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INTERREG ITALY-CROATIA PROJECT BIOBASED

D.2.1.1 Conversion pathways

(Version 1.0 – 15/05/2026)

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Document Control Sheet

Project number	ITHR0200448
Project acronym	BIOBASED
Project Title	Development support of blue biorefinery in the Adriatic Sea
Start of the project	01/04/2024
Duration	30 months

Related activity	Activity 2.1 - Valorisation of seaweeds and fisheries/aquaculture by-products with a Biorefinery Approach
Deliverable name	D.2.1.1 Conversion pathways
Type of deliverable	Report on the selection of different conversion pathways (at least two) for the valorisation/upcycling of seaweed biomass and IMTA biowaste
Deliverable Leader	UNIFG (LP)
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Language	English

Status	Completed

Disclaimer

This project has been funded within Interreg Italy – Croatia 2021-2027 Programme.

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Press-release overview

BIOBASED is turning seaweed and aquaculture side-streams into a practical resource for the Adriatic blue economy. Under Activity 2.1, researchers from Italy and Croatia are selecting green conversion pathways able to transform marine biomass into natural pigments, biopolymers, proteins, fatty acids, biostimulants and nutrient-rich products. The work links laboratory analysis with the needs of small and medium-sized enterprises, so that underused materials from seaweed cultivation, IMTA systems and aquaculture processing can become ingredients, materials and services with commercial value.

This deliverable, D.2.1.1 Conversion pathways, identifies the first portfolio of routes to be tested and optimised. The focus is on two complementary seaweed platforms. The red seaweed *Gracilaria gracilis* is treated as a pigment-and-agar feedstock, because it combines water-soluble phycobiliproteins with a strong agar fraction. The green seaweed *Ulva* is treated as an *Ulva*n-and-sugar feedstock, because it contains sulfated polysaccharides and a carbohydrate-rich residue that can be routed to fermentation or biostimulant applications.

The report follows a biorefinery logic: the most sensitive and highest-value products are recovered first, then the remaining material is processed into additional products, and the final residues are directed to energy, soil amendment or nutrient recovery. This cascade approach improves resource efficiency, reduces waste and supports new business opportunities for aquaculture producers, processors and technology providers in the Italy-Croatia programme area.

Executive summary

Deliverable D.2.1.1 is the technical output of Activity 2.1, "Valorisation of seaweeds and fisheries/aquaculture by-products with a Biorefinery Approach", in Work Package 2 of the BIOBASED project. Its purpose is to select and describe conversion pathways for the valorisation and upcycling of seaweed biomass and IMTA biowaste. The selected pathways are designed around the experimental composition of *Gracilaria gracilis*, *Ulva* laetevirens and *Ulva* sp. and around the project requirement to compare conventional and unconventional extraction technologies using green solvents and cascade processing.

The dataset included in the draft report confirms that *Gracilaria gracilis* is a strong candidate for a high-value-first cascade. The biomass contains a high galactan fraction associated with agar, protein values in the range of roughly 18-20% dry weight, and measurable phycobiliproteins. The analytical profile supports a sequence in which cold aqueous extraction of phycobiliproteins is performed first to preserve pigment integrity, followed by hot-water agar extraction and then residual biomass valorisation. This order is preferable to an agar-first sequence when the objective is to preserve R-phycoerythrin and related chromoproteins.

The *Ulva* samples show a different but complementary profile. They are dominated by carbohydrate fractions, with total sugars reaching 64.24% dry weight in one sample and with protein content varying widely across samples. The specific sugar analysis of the *Ulva* fraction shows glucose, rhamnose, xylose and uronic-acid-related components consistent with an *Ulva*n-rich matrix. This supports an *Ulva*n-first route followed by saccharification of residual cellulosic and glucan fractions into fermentable sugars or biostimulant substrates.

Four conversion routes are therefore selected for further testing. Two are core pathways: the *Gracilaria* pigment-to-agar cascade and the *Ulva* *Ulva*n-to-sugar cascade. Two are supporting pathways that improve whole-biomass use: recovery of proteins, lipids, phenolics and PUFA-rich minor fractions when technically and economically justified, and conversion

of residual solids and IMTA side-streams into NPK-rich products, biochar, compost, energy carriers or recirculated process streams. The selected portfolio satisfies the deliverable requirement of at least two conversion pathways and provides a flexible technology set for pilot optimisation.

The report recommends that pilot experiments prioritize a small number of measurable process variables: solid/solvent ratio, extraction time, temperature, pH, pretreatment intensity, water quality, drying method and sequence order. For every stage, both the extract and the residue should be characterised. This is critical because a cascade process can only be optimised when the effect of each step on downstream yields and product quality is known.

The expected output is not a single product but a staged product portfolio. For *Gracilaria*, the portfolio includes phycobiliprotein-rich extracts, agar fractions, protein and mineral-rich residues, and potential biochar or biostimulant products. For *Ulva*, the portfolio includes *Ulva*n polymer or oligosaccharides, fermentable sugar streams, protein-rich residues, phenolic fractions, minerals and final residue products. Together, these pathways support the BIOBASED objective of strengthening innovation capacities in the Adriatic blue economy by moving from biomass disposal toward integrated valorisation.

Summary of the selected conversion pathway portfolio for D.2.1.1.

Selected pathway	Feedstock	Primary products	Secondary/residue products	Selection rationale
Pathway 1	*Gracilaria gracilis*	Phycobiliproteins	Agar and residue products	Protects heat-sensitive pigments before hot-water agar extraction
Pathway 2	*Gracilaria gracilis* residue	Agar	Protein/mineral residues, biostimulants, biochar or digestion	Uses the dominant agar-forming fraction after pigment recovery
Pathway 3	*Ulva* spp.	*Ulva*n polymer/oligosaccharides	Residual sugars and protein-rich residues	Preserves the functional sulfated

				polymer before hydrolysis
Pathway 4	*Ulva* residue and IMTA biowaste	Sugars, nutrients, residue products	Closes the cascade and supports zero-waste valorisation	

Graphical abstract and pathway snapshot

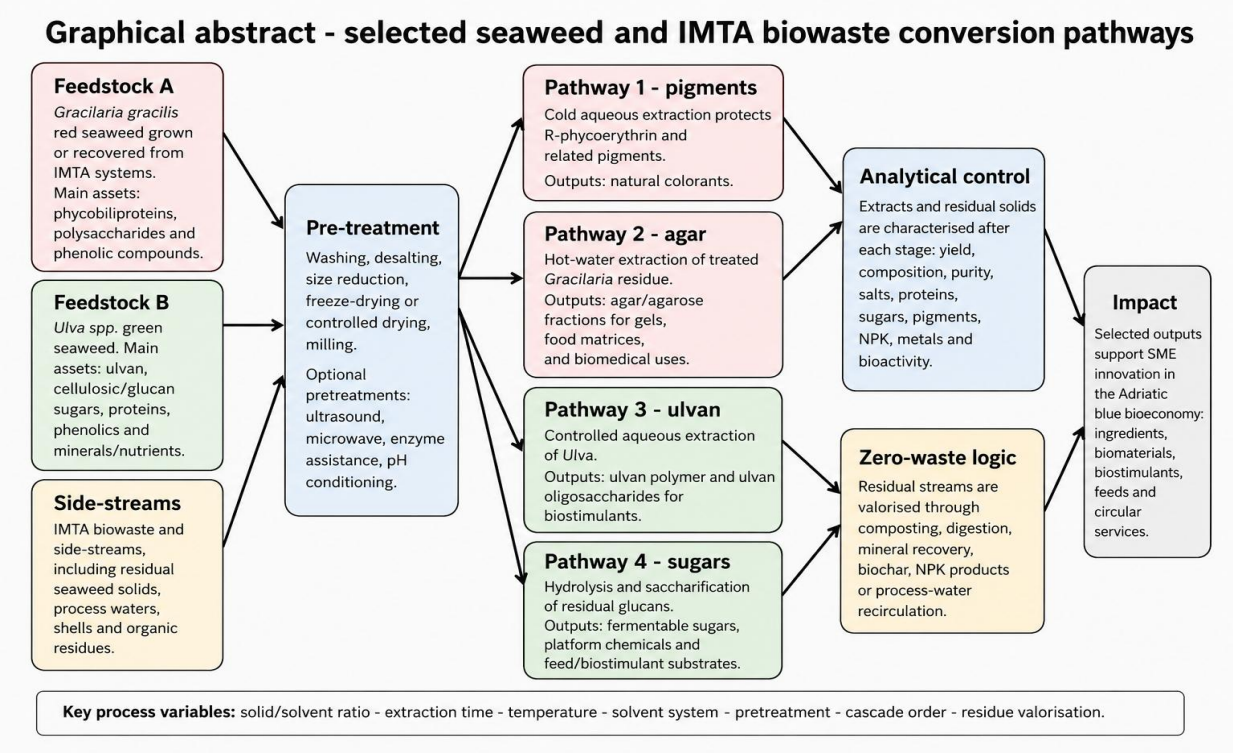

Figure 1. Graphical abstract representing the identified valorisation pathways for Gracilaria gracilis, Ulva and IMTA side-streams.

The graphical abstract summarises the pathway portfolio selected in this deliverable. It presents two species-specific streams that share a common pretreatment and analytical-control logic. *Gracilaria* is directed first to pigment extraction and then to agar recovery. *Ulva* is directed first to *Ulva*n extraction and then to residual carbohydrate conversion. Both species converge in the residue valorisation block, where remaining solids and IMTA biowaste are routed to nutrient, energy, biochar, compost or biostimulant options.

