey on the occasion of his 80th birthday

Beach wrack constitutes an unutilized, abundant renewable source of marine macro algae being rich in valuable biopolymers. This work investigates the revalorization of the brown algae *Fucus vesiculosus* collected from beach wrack as a source of alginate, the main polysaccharide in brown algae which constitutes a potential ingredient for the development of biopolymer films. Enzyme-assisted extraction has been investigated for cell disruption with commercial cellulase blends and proteases. The effect of enzyme type, enzyme activity, and extraction time on alginate yields, molecular weight distribution, functional groups, and purity has been studied.

Keywords: Alginate, Biomaterials, Biopolymers, *Fucus vesiculosus*, Macroalgae

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1 Introduction

Beach wrack is all the marine organic material casted and deposited onto the beach. This includes marine autotrophs such as micro and macro algae as well as seagrass, shells, and dead animals. Although beach wrack plays an important role in shoreline ecosystems by providing resources to organisms and stabilizing soft bottom substrates, its accumulation results in several problems not only from an environmental point of view, leading to eutrophication, but also from a socio-economic perspective, representing a high cost for the local communities and a possible disturbance of tourism [1]. Nevertheless, beach wrack represents a raw feedstock of macro algae which are a natural source of high-added value compounds [2].

Brown algae are rich in alginate, a marine biopolymer characterized by a high biocompatibility, film-forming properties, non-antigenicity, biodegradability, and availability [3]. These properties make this polysaccharide a potential ingredient for developing biopolymer films as readily biodegradable substitutes for synthetic, petroleum-based plastics. Nevertheless, only 1 to 2 % of the food packaging materials are nowadays made of natural polymers, even though their suitability has been shown for this application [4–6].

The main obstacle to gain access to alginate is the macro algae cell wall, constituted by diverse compounds which form a complex hydrocolloid matrix responsible for the mechanical strength and flexibility of the algae cell [7]. Therefore, cell disruption is necessary to extract targeted compounds. Enzyme-assisted extraction (EAE) is characterized by high selectivity, low energy requirements, and gentle conditions. Enzymes cleave specific substrates in the cell wall resulting in the release of polysaccharides from the cell wall. The importance of the cell disruption method and conditions is due to its direct effect on the yields and properties of the extracted compounds [8].

In this work, *Fucus vesiculosus* collected from beach wrack has been investigated as source of alginate to be used as potential ingredient for biopolymer films. EAE has been used for alginate extraction and the effect of the enzyme type, enzyme activity, and extraction time on alginate yields, molecular weight distribution, functional groups, and purity has been studied.

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2 Experimental

2.1 Biomass Collection and Pretreatment

Brown macro algae *F. vesiculosus* was collected in April 2021 by the company Hanseatische Umwelt CAM GmbH from the coast of the Baltic Sea (Germany). Prior to EAE, biomass underwent a mild pretreatment to soften the macroalgae tissue walls and remove pigments and lipids [9, 10]. Washed and dried algae were milled to a particle size of < 5 mm in a Retsch ZM 200 mill (Retsch, Germany). A fine powder was obtained, which was then treated with 85 % (v/v) ethanol (1:10 biomass/ethanol) during 2 h at room temperature followed by a centrifugation step (10 000 min^{-1}, 4 °C, 10 min) with an Avanti J-25 centrifuge (Beckman Coulter, USA) equipped with a JA-10 rotor. After repeating this step twice, the supernatant was discarded, and the biomass was treated with pure acetone (1:10 biomass/acetone) and stirred overnight at room temperature and 150 min^{-1}. After a final centrifugation step, the pretreated biomass was dried at room temperature in a fume hood and stored at room temperature until further use.

2.2 Biochemical Composition of the Biomass

The macroalgae biomass was first characterized by analyzing the biochemical composition including the quantification of total ash content, total lipid content, total protein content, total phenolic compounds, and the monosaccharides arabinose, fucose, galactose, glucose, mannose, and xylose. For all analytical experiments, samples were lyophilized in a two-step procedure including main drying and final drying in a laboratory freeze dryer (Martin Christ Gefriertrocknungsanlagen GmbH, Germany; model: Alpha 1-2 LDplus). Finally, samples were grounded to ≤ 0.5 mm particle size. All measurements were performed in triplicates.

2.2.1 Ash

For the determination of the ash content, a laboratory muffle furnace was used according to DIN EN ISO 18122 at 550 °C [11]. The samples were incinerated, cooled down in a desiccator, and then analyzed gravimetrically. The results are expressed as mass ratio of heated macro algae mass to original macro algae mass.

2.2.2 Lipids

Analysis of the total lipid content was performed by a modified method based on Ryckebosch and Foubert [12]. A chloroform-methanol mixture in a volumetric ratio of 1:1 was used as extraction solvent. For lipid extraction, 2 g of biomass were weighed in a cellulose extraction thimble and placed in a soxhlet apparatus. The round flask was filled with 160 mL of solvent mixture and brought to boiling. In total, 25 extraction cycles were performed. After cooling down, 40 mL of deionized water were added and the flask was placed in a shaker for 30 min at the highest rate to separate all non-lipid components in the polar phase. The mixture was transferred to a separatory funnel until the phases were completely separated. The chloroform layer was then passed through a funnel with a Whatman No. 1 cellulose filter prepared with a layer of anhydrous sodium sulfate to remove the non-lipid contaminants. The solvent was then removed in a rotary evaporator and the round-bottom flask was placed in an oven at 105 °C for 2 h of final drying. After cooling down in a desiccator, the total lipid content was determined gravimetrically and expressed as mass of total lipids per mass of original macro algae biomass.

2.2.3 Proteins

The protein content was determined using high performance liquid chromatography (HPLC) by chromatographic quantification of the proteinogenic amino acids according to Lamp [13] with an Agilent Infinity 1260 HPLC Series (Agilent Technologies, USA) with fluorescence detector and an LC-Poroshell HPH-C18 separation column (4.6×100 mm, 2.7 µm; Agilent Technologies; Part No: 695975-702). The HPLC was operated in gradient mode with a flux rate of 1.5 mL min^{-1} and a column temperature of 40 °C. The mobile phase is operated according to the injection and gradient program described by Lamp [14]. For hydrolysis, 0.3 g of biomass were weighed in and 25 mL of 6 M HCl were added. The mixture was incubated for 24 h at 110 °C. The protein content is expressed as the sum of the mass of amino acids per mass of original biomass.

2.2.4 Total Phenolic Compounds

Total phenolic compounds were determined by the Folin-Ciocalteu assay [15]. For the preparation of the sample extract, 1 g of freeze-dried sample was crushed in liquid nitrogen and extracted with 50 mL of 50 % (v/v) methanol by continuous agitation for 1 h in an orbital shaker. The extract was then filtered with filter paper and used directly for total phenolic compounds determination [16]. After sample preparation, 20 µL of extract were mixed with 180 µL of Folin-Ciocalteau's reagent and incubated for 90 min at room temperature in the dark. The absorbance was subsequently measured at 765 nm in an UV-vis spectrophotometer UV-1280 (Shimadzu, Japan). Calibration was performed with a set of gallic acid standards with concentrations between 10 and 300 µg mL^{-1}.

2.2.5 Saccharides

Monosaccharides were determined on an Agilent Infinity 1260 HPLC Series (Agilent Technologies, USA) with a refractive index detector and an Agilent Hi-Plex H separation column (7.7×300 mm, 8 µm; Agilent Technologies; Part No: PL1170-6830). The HPLC was operated in isocratic mode with a mobile phase (5 mM H_2SO_4) flux of 0.5 mL min^{-1}, a