The diagram also shows the process variables that must be controlled during Activity 2.1: solid/solvent ratio, extraction time, temperature, solvent system, pretreatment and cascade

order. These variables are not isolated laboratory parameters. They determine both product yield and downstream residue quality. For that reason, the pathway selection in this report is based on whole-cascade performance rather than single-step extraction yield.

1. Project context and deliverable reference

The BIOBASED project, identified in the signed application form as ITHR0200448, addresses the development of blue biorefinery capacity in the Adriatic Sea. The project connects seaweed production in Integrated Multi-Trophic Aquaculture systems with the valorisation of seaweeds, fisheries by-products and aquaculture side-streams. Its practical ambition is to support producers and processors in Italy and Croatia by transferring research knowledge into greener products, processes and services.

Work Package 2 is dedicated to the introduction of the biorefinery approach in Adriatic aquaculture. The work package aims to analyse conditions for process industrialisation and to support the emergence of a chain of producers and processors. Activity 2.1, the specific activity behind this report, concerns the identification of green technologies for converting co-products and by-products into classes of commercial value. This includes sequential extraction, comparison of conventional and unconventional techniques, use of green solvents and analysis of extracts and residues after each stage.

The present deliverable is D.2.1.1 "Conversion pathways - Report on the selection of different conversion pathways (at least two) for the valorisation/upcycling of seaweed biomass and IMTA biowaste". The deliverable therefore has a selection function: it does not merely describe what could theoretically be done with seaweed biomass; it establishes which routes should be prioritised for BIOBASED testing, optimisation and dissemination.

The activity description establishes a clear technical boundary. Target products include phycobiliproteins, lipophilic pigments, proteins, polyunsaturated fatty acids, polysaccharides, biostimulants and nutrients such as NPK. The process boundary includes raw material pretreatments, extraction technologies, solvent choice, yield, solid/solvent ratio, extraction time, extraction temperature and residue composition. These parameters form the basis of the pathway selection matrix used throughout this report.

The report is also written for a mixed audience. The technical chapters are intended for researchers, bioprocess engineers and project partners who will define and execute laboratory and pilot work. The introductory sections and the summary boxes are intended for SMEs, communication staff and local stakeholders who require a clear explanation of how seaweed and IMTA side-streams can move from low-value biomass to product classes with market relevance.

The selection of pathways is built on the experimental evidence already available in the draft report. This evidence includes macromolecular composition, elemental composition, amino-acid profiles, lipid and unsaponifiable fractions, phenolic content, phycobiliprotein content, agar-related sugars, *Ulva*n-related sugars and biomass productivity estimates from IMTA configurations. Rather than treating these data as isolated analytical results, this deliverable interprets them as decision variables for a cascade biorefinery.

The main recommendation is to prioritise two core pathways: a *Gracilaria gracilis* route based on cold recovery of phycobiliproteins followed by agar extraction, and an *Ulva* route based on *Ulva*n extraction followed by hydrolysis of residual carbohydrate fractions. Residual streams from both routes should be integrated into nutrient, biostimulant, biochar, compost, energy or anaerobic digestion routes. This gives BIOBASED an integrated portfolio rather than a single-product extraction scheme.

- Activity: 2.1 - Valorisation of seaweeds and fisheries/aquaculture by-products with a Biorefinery Approach.
- Deliverable: D.2.1.1 - Conversion pathways.
- Lead contribution: UNIFG, with involvement of CNR_IRBIM and UNIDU as reported in the application form.
- Core selected pathways: *Gracilaria* pigment-to-agar cascade and *Ulva* *Ulva*n-to-sugar cascade.

2. Biorefinery rationale for Adriatic seaweed and IMTA side-streams

A seaweed biorefinery differs from a conventional extraction plant because it is organised around whole-biomass use. In a conventional single-product process, a valuable molecule is removed and the remaining biomass is often treated as a waste. In a biorefinery, the residue from one operation is the feedstock for the next. This distinction is particularly important for marine biomass, where water, salts, proteins, polysaccharides, minerals and pigments are all present in the same matrix and where processing conditions for one product can strongly affect downstream fractions.

Macroalgae are suitable for this approach because their biochemical architecture is inherently multifunctional. Red algae such as *Gracilaria* contain sulfated galactans, water-soluble pigments, proteins, minerals and minor lipids. Green algae such as *Ulva* contain sulfated rhamno-uronic polysaccharides, cellulosic/glucan fractions, proteins, pigments, phenolics and mineral nutrients. These classes do not have the same market value, stability or extraction requirements; therefore, a cascade order must be chosen deliberately.

The Adriatic context strengthens the case for an integrated cascade. IMTA systems can produce seaweed biomass while also providing ecosystem services such as nutrient capture and diversification of aquaculture production. Side-streams from aquaculture and seafood processing can also contain mineral, protein and organic fractions suitable for valorisation. When seaweed and IMTA residues are managed together, the processing model can reduce waste and create local product opportunities instead of relying on disposal.

The first design principle is to protect labile high-value compounds. Phycobiliproteins are sensitive to temperature, pH, light and oxidative stress, so they should be extracted under mild aqueous conditions before harsher treatments are applied. Lipophilic pigments and PUFA-rich fractions can also be degraded by oxygen and heat. These products are often present in lower mass yield than structural polysaccharides, but they can contribute disproportionately to economic feasibility when suitable purity grades are reached.

The second design principle is to route structural carbohydrates to their most appropriate product form. In *Gracilaria*, the agar fraction is a major value driver because it has established uses in food, microbiology and biotechnology. In *Ulva*, the *Ulva*n fraction is scientifically and commercially attractive because its sulfated structure gives it functionality beyond simple carbohydrate content. Residual glucans, once depleted of higher-value fractions, can be hydrolysed to sugars or directed to biostimulants and fermentation routes.

The third design principle is to maintain residue quality. Strong acid treatment, uncontrolled thermal processing or excessive salt carry-over may improve an upstream extraction yield but damage downstream valorisation. For this reason, BIOBASED should evaluate not only the concentration of target compounds in each extract but also the remaining protein, carbohydrate, ash, salt and mineral composition of each residue. This residue-centred view is essential for determining whether a cascade is truly sustainable.

The fourth design principle is process simplicity. SMEs require technologies that are technically robust, safe and compatible with realistic investment capacity. Water extraction, mild pH control, ultrasound-assisted extraction, microwave-assisted extraction and enzyme-assisted steps are more relevant to project uptake than highly specialised methods that cannot be scaled or maintained locally. The selected pathways therefore favour process steps that can be tested at laboratory scale and translated toward pilot demonstrations.

The fifth design principle is graded product quality. Not every extract has to be purified to pharmaceutical or analytical grade. Food, feed, cosmetic, agricultural and material applications can often tolerate different specifications and may be more accessible to early-stage SMEs. A biorefinery portfolio should therefore define multiple grades for the same molecule class: crude functional extracts, semi-purified ingredients, refined polymers and high-purity speciality products.

3. Feedstock basis: *Gracilaria gracilis*, *Ulva* and IMTA biowaste

Gracilaria gracilis is a red macroalga of particular interest because it combines a structural polysaccharide platform with water-soluble pigment proteins. In practical terms, it is not simply an agar feedstock. It is a biomass that can deliver phycobiliprotein-rich extracts, agar fractions, proteins, minerals and residue products if the processing order is designed correctly. The experimental draft confirms this dual value by reporting both agar-related composition and measurable R-phycoerythrin, allophycocyanin and phycocyanin fractions.

The *Gracilaria* route is attractive for cascade processing because the major target products have different extraction windows. Phycobiliproteins are recovered in aqueous media under mild conditions, while agar requires hot-water treatment and often benefits from conditioning steps that improve gel quality. If hot extraction is performed first, pigment proteins are likely to be denatured or lost. If cold pigment extraction is performed first, the biomass residue can still be used for agar recovery. This logic strongly supports pigment-first processing.

Ulva laetevirens and *Ulva* sp. represent a green seaweed platform with a different emphasis. The draft data show high carbohydrate fractions and variable protein content. The key polysaccharide is *Ulva*n, a sulfated heteropolysaccharide associated with rhamnose, uronic acids, xylose and sulfate groups. *Ulva*n is not just a bulk carbohydrate; it is a functional polymer whose molecular weight, sulfation pattern and purity can influence material and biological properties. This makes extraction conditions especially important.

The *Ulva* route is also relevant for resource efficiency because the residue after *Ulva*n extraction can still contain glucose-rich cellulosic and glucan fractions. These fractions can be hydrolysed to fermentable sugars or converted into biostimulant substrates. The presence of rhamnose, xylose and uronic acids requires caution, because conventional fermenting microorganisms do not use all of these sugars efficiently. Therefore, fermentation should be considered as one of several outlets, not as the only residue route.

IMTA biowaste and aquaculture by-products broaden the scope beyond seaweed. In this report, IMTA biowaste is considered as the set of side-streams generated around seaweed and shellfish cultivation and processing: residual seaweed solids after extraction, mixed organic residues, saline process waters, shell-rich fractions, epiphytes and non-target biomass. The exact composition of these streams should be measured case by case, but they can be incorporated into the same cascade logic through nutrient recovery, mineral products, composting, biochar and digestion.