sample injection volume of 20 µL, a column temperature of 55 °C, and a detector temperature of 55 °C. Arabinose, fucose, galactose, glucose, mannose, and xylose were chromatographically quantified after hydrolysis. The two-step hydrolysis was carried out as described in the National Renewable Energy Laboratory (NREL) procedure for the determination of structural carbohydrates in biomass by Sluiter [17]. Briefly, 3 mL of 72 % (v/v) sulfuric acid were subjected to 0.3 g of biomass in a pressure tube. Samples were then incubated for 60 min at 30 °C. After this first hydrolysis step, 84 mL of deionized water were added to dilute the sulfuric acid to 4 % (v/v). Samples were mixed by shaking and then placed in an autoclave for 60 min at 121 °C. After cooling down, samples were neutralized to pH 5–6 using calcium carbonate and then centrifuged and passed through a 0.45 µm filter. Since only saccharides specifically searched for using known authentic standards can be quantified on the HPLC column, only these monomers were analyzed according to Sluiter [17]. Galactose, mannose, and xylose were determined as a sum parameter due to overlapping peaks on this column type. After determining the monosaccharide concentration in the hydrolysate, a recalculation considering potential losses of the monosaccharides during hydrolysis is applied according to the NREL procedure [17]. The detected monosaccharides are expressed as mass of detected monosaccharide per mass of original biomass.

To calculate the concentration of polymeric sugars such as cellulose, an anhydro correction factor is needed considering the addition of water to monosaccharides during hydrolysis. This factor is 0.88 (= $M_{C5,Polymer}/M_{C5,Monomer}$ = 132:150) for C_5 sugars (arabinose, xylose) and 0.9 (= $M_{C6,Polymer}/M_{C6,Monomer}$ = 162:180) for C_6 sugars (galactose, mannose, glucose) [17]. For fucose, a C_6 sugar, it must also be taken into account that in brown algae it is a molecule of the sulfated polysaccharide fucoidan and that the sulfate groups are most likely also cleaved during acid hydrolysis [18]. However, to be able to make a valid statement regarding the polymeric sugars in the biomass, the sulfate content or the fucoidan content of the samples must first be clearly determined and the three monosaccharides xylose, mannose, and galactose must be separated on a different chromatographic separation column for individual quantification. In this work, only the monomeric sugars that have been detected are quantified and presented (Tab. 1).

2.3 Design of Experiments for Enzyme Activity and Extraction Time Optimization

Design of experiments (DoE) was applied to evaluate the alginate yields as a function of extraction time and enzyme activity using the response surface methodology (RSM). The model used is an adjustment to the Box-Behnken design [19]. As input parameters, the extraction time and enzyme activity were selected. The extraction time ranged from 3 to 15 h and the enzyme activity from 40 to 500 U and 100 to 1000 U for cellulase blends and proteases, respectively. Intermediate values were randomized by the software. The output parameter was the total alginate yield.

2.4 Enzyme Activity Assays

The cellulase activity was determined by the 3,5-dinitrosalicylic acid (DNS) assay, based on the quantification of glucose monomers released after enzymatic hydrolysis of carboxymethyl cellulose (CMC) [20]. For the reaction, 200 µL of CMC were mixed with 200 µL of sodium acetate buffer (0.1 M), 50 µL of dH_2O, and 50 µL of enzyme. After 15 min reaction (50 °C and pH 4.5), the tubes were placed on ice for 10 min to stop the reaction. 500 µL DNS reagent were added and the solution was boiled in a water bath for 10 min. After cooling to room temperature, the absorbance was determined at 546 nm. The cellulase activity was measured based on a glucose standard calibration curve. One unit of activity (U) was defined as the amount of enzyme that could release 1 µmol of glucose within 1 min of reaction [21].

The activity of the proteases was determined by the azocasein assay, based on the hydrolysis of the protein casein and the quantification of released peptides loaded with the azo dye. For the reaction (50 °C and pH 7.5), 140 µL of azocasein solution were mixed with 120 µL of enzyme. The reaction was stopped after 15 min by adding 600 µL trichloroacetic acid (10 % (w/v)). The solution was left on ice for 5 min and centrifuged at 13 000 rpm at 4 °C for 10 min. 800 µL of the supernatant were pipetted into a cuvette and neutralized with 200 µL NaOH (1 M). Absorbance was measured at 420 nm. One unit of activity (U) was defined as the amount of enzyme which yielded an increase in A_{420} of 0.01 [22, 23].