Feedstock variability is a central challenge. Seaweed composition changes with species, season, salinity, light, nutrient availability, cultivation density and post-harvest handling. The same nominal species can therefore behave differently in extraction. The draft data illustrate this point by showing variable protein and carbohydrate contents across *Ulva* samples and moderate variation across *Gracilaria* samples. The selected pathways must therefore be resilient rather than dependent on a single fixed composition.

The pathway selection in this deliverable uses the available data as a baseline but assumes that further sampling will refine the mass balance. This is appropriate for Activity 2.1, which is expected to test and optimise technologies. The report therefore distinguishes between confirmed analytical observations from the draft and process recommendations to be validated during pilot work.

Figure 2. Gracilaria gracilis biomass in marine conditions, included from the draft report image material.

Figure 3. Ulva and Gracilaria handling in the IMTA context, included from the draft report image material.

4. Experimental data as pathway-selection evidence

The experimental dataset shows that the two biomass groups should not be processed with the same priority order. *Gracilaria* samples are characterised by a stable combination of sugars, proteins and lipids, with sufficient pigment content to justify a mild first extraction. *Ulva* samples are more carbohydrate-dominant and show greater variability in protein content. These differences create two complementary conversion platforms: a *Gracilaria* high-value extract and agar platform, and an *Ulva Ulva*n and sugar platform.

The macromolecular data indicate that sugars are the dominant fraction in all samples but are especially prominent in *Ulva* laetevirens. One *Ulva* laetevirens sample reaches 64.24% dry weight total sugars, which is a strong signal for polysaccharide and carbohydrate conversion. *Gracilaria* samples show sugars around 40-42% dry weight and proteins around 18-20% dry weight, indicating a balanced matrix where both proteinaceous pigments and agar can be relevant.

The lipid dataset confirms that neither *Gracilaria* nor *Ulva* should be treated primarily as an oil crop. Lipid values are too low for a biodiesel-first route. However, the presence of unsaponifiable fractions and FAMEs can still be relevant in a cascade if high-value lipidic components, pigments or PUFA-rich fractions are recovered in small volumes. The correct interpretation is therefore not "lipids are irrelevant" but "lipids are secondary products, not the core pathway driver".

The elemental composition and C/N ratios provide information for residue routing. Samples with balanced C/N ratios can support microbial conversion, digestion or composting more easily than highly carbon-rich materials. A high C/N ratio in one *Ulva* laetevirens sample suggests carbohydrate dominance and potential need for nitrogen balancing if anaerobic digestion or fermentation is selected. This is important because downstream operations must be designed around residues, not only primary extracts.

The phenolic content is modest but relevant for functional extracts. Phenolic compounds may contribute antioxidant capacity and can influence product positioning for food, feed, cosmetic or agricultural applications. Because the total phenol values are not the main mass component, phenolics should be considered a supporting co-product or quality marker rather than a stand-alone extraction target at this stage.

The phycobiliprotein data from *Gracilaria* are particularly important for pathway order. R-phycoerythrin is present at higher levels than allophycocyanin and phycocyanin in the two analysed *Gracilaria* cases. Since these proteins are water-soluble and heat-sensitive, their recovery should precede any hot extraction, alkaline treatment or severe pretreatment. This justifies the first stage of the *Gracilaria* cascade.

The specific carbohydrate tables confirm the product identity of the two core polysaccharide routes. The *Gracilaria* agar-related table is dominated by galactose and associated products, with a total reported fraction of 70.53% dry weight in the analysed agar fraction. The *Ulva* table shows glucose, rhamnose, xylose and uronic-acid-related compounds with a total of 46.27% dry weight in the *Ulva*n-related fraction. These results support product-specific extraction rather than generic carbohydrate recovery.

The biomass productivity estimates included in the draft are useful because they translate laboratory yields into operational quantities. They show differences between mussel-associated and oyster-associated configurations, between densities and between seasons. Such estimates should be treated as first planning values for pilot campaigns. They also underline the need to connect extraction yield with biomass availability, because a high-value compound may still require sufficient feedstock flow to be commercially meaningful.

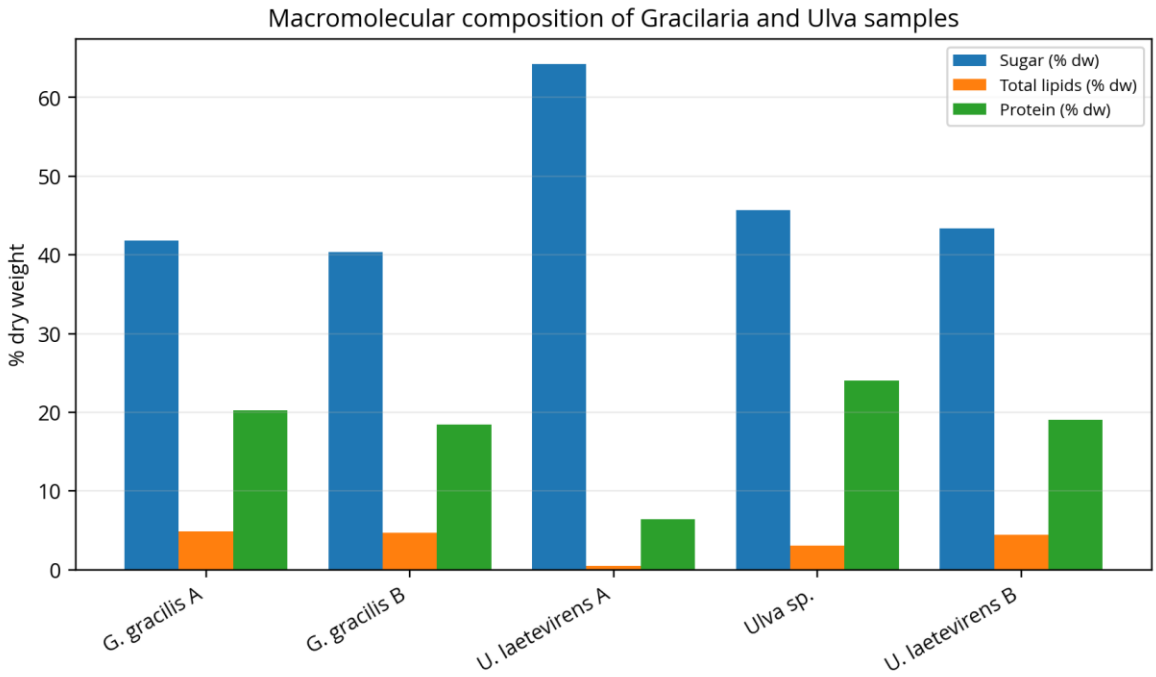

Figure 4. Macromolecular composition of Gracilaria gracilis, Ulva laetevirens and Ulva sp. based on data extracted from the draft report.

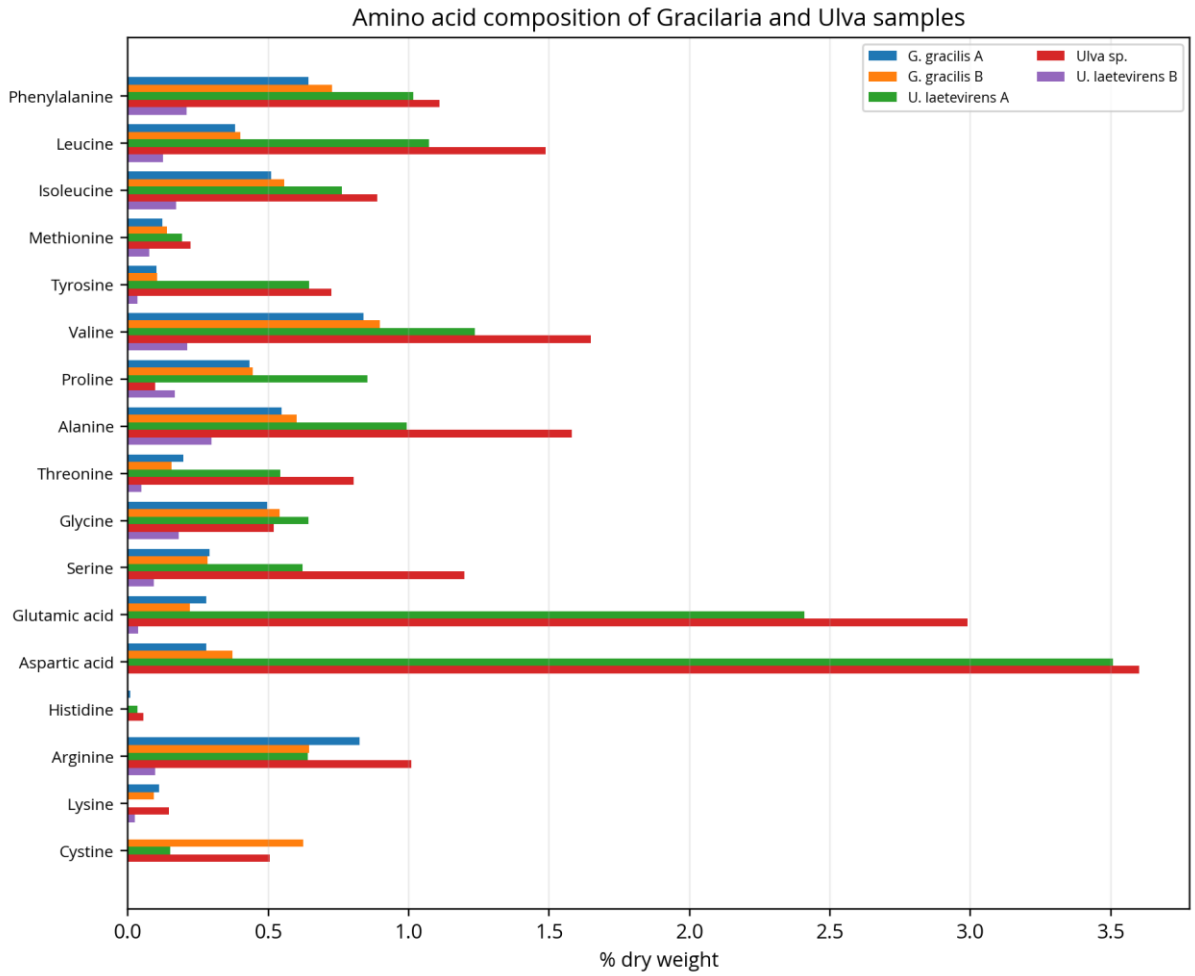

Figure 5. Amino acid composition of Gracilaria and Ulva samples based on data extracted from the draft report.