2.5 Enzyme-Assisted Extraction and Alginate Purification

Two commercial cellulase blends (Viscozyme L and Celluclast 1.5L) and two commercial proteases (Alcalase 2.4L and Neutrase 0.8L) from Sigma-Aldrich (Germany) were used for alginate enzyme-assisted extraction. EAEs with cellulase blends were performed at pH = 4.5 and 50 °C in acetate buffer. For proteases, pH = 7.5 and 50 °C in Tris-HCl were used.

For alginate EAE, 3 g of pre-treated biomass were incubated with 90 mL of the corresponding buffer at 50 °C and 150 min^{-1} in a Köttermann 2737 (Köttermann GmbH, Germany) incubator. Extraction time and enzyme activity were varied according to the design of experiments protocol. The reaction was stopped by boiling the mixture at 100 °C for 10 min in a water bath and, subsequently, alginate-selective extraction was performed according to Abraham [24] with

minor adjustments. Briefly, alginate was first converted into its water-insoluble form calcium alginate by addition of a 10 % (w/v) calcium chloride solution (1:20) and separated from the water-soluble fractions by centrifugation (10 000 min^{-1}, 4 °C, 20 min). The obtained pellet was homogenized in 50 mL of deionized water (1:20) and pH was adjusted to a value below 2 using 1 M HCl to convert the calcium alginate into alginic acid. The solution was stirred for 3 h at room temperature. After phase separation by centrifugation (10 000 min^{-1}, 4 °C, 20 min), alginic acid in solid form was obtained. This was homogenized in 50 mL of deionized water (1:20) and pH was adjusted to 10 with 1 M NaOH. Finally, 96 % (v/v) ethanol was added (1:2 biomass/ethanol) and the mixture was stirred overnight at 4 °C. After a final centrifugation step (10 000 min^{-1}, 4 °C, 20 min), purified sodium alginate was frozen at –20 °C overnight and freeze-dried.

2.6 Alginate Characterization

2.6.1 Molecular Weight Distribution

The molecular weight distribution was analyzed using a gel permeation chromatography (GPC) system equipped with two PL aquagel-OH Mixed-H 8 µm 300×7.5 mm columns from Agilent (Agilent Technologies, USA). Freeze-dried sodium alginates were dissolved in 0.1 M sodium nitrate at a concentration of 1.5 mg mL^{-1}. The solution was then centrifuged at 4500 min^{-1} for 10 min in a Universal 320 R centrifuge by Hettich Zentrifugen and the supernatant was injected into the system. The analysis was conducted at 30 °C with 0.1 M sodium nitrate as mobile phase at a flow rate 1 mL min^{-1}. The calibration of the columns was performed with a pre-weighed calibration kit EasiVial polyethylene oxide (PEO)/polyethylene glycol (PEG) (Sigma-Aldrich, Germany). Measurements were performed in duplicates.

2.6.2 ATR-FTIR Analysis

The functional groups of alginates were determined with attenuated total reflection-Fourier transformation infrared (ATR-FTIR) spectroscopy. Samples were scanned at wave numbers ranging from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1} using a Vertex70 spectrometer from Bruker Instruments (Germany) with OPUS 8.5 software.

2.6.3 Purity: Protein and Total Phenolic Compounds Content

The extent of alginate purity was determined by measuring its content in proteins and total phenolic compounds with the Bradford [25] and Folin-Ciocalteu [15] assay, respectively.

3 Results and Discussion

3.1 *Fucus vesiculosus* Biochemical Composition and Alginate Extraction Yields

The biochemical composition of the used brown macro algae *F. vesiculosus* biomass (100 g dw) is shown in Tab. 1. The ash content of the biomass is almost 20.1 % dw and the protein content is 12.7 % dw. Lipids were determined to be 2 % dw and total phenolic compounds represent 7.96 % dw. The recovered monosaccharides after hydrolysis sum up to 24.8 % dw without anhydro correction. The remaining 32.4 % of the total mass balance are most likely attributed to alginate and other polysaccharides that were not quantified using this HPLC method.