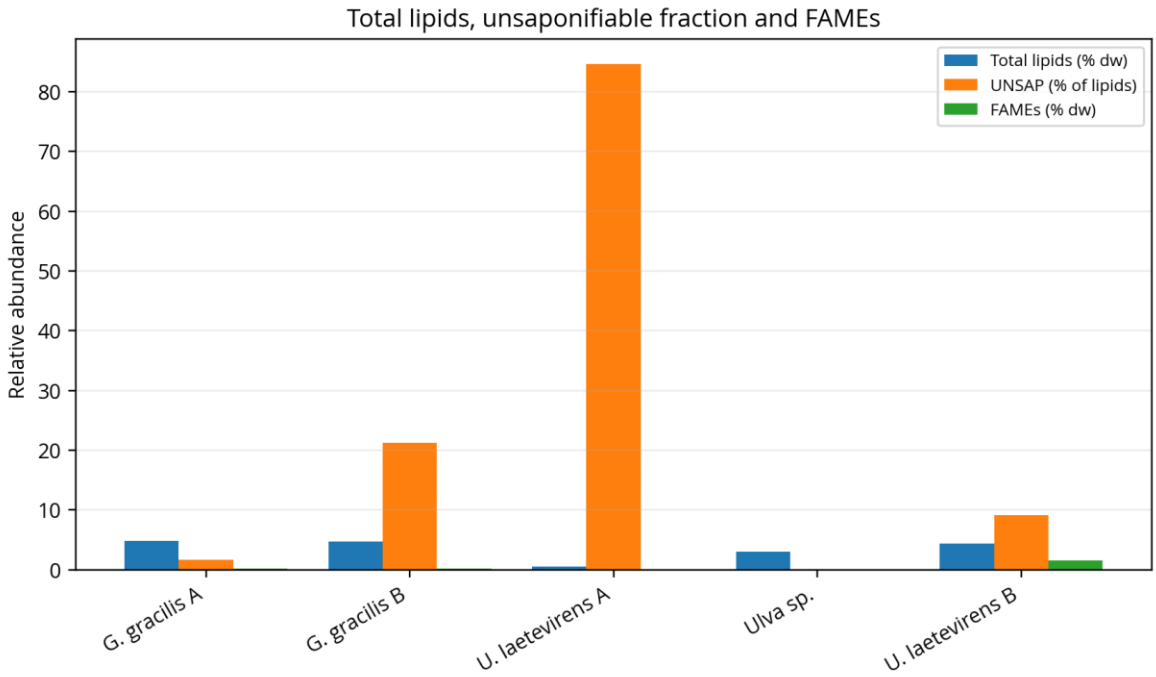

Figure 6. Total lipids, unsaponifiable fraction and FAMEs based on data extracted from the draft report.

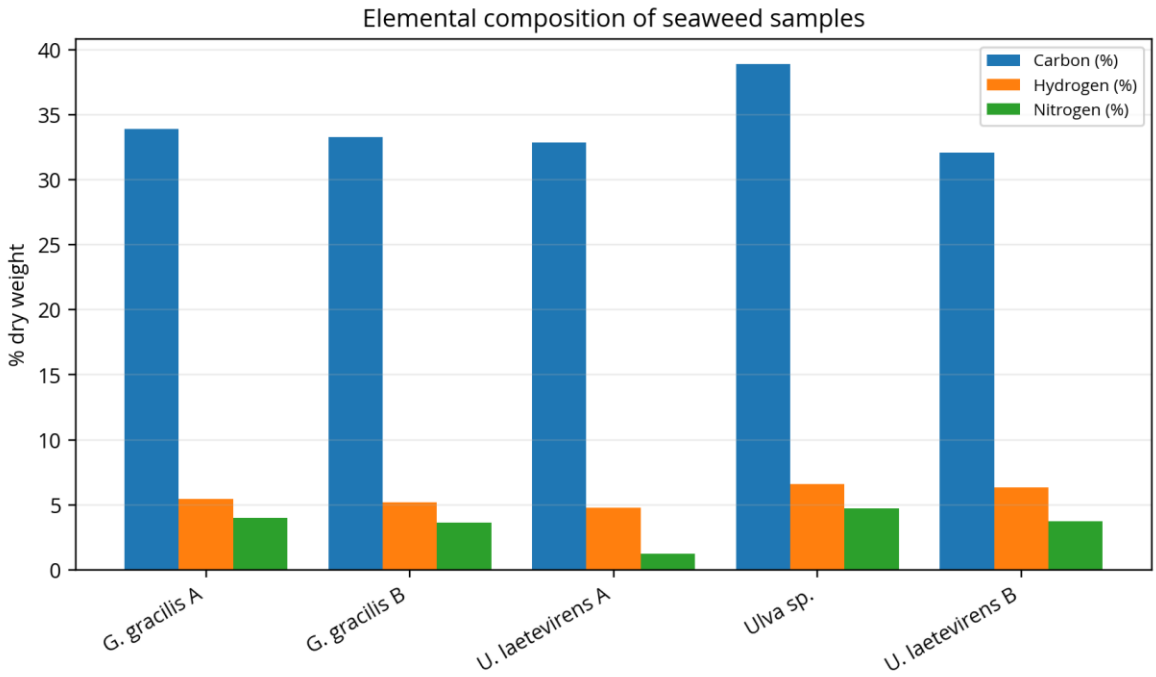

Figure 7. Elemental composition of the seaweed samples based on data extracted from the draft report.

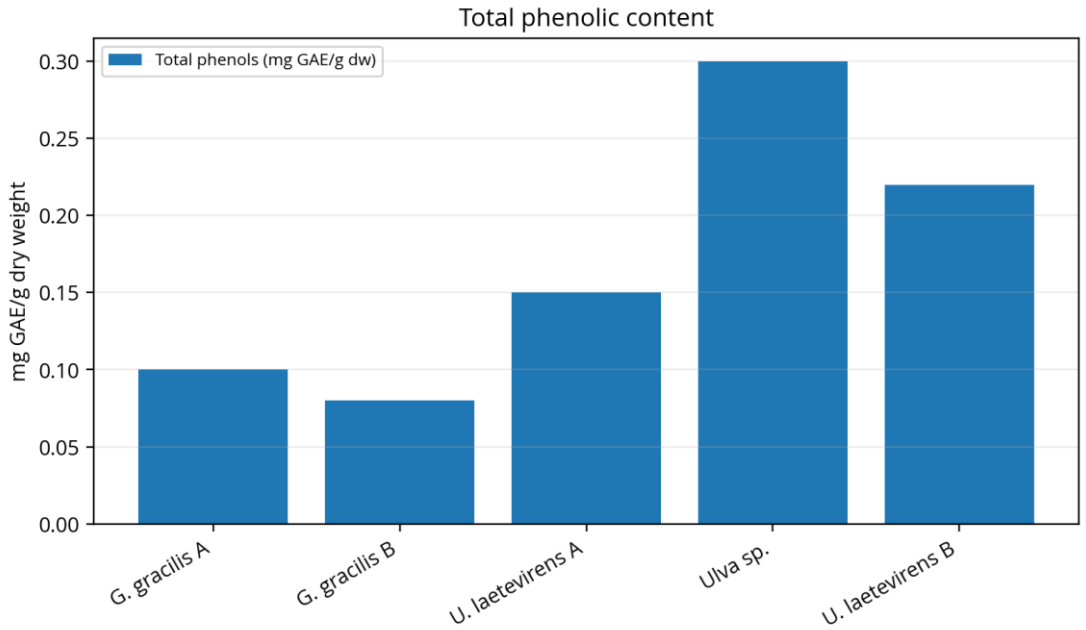

Figure 8. Total phenolic compounds in Gracilaria and Ulva samples based on data extracted from the draft report.