Table 1. Biochemical composition of the investigated *Fucus vesiculosus* biomass.

Component	Amount [% dry weight]
Ash	20.1 ± 0.01
Lipids	2 ± 0.01
Proteins	12.7 ± 0.25
Total phenolic compounds	7.96 ± 0.37
Monosaccharides	24.8 ± 0.08
Arabinose	0
Fucose	13.5 ± 0.21
Glucose	7.3 ± 0.04
Galactose/mannose/xylose	4 ± 0.08

The influence of the extraction time and enzyme activity on the alginate extraction efficiency was studied using DoE. Modulation of the yields was done with linear fit, sum of squares, and quadratic fit. Significance was given by the p-value, which was < 0.05.

The results showed that both extraction time and enzyme activity strongly influenced the extraction yield, showing a similar behavior for the four enzymes studied: higher extraction time and enzyme activity results in higher alginate yields (Fig. 1). Nevertheless, significant differences were not observed between the different enzymes tested, reaching the highest experimental extraction yield (9.60 ± 1.03 %) with Alcalase 2.4L (15 h, 824.5 U), followed by Viscozyme (9.19 ± 0.75 %, 15 h, 500 U), Neutrase 0.8L (8.76 ± 0.22 %, 15 h, 824.5 U), and Celluclast 1.5L (8.75 ± 0.17 %, 15 h, 311.4 U).

Enzymatic cell disruption is based on the conversion of specific enzyme substrates, e.g., cellulose, fucoidan, laminarin, and proteins, found in the cell wall matrix for cell wall rupture and release of the target compounds. During the enzymatic reaction, process conditions such as low pH,