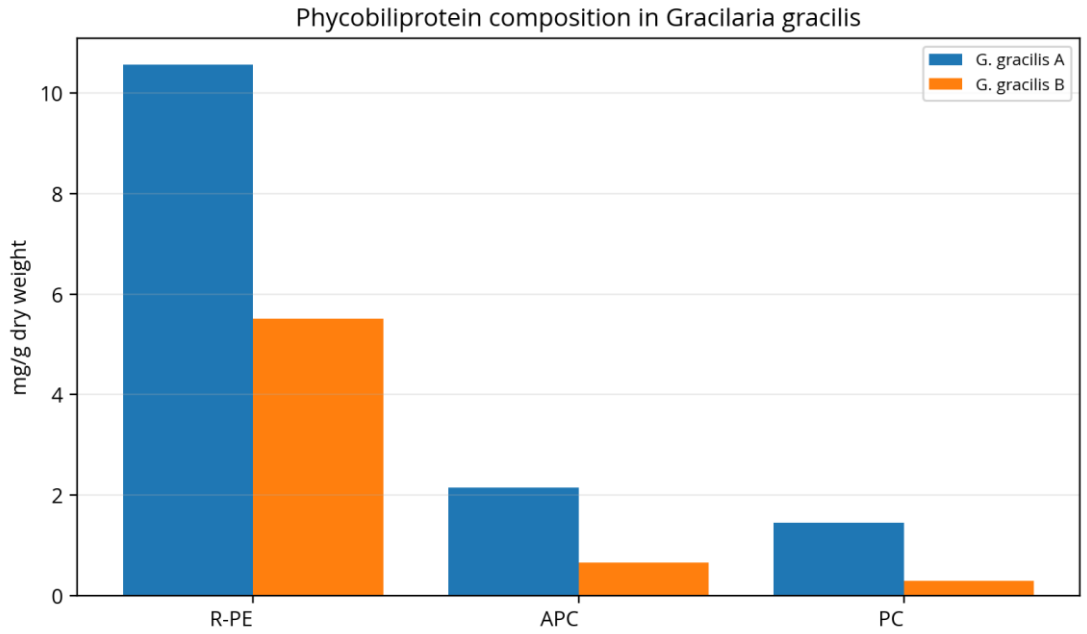

Figure 9. Phycobiliprotein composition in Gracilaria gracilis based on data extracted from the draft report.

Table 1. C:N ratios and process interpretation for Gracilaria and Ulva samples.

Species	C:N ratio	Biorefinery interpretation
Gracilaria gracilis	8.5	Balanced C/N; suitable for protein recovery and anaerobic digestion of residues
Gracilaria gracilis	9.2	Balanced C/N; suitable for protein recovery and anaerobic digestion of residues
Ulva laetevirens	26.3	Carbohydrate-rich sample; suitable for carbohydrate conversion and *Ulva*n-first processing
Ulva laetevirens	8.6	Balanced C/N; suitable for protein recovery and digestion of residues
Ulva sp.	8.3	Balanced C/N; suitable for protein recovery and digestion of residues

5. Green technology selection criteria

The technology selection for Activity 2.1 should be guided by green chemistry, product quality and scale-up realism. A green technology is not defined only by the absence of toxic solvents. It must also reduce energy demand, minimise water consumption, avoid unnecessary neutralisation steps, maintain product quality, enable residue use and remain safe for operators. In a cascade biorefinery, a technology that gives a high yield but destroys downstream residues may be less sustainable than a milder process with slightly lower primary yield.

Conventional aqueous extraction remains the benchmark for both *Gracilaria* and *Ulva* because water is compatible with food, feed, agricultural and many cosmetic applications. For phycobiliproteins, cold buffered water can preserve protein conformation. For agar and *Ulva*n, water extraction is the natural starting point because both target fractions are hydrophilic polymers. The role of unconventional technologies is therefore to improve kinetics, reduce extraction time or increase accessibility, not to replace the need for product-specific chemistry.

Ultrasound-assisted extraction can improve mass transfer by cavitation, cell wall disruption and boundary-layer thinning. For pigments and proteins, ultrasound should be mild enough to avoid denaturation or excessive foaming. For polysaccharides, it can reduce extraction time but may also decrease molecular weight if applied too intensely. This is particularly relevant for *Ulva*n, where molecular weight is linked to material performance and some biological properties.

Microwave-assisted extraction can accelerate heating and improve penetration of water into biomass. It may be useful for agar or *Ulva*n extraction where controlled thermal treatment is required. However, it must be carefully assessed for phycobiliproteins and PUFA-rich fractions because thermal hotspots can damage labile compounds. A sequence in which

microwave is reserved for polysaccharide extraction after pigment removal is therefore preferable for the *Gracilaria* route.

Enzyme-assisted extraction is relevant when the target is embedded in a robust cell wall matrix or when the objective is selective depolymerisation. Enzymes can improve liberation of pigments or proteins under mild conditions and can generate oligosaccharides from *Ulva*n or residual glucans. The main limitations are enzyme cost, reaction time, sensitivity to salts and the need to control downstream purity. Enzyme-assisted steps should therefore be tested as targeted improvements rather than default operations for every stream.

Solvent selection should follow the expected product class. Water and food-compatible aqueous buffers are preferred for phycobiliproteins, proteins, *Ulva*n and agar. Ethanol or other low-toxicity solvents may be considered for phenolics and certain pigments if the product destination allows them. Chloroform/methanol protocols remain useful for analytical lipid determination but are not appropriate as the default process route for an SME-oriented green biorefinery. Analytical methods and production methods should therefore be clearly separated.

Pretreatment selection must consider what the pretreatment does to both the target and the residue. Washing and desalting reduce salt load and improve downstream quality. Freeze-drying preserves labile compounds but may be expensive at scale, while controlled low-temperature drying may be more realistic. Milling increases surface area but can accelerate oxidation. Alkaline conditioning may improve agar properties but can damage proteins. These trade-offs should be explicitly recorded in the experimental worksheet.

A practical technology screening should score each option against yield, product integrity, selectivity, energy intensity, solvent safety, processing time, residue quality, equipment availability and scalability. These criteria are used later in this report to justify the selected pathways and to define which parameters should be optimised during Activity 2.1 experiments.

Table 2. Key optimisation variables for Activity 2.1 extraction trials.

Variable	Main stage	Why it matters	Suggested response variable
Solid/solvent ratio	All extraction stages	Yield, concentration, water use	g dry biomass per mL solvent; recovery per kg biomass
Time	All extraction stages	Mass transfer and degradation	Target concentration versus time; stability
Temperature	Pigments, agar, *Ulva*n	Protein integrity and polymer recovery	Yield plus purity/gel/molecular-weight indicators
pH	Pigments, proteins, *Ulva*n	Charge, solubility, stability	Target yield, purity, residue quality
Pretreatment intensity	UAE, MAE, enzymes, milling	Cell disruption, degradation risk	Yield increase versus energy/time/residue damage
Drying method	Raw biomass preparation	Stability and cost	Moisture, pigment retention, energy estimate

Figure 10. Laboratory workflow and sample preparation images included from the draft report.

Figure 11. Liquid-liquid lipid extraction workflow included from the draft report.

6. Selected core pathway 1 - *Gracilaria* pigment-to-agar cascade

The first core conversion pathway selected for BIOBASED is the *Gracilaria* pigment-to-agar cascade. The route begins with cleaned and milled *Gracilaria gracilis* biomass and applies a mild aqueous extraction to recover water-soluble phycobiliproteins. The extraction residue is then used for hot-water agar extraction. The remaining solid and liquid side-streams are routed to protein, mineral, biostimulant, compost, digestion or biochar options depending on composition and local needs.

The scientific logic is straightforward. Phycobiliproteins are valuable but labile, whereas agar is abundant and thermally extracted. A hot agar-first process would make pigment recovery unlikely. A pigment-first process preserves the high-value proteinaceous fraction while leaving the agar-forming polysaccharide matrix available for subsequent extraction. This is the main reason this pathway is selected as the priority *Gracilaria* route.

The first operation should be a mild washing and desalting stage. Salt reduction improves extraction reproducibility, decreases interference in protein measurement and helps later product formulation. Washing must be gentle enough to avoid pigment loss and should be performed with controlled water volumes to prevent excessive wastewater generation. Process water can be analysed for soluble salts, nitrogen and organic matter to assess potential recirculation or nutrient recovery.

The pigment extraction stage should use cold water or a food-compatible buffer, controlled pH, limited light exposure and short residence time. Solid/solvent ratio should be varied systematically because it affects both yield and downstream concentration. Too much solvent may improve extraction completeness but increases concentration costs. Too little solvent may reduce mass transfer and increase viscosity. The preferred operating window should be based on pigment yield per kilogram biomass and pigment concentration per litre extract.

Clarification is critical after pigment extraction. Centrifugation, filtration or membrane separation may be needed to remove cell debris. The crude extract can be evaluated as a lower-purity functional colourant, while a semi-purified fraction can be obtained using ammonium sulfate precipitation, membrane concentration or chromatographic polishing if higher-value applications are pursued. For Activity 2.1, the recommended first comparison is not full purification but yield and stability of crude and semi-purified extracts.

After pigment removal, the solid residue should be characterised before agar extraction. This intermediate analysis is important because the first extraction may remove proteins, salts and soluble sugars that influence agar yield and gel quality. The residue should be assessed for moisture, ash, protein, carbohydrate and colour. If the residue is too wet, direct hot-water extraction may still be preferable to energy-intensive drying, but the mass balance must be corrected to dry weight.

The agar extraction stage should use hot water and defined time-temperature conditions. Optional alkaline pretreatment can be evaluated if the objective is to improve gel strength or reduce sulfate content, but it should be tested against residue quality and chemical consumption. The crude agar extract is filtered, cooled, gelled, recovered and dried. Gel strength, gelling temperature, melting temperature, colour and ash content should be considered product-quality indicators alongside mass yield.