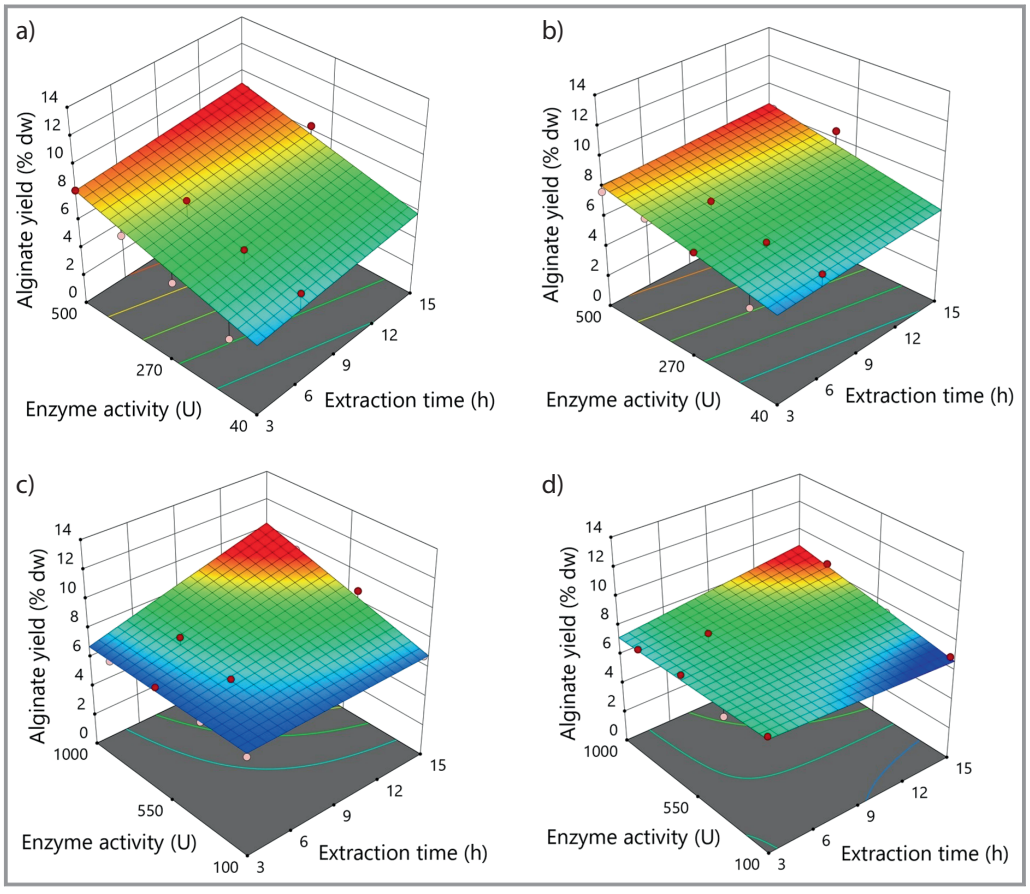

Figure 1. Alginate yields in dependency of extraction time and enzyme activity extracted with a) Viscozyme L, b) Celluclast 1.5L, c) Alcalase 2.4L, and d) Neutrase 0.8L. Dots indicate the experimental results above and below the model surface.

elevated temperatures, or shear stress also contribute to cell breakage explaining the increased extraction yields with higher extraction times and enzyme activity.

Alginate extraction yield is influenced by several factors such as the macro algae species, growth conditions as well as extraction and purification method. Borazjani [26] obtained a maximum alginate yield of 3.50 % when performing enzymatic extraction with Alcalase (5 % enzyme (w/w), pH 8, 50 °C, 24 h) followed by 3.47 % with Cellulase (5 % (w/w), pH 8, 50 °C, 24 h) from the brown macro algae *Sargassum angustifolium*. Rostami [27] extracted 6.60 % alginate from *Colpomenia peregrina* by EAE with Cellulase (5 % (w/w), pH 4.5, 50 °C, 24 h) and 3.80 % with Alcalase (5 % (w/w), pH 8, 50 °C, 24 h). Compared to these values, the maximum yields of sodium alginate in this study (8.75–9.19 %) were shown to be higher. To further increase the efficiency of the process, other reaction conditions like temperature, pH, and enzyme combinations must be optimized.

3.2 Alginate Structure and Chemical Composition

3.2.1 Molecular Weight Distribution

Fig. 2 shows the box plots of the average molecular weights of all extracted alginates determined by gel permeation chromatography.

As shown in Fig. 2, a wide range of molecular weight values were registered, reflecting a high heterogeneity in the alginate size as well as a high influence of the studied parameters. This high heterogeneity has been shown by different works in which the reported M_w values for alginate differ between macro algae species but also due to the season of collection, source, extraction method, and process conditions. Reported M_w values for macro algae sodium alginate show a broad spectrum ranging from 127 in *Cystoseira barbata* to 719 kDa in *Laminaria digitata* [10, 26, 28]. The alginate gel formation ability is increased with increased molecular weight. Furthermore, longer alginate chains lead to stronger films with lower solubility and maximum homogeneity. On the contrary, lower molecular weight results in a higher number of reactive positions

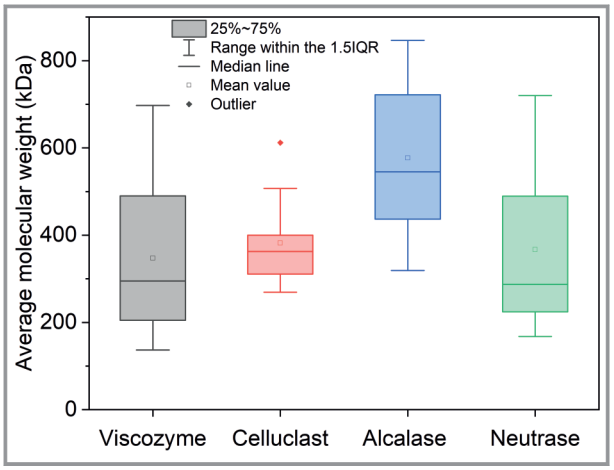

Figure 2. Box plots of average molecular weight (M_w) values of all extracted alginates determined by gel permeation chromatography. IQR, interquartile range.

available for hydrolysis in the polymer, which increases the degradation speed of biopolymer films [29]. Therefore, high molecular weight alginates, as those obtained with Alcalase 2.4L at 869.5 U and 3 h, are desired in the formation of biopolymer films.