The pathway creates several product-grade options. A crude pigment extract could be positioned for colour or antioxidant screening. A semi-purified R-phycoerythrin-rich fraction could be assessed for higher-value uses. Agar can be developed as food-grade, technical-grade or biomaterial-grade material depending on purity. Residual solids can be used for biostimulants, compost, anaerobic digestion or thermochemical conversion. This portfolio approach increases resilience compared with relying on a single product.

The main technical risks are pigment degradation, microbial contamination in aqueous extracts, agar quality variability and excessive water use. These risks can be mitigated

through cold processing, rapid clarification, hygienic handling, standardised biomass drying, process water monitoring and mass-balance-based optimisation. The route is therefore technically realistic but requires disciplined process control.

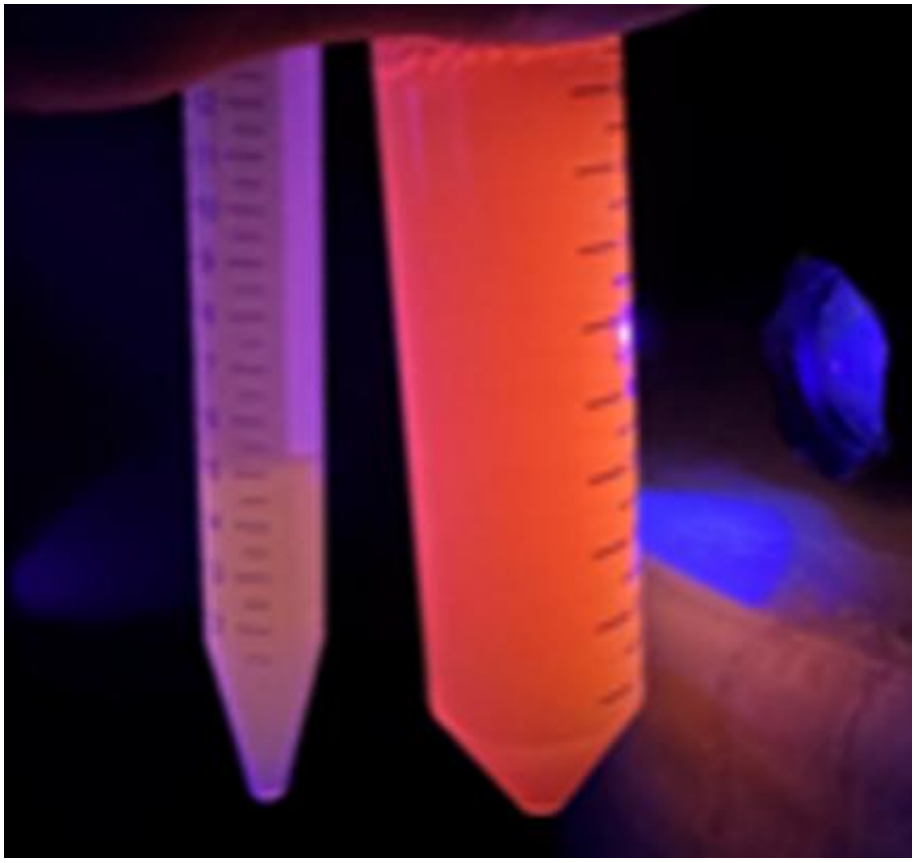

Figure 12. Phycobiliprotein extracts from Gracilaria gracilis included from the draft report.

Figure 13. Agar fraction recovered from Gracilaria gracilis included from the draft report.

Table 3. Composition of agar-related fraction from Gracilaria gracilis.

Structural carbohydrate / derivative	g/g dry weight	% dry weight
Galacturonic acid	0.0574	5.74
Glucose	0.0805	8.06
Galactose	0.3958	39.58
Rhamnose	0.0273	2.73
Arabinose	0.0270	2.70
Levulinic acid	0.0897	8.97
5-HMF	0.0274	2.74
Total	0.7052	70.53

7. Selected core pathway 2 - *Ulva Ulva*n-to-sugar cascade

The second core conversion pathway selected for BIOBASED is the *Ulva Ulva*n-to-sugar cascade. The route begins with washed and size-reduced *Ulva* biomass, proceeds through controlled aqueous extraction of *Ulva*n, and then converts the remaining carbohydrate-rich residue into sugars, oligosaccharides, biostimulant substrates or microbial conversion feedstocks. Final residues are directed to nutrients, compost, digestion or biochar depending on composition.

The selection is justified by the analytical profile of the *Ulva* samples. High total sugars, combined with the *Ulva*n-related sugar fraction, indicate that the biomass contains both a functional sulfated polymer and a residual carbohydrate pool. These are not interchangeable products. *Ulva*n value depends on preserving polymer structure and sulfation, while sugar conversion depends on efficient depolymerisation. The cascade must therefore separate polymer extraction from residue hydrolysis.

The first processing step should be washing and desalting. *Ulva* can retain salts and sand, which interfere with polymer quality, ash content and downstream fermentation. However, excessive freshwater washing can leach soluble components. The washing stage should therefore be measured through conductivity, ash reduction and dry-matter loss. If desalting water contains nutrients, it should be assessed for reuse or integration into biostimulant formulation.

*Ulva*n extraction should be carried out in water under controlled temperature and pH. Higher temperature can increase extraction yield but may reduce molecular weight. Acidic or alkaline conditions can change polymer properties and should be avoided unless the intended product is an oligosaccharide or modified polymer. For Activity 2.1, the key comparison should include conventional hot-water extraction, ultrasound-assisted extraction and microwave-assisted extraction under matched solid/solvent ratio and extraction time.

The recovered *Ulva*n fraction should be evaluated using yield, sugar composition, sulfate content, uronic acid content, molecular-weight distribution, ash and colour. A crude *Ulva*n extract may be suitable for agricultural or material screening, while biomedical or high-purity applications would require more demanding purification. The report therefore recommends a graded quality strategy instead of assuming that every batch must meet the highest specification.

After *Ulva*n recovery, the residue still has value. Glucose-rich cellulosic and glucan fractions can be hydrolysed enzymatically or by dilute acid. Enzymatic hydrolysis is milder and can reduce inhibitor formation, while dilute acid can be faster but may generate furans, salts and degradation products. For the first pilot experiments, enzymatic hydrolysis should be tested as the preferred green route, with dilute acid as a benchmark where appropriate.

The fermentable sugar stream should be interpreted with caution. Glucose can be used by many conventional microorganisms, but rhamnose, xylose and uronic acids require specialised strains, engineered strains or mixed-culture strategies. A simple ethanol route may therefore underuse the sugar mixture. More flexible outlets include lactic acid, organic acids, microbial biomass, biostimulant substrates or *Ulva*n-derived oligosaccharide products.

The *Ulva* cascade has a strong circularity profile because product streams can be aligned with different purity requirements. *Ulva*n polymer and oligosaccharides can serve higher-value applications; sugar hydrolysates can support fermentation or agricultural products; protein-rich residues can be assessed for feed or biostimulant uses; minerals can support NPK products. This makes the route suitable for SMEs that may enter the value chain at different levels of refinement.

The main risks are polymer degradation, high ash content, fermentation inhibitors, seasonal variability and uncertain market specifications for *Ulva*n. Mitigation requires mild extraction, desalting control, residue analysis, small-scale hydrolysis screening and early engagement

with potential end users. The pathway is selected because it combines high carbohydrate availability with multiple downstream options rather than because any single product is guaranteed to dominate revenue.

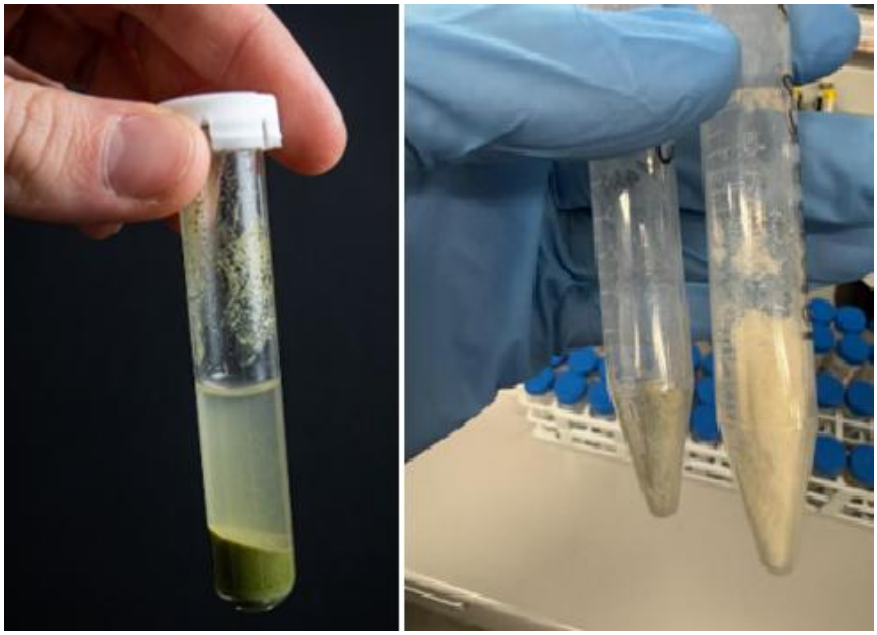

Figure 14. Ulvan extracts/fractions from Ulva included from the draft report.

Table 4. Composition of the Ulvan-related sugar fraction from Ulva sp.