In this work, the highest average molecular weight (847 ± 4.59 kDa) was obtained with Alcalase 2.4L (869.5 U, 3 h) followed by Neutrase 0.8L (M_w = 720 ± 0.35 kDa, 343 U, 3 h). This could be due to the different pH value used in the EAE with proteases (pH = 7.5) in contrast to that used with the cellulase blends (pH = 4.5). Acidic pH could have led to the hydrolysis of alginates into smaller polymers, thus reducing their average molecular weight. This behavior has also been observed in other studies in which more acidic extraction media leads to the production of medium to lower molecular size alginates [24, 29]. This high heterogeneity in the extracted alginate indicates that depolymerization of alginate during the extraction or purification process may have occurred [10].

3.2.2 ATR-FTIR Analysis

ATR-FTIR spectroscopy was used to study the effect of the different enzyme types and process parameters on the chemical structure and functional groups of the extracted alginates. The FTIR spectra of the extracted alginates from *F. vesiculosus* with reference to commercial sodium alginate are shown in Fig. S1 in the Supporting Information.

In general, the infrared spectra in the range of 4000 to 500 cm^{-1} of all extracted alginates are very similar to that of the commercial standard, showing similar positions of the characteristic bands reported in literature for pure alginate [26, 30, 31]. The broad signal at 3430 cm^{-1} is attributed to the stretching vibrations of hydroxyl groups O–H and the small signal at 2930 cm^{-1} corresponds to the stretching vibration of C–H. The peak at 1600 cm^{-1} is attributed to the O–C–O carboxylate asymmetric stretching and the peak at 1400 cm^{-1} corresponds to C–OH deformation vibration with symmetric stretching vibration of O–C–O. The peak at 1020 cm^{-1} is attributed to the C–O group. The fingerprint (or anomeric region) is found between 950 and 750 cm^{-1} and is assigned to uronic acid residues.

These results revealed that the use of different enzyme types, enzyme activity, and extraction times did not cause significant shifts in the characteristic peaks of pure alginate and, therefore, the chemical structure was not changed. Alginate with a high purity degree was enzymatically extracted with the four enzymes despite small signals appearing in some regions, which could be attributed to small impurities. Alginate gelling and crosslinking properties, and therefore, its capacity to form strong films, are highly influenced by its chemical structure and purity. To preserve these properties, it is necessary to apply extraction methods that result in high-purity alginates without altering its chemical structure, which was achieved with our enzymatic process.

3.2.3 Purity: Protein and Total Phenolic Compounds Content

The extent of alginate purity was further studied by measuring the level of protein and total phenolic compounds in all extracted alginates. These constitute the main molecules associated to alginate in the macro algae cell walls. The box plots in Fig. 3 represent the protein (Fig. 3a) and phenolic compounds (Fig. 3b) contents (as % of alginate dry weight) of all extracted alginates. The plots show that the use of different enzymes and process parameters resulted in alginates having different amounts of both proteins and phenolic compounds. Neutrase 0.8L (100 U, 3 h) led to the highest protein level (1.53 % of alginate dry weight). Considering that the initial protein content in *F. vesiculosus* biomass was 12.7 ± 0.25 % of the biomass dry weight, it can be confirmed that a low protein content was achieved in all alginate samples, reaching removal values of up to 98.6 % (Viscozyme L, 247 U, 3 h). Regarding total phenolic compounds, all extracted alginates contained amounts ranging from 0.22 (Viscozyme L, 488.5 U, 15 h) to 3.76 % of alginate dry weight (Celluclast 1.5L, 500 U, 3 h and Neutrase 0.8L, 595 U, 3 h, respectively). The initial content of total phenolic compounds in *F. vesiculosus* biomass was 7.96 ± 0.37 %, which means that reduction levels between 52.7 and 97.2 % were achieved.