Sugar or derivative	g/g dry weight	% dry weight
4-O-beta-D-glucuronosyl-L-rhamnose	0.0037	0.37
Glucuronic acid	0.0174	1.74
Iduronic acid	0.0162	1.62
Glucose	0.1678	16.78
Xylose	0.0519	5.19
Rhamnose	0.1124	11.24
Glucuronic acid lactone	0.0934	9.34
Total	0.4627	46.27

8. Supporting pathway 3 - proteins, lipophilic pigments, PUFA and phenolics

The third pathway is a supporting route that targets proteins, lipophilic pigments, PUFA-rich minor fractions and phenolic compounds. It is not selected as a primary pathway because the draft data indicate that structural carbohydrates and phycobiliproteins are stronger drivers for *Gracilaria* and *Ulva*. Nevertheless, these minor and medium-yield fractions can improve the overall value of the cascade if recovered without compromising the two core routes.

Protein recovery is relevant in both seaweed groups but for different reasons. In *Gracilaria*, protein extraction overlaps with phycobiliprotein recovery because pigments are chromoproteins. In *Ulva*, protein content is variable but can be high enough in some samples to justify consideration of protein ingredients, hydrolysates or feed applications. The amino-acid data show high glutamic and aspartic acid values in some *Ulva* samples, which may influence taste, nutritional value and hydrolysate functionality.

Protein extraction should be considered after the protection of priority targets. In *Gracilaria*, a mild first extraction already recovers a proteinaceous pigment fraction. Additional protein hydrolysis of residues could be considered after agar extraction if the residue quality remains acceptable. In *Ulva*, protein extraction can be integrated before or after *Ulva*n extraction depending on the chosen pH and temperature. However, strong alkaline protein extraction may alter *Ulva*n and should be tested carefully.

Lipophilic pigments and PUFA-rich fractions should be approached as specialty side-products. The lipid content is not sufficient for a fuel-first strategy, but small lipid fractions may contain compounds of interest for nutraceutical, cosmetic or feed applications. Analytical solvent protocols can quantify these compounds, but production-scale extraction should use safer solvents or supercritical/food-compatible methods if the product destination requires it.

Phenolic compounds can contribute antioxidant capacity and may support the positioning of crude extracts. The draft phenolic values are modest, with variation across samples. A phenolics-only extraction is not recommended as a first BIOBASED route, but phenolic content should be measured in aqueous, hydroethanolic or residual extracts because it can improve the functional profile of biostimulant or cosmetic ingredients.

The supporting pathway is best implemented as a screening layer. During the first pilot experiments, each core pathway should collect side samples for protein, lipid, phenolic and pigment measurements. If a fraction shows high value, a targeted recovery step can be added. If it does not, the fraction should be left in the residue for nutrient, feed, digestion or biostimulant routes. This avoids unnecessary complexity in early process development.

The advantage of this approach is flexibility. It acknowledges that biomass quality varies seasonally and that not every harvest will justify the same product slate. A protein-rich *Ulva* batch may be routed differently from a carbohydrate-rich *Ulva* batch. A *Gracilaria* batch with high pigment content may be processed with stronger emphasis on R-phycoerythrin stability. This adaptive model is more robust for SMEs than a rigid, one-size-fits-all process.

9. Supporting pathway 4 - residues and IMTA biowaste to nutrient and material products

The fourth pathway closes the cascade by valorising residues and IMTA side-streams. A biorefinery process cannot be considered complete if its final residues create a disposal problem. Residual solids, process waters and mineral-rich fractions should be characterised and directed to the most appropriate outlet. The objective is not simply waste reduction; it is the creation of lower-value but higher-volume products that stabilise the economics and environmental performance of the overall system.

Seaweed residues after extraction can retain proteins, minerals, structural carbohydrates and fixed carbon. Depending on composition and regulatory constraints, these residues can be evaluated for biostimulants, soil amendments, compost, anaerobic digestion, biochar or energy recovery. The residue route should be selected after measuring ash, nitrogen, phosphorus, potassium, salts, moisture, metals and residual organic matter. This is especially important for marine biomass because salt and metal contents can limit some agricultural uses.

Process waters should not be ignored. Washing, extraction and precipitation steps can generate saline streams containing soluble organic matter and nutrients. Instead of treating these waters as automatic waste, BIOBASED should evaluate recirculation, nutrient recovery, concentration, use in biostimulant formulation or controlled disposal. Water management is likely to be one of the main scale-up constraints because green extraction often relies on water as solvent.

Shell-rich aquaculture side-streams, if included in later project work, may offer mineral products based on calcium carbonate. They should be treated separately from seaweed organic residues because their chemistry and processing routes are different. Potential uses include liming materials, fillers, construction-related powders or composite ingredients, but these routes require specific quality, hygiene and regulatory assessment. In this deliverable,

shell streams are included as an IMTA biowaste category for future integration rather than as a primary experimental pathway.

Biochar production from residual solids can be considered when extraction residues are unsuitable for feed or direct agricultural use. Biochar can stabilise carbon, concentrate minerals and produce a solid material with potential soil-amendment value. However, pyrolysis must be evaluated for salt content, ash behaviour, emissions and energy balance. Biochar is therefore an option for residue management, not a universal solution.

Anaerobic digestion can convert residual organic matter into biogas while leaving a digestate that may contain nutrients. The suitability of seaweed residues depends on C/N ratio, salt concentration, sulfur compounds and degradability after extraction. The C/N ratios in the draft suggest that some residues could be suitable, while high-carbohydrate *Ulva* fractions may require nitrogen balancing. Digestion should be tested on residues, not only raw biomass, because extraction changes biodegradability.

Composting and biostimulant routes may be more accessible for SMEs than energy routes in some local contexts. They require lower capital intensity and can connect directly to agricultural users. Nevertheless, product safety and consistency are essential. The residue must be evaluated for salinity, metals, pathogens, stability and agronomic performance. A biostimulant claim should be supported by bioassays, not only by nutrient content.

This residue pathway is selected because it operationalises the circular-economy ambition of BIOBASED. It ensures that the cascade is not limited to high-value extracts but also addresses the fate of the larger residual mass. It is also the route most directly linked to waste minimisation and potential NPK recovery from seaweed and IMTA side-streams.

Table 5. Cascading biorefinery for Gracilaria gracilis, Ulva and residual solids.

Biomass/fraction	Main organic structures	Extraction/conversion	Products
Gracilaria phycobiliproteins	Chromopeptide proteins	Cold aqueous extraction; optional UAE	R-PE/APC/PC pigments, colorants, nutraceutical ingredients
Gracilaria agar	Sulfated galactans	Hot-water extraction; optional alkaline conditioning	Agar for gels, food, microbiology and biomaterials
Ulva Ulvan	Sulfated rhamno-uronic polysaccharide	Aqueous extraction; pH and temperature control	*Ulva*n polymer and *Ulva*n oligosaccharides
Ulva glucose/cellulosic fraction	beta-1,4-glucan and residual sugars	Enzymatic or dilute-acid saccharification	Fermentable sugars and platform molecules
Residual solids from both biomasses	Fixed carbon, minerals, residual proteins	Composting, digestion, pyrolysis, combustion, biochar	Energy carriers, soil amendments, NPK-rich products

10. Cascade order and mass-balance logic

The order of operations is the central design decision in a biorefinery. A cascade process is not simply a list of possible products; it is a sequence in which the first step changes the feedstock available to the second step. The selected BIOBASED pathways therefore use a high-value-first and sensitivity-first logic. Labile molecules are recovered before harsh treatments, abundant polymers are extracted after mild steps, and residues are processed only after their remaining value has been assessed.

For *Gracilaria*, the preferred sequence is washing and size reduction, cold aqueous pigment extraction, clarification and stabilisation of the pigment extract, hot-water agar extraction from the pigment-depleted residue, gel recovery or polymer isolation, residue characterisation and final residue valorisation. This sequence protects phycobiliproteins while retaining the agar route. It also produces analytical checkpoints after both primary stages.

For *Ulva*, the preferred sequence is washing and desalting, controlled *Ulva*n extraction, polymer recovery or concentration, residue characterisation, enzymatic or mild chemical hydrolysis of residual carbohydrates, sugar or oligosaccharide recovery, and final residue valorisation. This sequence preserves *Ulva*n functionality before depolymerising the remaining biomass. It also avoids treating all carbohydrates as a single undifferentiated sugar pool.

Mass balance should be reported on a dry-weight basis at every step. For each kilogram of dry biomass, the process should record extract mass, residue mass, target concentration, recovery yield, ash, moisture and key compositional indicators. Without mass balance, it is impossible to know whether an apparent yield improvement is genuine or merely a dilution, moisture or ash effect. The same principle applies to product quality: crude extract mass should not be confused with purified target mass.

A useful pilot mass balance should include water and energy flows as well as biomass flows. Water use is a critical parameter for aqueous extraction, while energy use is important for drying, heating, microwave processing, concentration and solvent recovery. Because Activity 2.1 emphasises green technologies, the best pathway is not always the pathway with the highest extraction yield; it is the pathway with the best combination of yield, quality, resource use and residue value.

The experimental design should therefore use a cascade yield metric. For example, a *Gracilaria* test should report phycobiliprotein yield, agar yield and final residue value together. An *Ulva* test should report *Ulva*n yield, residual sugar yield and residue quality together. This prevents optimisation of one step at the expense of the whole biomass.

The graphical abstract in this report summarises the cascade logic. It shows that both seaweed groups pass through pretreatment and analytical control, then diverge into species-specific high-value routes before converging again at residue valorisation. This structure reflects the practical requirement of a shared pilot methodology with species-specific process parameters.