These results show that the alginate extraction and downstream used in this work is effective in removing high levels of the main alginate contaminants, which was also observed in the ATR-FTIR analysis. This is also attributed to the pretreatment step since ethanol precipitates proteins and unbinds phenolic compounds of the macro algae cell walls. Nevertheless, the presence of proteins and low molecular weight phenols is still reported as the main drawback of polysaccharides EAE [26, 32].

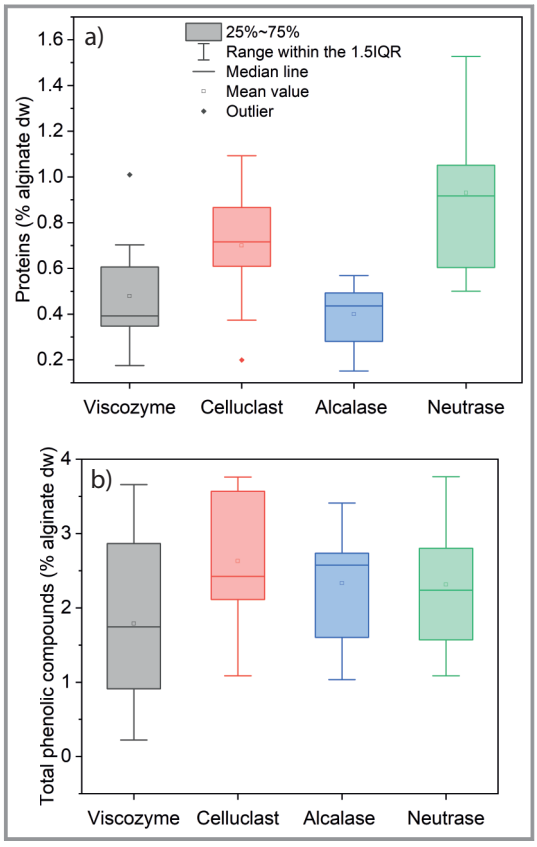

Figure 3. Box plots of the a) protein and b) total phenolic compounds content as % of alginate dry weight determined for all the extracted alginates. IQR, interquartile range.

For the development of biopolymer films, impurities should be as low as possible to avoid unwanted side effects such as changes in the stability of the material or faster material degradation, as reported for phenols by Rosiak [29].

4 Conclusions

Enzyme-assisted extraction of alginate from *F. vesiculosus* biomass has been performed in this work. Different enzyme types, enzyme activity, and extraction time values have been employed to study the effect of these parameters on the yields and main properties of the extracted alginates. For all enzymes, longer extraction time and higher activity led to higher alginate yields, reaching a maximum of 9.60 % dw with Alcalase 2.4L after 15 h of extraction and 824.5 U of enzyme activity. The characterization of the extracted alginates showed that the extraction and purification processes used in this work effectively removed up to 98.6 and 97.2 % of proteins and phenolic compounds, respectively, without structural modification of the extracted polysaccharides. The simultaneous use of different enzyme types or the combination of EAE with mechanical or hydrothermal processes could lead to an improvement of the extraction yields.

The successful transition from the crude oil era to a circular bioeconomy will depend on the efficient conversion of biological resources by sustainable and cost-effective technologies for the production of novel bioproducts.

Supporting Information

Supporting Information for this article can be found under DOI: https://doi.org/10.1002/cite.202200173.

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Symbol used

M_w	[kDa]	average molecular weight

Abbreviations

ATR-FTIR	attenuated total reflection-Fourier transformation infrared
DoE	design of experiments
dw	dry weight
EAE	enzyme-assisted extraction
GPC	gel permeation chromatography
HPLC	high performance liquid chromatography
RSM	response surface methodology

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