Figure 15. IMTA biomass collection and preparation context included from the draft report.

11. Process variables to be optimised during Activity 2.1

The activity description identifies four core process variables: yield of target compounds, solid/solvent ratio, extraction time and extraction temperature. These variables should be treated as the minimum experimental set. Additional variables such as pH, particle size, drying method, pretreatment intensity, light exposure, oxygen exposure and biomass storage time should be recorded because they can strongly influence labile compounds and polymer quality.

Solid/solvent ratio affects mass transfer, extract concentration, equipment size and downstream concentration cost. A low biomass loading may increase apparent extraction completeness but generate dilute extracts and high water use. A high biomass loading may improve concentration but limit mixing. Activity 2.1 should therefore report both recovery per kilogram biomass and concentration per litre extract.

Extraction time should be optimised for each product class. For phycobiliproteins, prolonged extraction may increase protein degradation or microbial risk. For agar and *Ulva*n, longer extraction can increase polymer recovery but may change molecular weight or colour. Time should therefore be evaluated together with product integrity indicators, not only total recovered mass.

Temperature is one of the most consequential variables. Pigment proteins require low-temperature handling, while agar extraction requires elevated temperature. *Ulva*n extraction often benefits from heating but can suffer depolymerisation if conditions are too harsh. The selected pathways therefore separate cold pigment extraction from hot polysaccharide extraction and recommend controlled thermal windows for *Ulva*.

Pretreatment should be tested as an enhancement, not assumed to be beneficial. Ultrasound, microwave, enzyme assistance, pH conditioning and milling can improve access to intracellular or cell-wall-bound compounds. However, they can also increase degradation,

energy demand or equipment cost. Each pretreatment should be compared with a conventional benchmark under the same biomass batch and analytical method.

Product stability should be included as an optimisation variable. A pigment extract that has a high initial yield but loses colour within a few days may be less useful than a lower-yield extract that remains stable. Similarly, an *Ulva*n extract with high mass recovery but degraded molecular weight may not meet material specifications. Stability tests should include storage temperature, light exposure, pH and microbial control.

Residue quality must be measured after each extraction. If a condition improves upstream yield but makes the residue unsuitable for downstream conversion, the overall cascade may be less attractive. For example, severe acid hydrolysis may increase sugar release but generate inhibitors and salts. Alkaline conditioning may improve agar properties but affect protein residues. These effects should be captured in the selection matrix.

12. Analytical characterisation plan

Analytical characterisation is the backbone of Activity 2.1. The project description requires chemical composition of extracts and residues after extraction. This means that every extraction experiment should generate two analytical objects: the product stream and the depleted biomass. Both should be analysed because the value of a cascade depends on what remains available after each step.

For raw biomass, the minimum analytical set should include moisture, ash, proteins, total lipids, total carbohydrates, elemental composition, total phenolic content, target polysaccharide indicators, pigments and mineral nutrients. These data establish the feedstock baseline and allow comparisons across species, seasons and pilot sites. Without this baseline, yield differences could be incorrectly attributed to technology rather than biomass quality.

For phycobiliprotein extracts, the plan should include absorbance spectra, R-phycoerythrin, allophycocyanin and phycocyanin estimates, total protein, purity index, colour stability, pH, conductivity and microbial indicators where relevant. If the extract is intended for higher-purity applications, fluorescence and chromatographic purity can be added. For early screening, absorbance-based quantification and stability are sufficient to rank conditions.

For agar extracts, the analytical plan should include yield, galactose-rich fraction, sulfate/ash indicators where available, gel strength, gelling temperature, melting temperature, colour and moisture. Gel quality is as important as mass yield because agar value depends on performance. Residue composition after agar extraction should also be measured to determine whether digestion, composting or biochar remains feasible.

For *Ulva*n extracts, key indicators include yield, rhamnose, uronic acids, xylose, glucose, sulfate, ash, molecular-weight distribution and viscosity. Depending on application, antioxidant or bioactivity assays may be added. Because *Ulva*n is a family of polymers rather than a single molecule, structural indicators are more informative than total mass alone.

For sugar hydrolysates, the analytical plan should include glucose, xylose, rhamnose, uronic acids, total soluble sugars, inhibitors such as 5-HMF or furans where relevant, salts, pH and residual solids. These parameters determine whether fermentation is realistic and which microorganisms or product routes are appropriate. A hydrolysate should not be labelled fermentable solely because it contains sugars.

For residue and IMTA side-stream products, characterisation should include moisture, ash, carbon, nitrogen, phosphorus, potassium, salinity, metals, organic matter stability and basic agronomic or bioactivity indicators. This is essential if residues are intended for biostimulants, soil amendments or compost. Safety and regulatory suitability must be assessed before any application claims are made.

13. Product classes and target specifications

The selected pathways generate several product classes of commercial value. The activity description specifically mentions phycobiliproteins, lipophilic pigments, proteins, PUFA, polysaccharides, biostimulants and nutrients. This report maps those target classes to the most suitable biomass stream and process position. The result is a portfolio in which not all products are pursued with the same intensity but all are considered within the cascade.

Phycobiliproteins are the most sensitive *Gracilaria* target. Crude extracts may be evaluated as natural colourants or functional antioxidant extracts. Higher-purity R-phycoerythrin-rich fractions require additional clarification and purification. Product specifications should include colour, absorbance ratios, protein content, stability, pH and microbiological quality. The market destination determines whether crude, semi-purified or purified material is realistic.

Agar is the main structural product from *Gracilaria*. The most important specifications are not only extraction yield but gel strength, clarity, ash, sulfate content and thermal gel

behaviour. Food, technical, microbiological and biomaterial grades have different requirements. BIOBASED should initially evaluate whether locally produced agar can meet functional specifications rather than immediately targeting the most purified grade.

*Ulva*n is the main structural product from *Ulva*. Specifications should include rhamnose and uronic-acid content, sulfate content, ash, molecular-weight profile, viscosity and colour. Different applications may favour different molecular weights. Agricultural and material applications may tolerate crude or semi-purified *Ulva*n, while biomedical applications require much stricter purity and safety standards.

Protein products can be developed as crude protein extracts, hydrolysates, feed ingredients or functional peptide fractions. The amino-acid profile from the draft suggests that *Ulva* can contain relevant acidic amino acids, while *Gracilaria* protein recovery is linked to pigments. Protein product development must consider digestibility, salt, colour, odour, safety and regulatory status.

Lipophilic pigments and PUFA-rich fractions should be screened as specialty ingredients. They may have value in feed, nutraceuticals or cosmetics, but they require careful oxidation control and safe extraction. Given the low lipid content in the analysed samples, these fractions should remain secondary and opportunistic unless future biomass batches show higher lipid or pigment values.

Biostimulant and nutrient products provide a practical outlet for residues and lower-purity streams. They can use mineral, organic and oligosaccharide fractions without requiring the same purity as food or pharmaceutical ingredients. However, efficacy must be demonstrated through plant or microbial assays, and safety must be controlled through salinity, metals and microbiology. These products are therefore attractive but not automatically simple.

Table 6. Product-class map for the selected pathways.

Product class	Source	Process stage	Potential applications	Critical quality indicators
R-PE/APC/PC-rich extracts	*Gracilaria*	Cold aqueous extraction	Natural colour, fluorescence, antioxidant screening	Yield, purity index, stability, microbiology
Agar	*Gracilaria* residue	Hot-water extraction	Food gels, technical gels, biomaterials	Yield, gel strength, ash, colour, gelling temperature
*Ulva*n polymer	*Ulva*	Controlled aqueous extraction	Biostimulants, films, hydrogels, functional ingredients	Yield, sulfate, uronic acids, MW, ash
Sugar hydrolysate	*Ulva* residue	Enzymatic or mild acid hydrolysis	Fermentation, biostimulants, platform chemicals	Glucose, xylose, rhamnose, inhibitors, salts
Protein/hydrolysate	Selected extracts or residues	Aqueous/enzymatic extraction	Feed, functional ingredients, biostimulants	Protein, amino acids, digestibility, salt
Residue products	Both species + IMTA biowaste	Compost, digestion, pyrolysis, nutrient recovery	Soil amendments, energy, NPK products	C/N, NPK, salinity, metals, stability

14. Market-facing interpretation without overclaiming

A deliverable on conversion pathways should connect scientific feasibility with market logic, but it should avoid overclaiming. The presence of a compound in seaweed does not automatically create a profitable product. Market value depends on purity, functionality, volume, consistency, regulatory acceptance, logistics and the ability of SMEs to produce and sell the material. For this reason, this report uses product classes rather than fixed revenue projections as the main selection criterion.

Phycobiliproteins have high potential because they combine colour and fluorescence with natural origin. However, high-value markets require stability and purity that may be difficult to reach in early pilot work. The recommended strategy is staged: first demonstrate crude extraction and stability, then evaluate semi-purification, then consider higher-purity applications only if yields and quality justify the investment.

Agar has a more established market and is therefore a practical anchor product for *Gracilaria*. Its challenge is not market existence but quality and competitiveness. Locally produced agar must meet functional specifications and be costed against extraction energy, drying, filtration and biomass availability. A technical or food-grade product may be more realistic than molecular-biology-grade agarose in the first phase.

*Ulva*n is promising but less standardised than agar. Its market development depends on demonstrating functionality in materials, biostimulants, cosmetics, nutraceuticals or biomedical research. Because specifications vary, BIOBASED should engage potential end users early and produce application-oriented samples rather than a generic powder with no defined use.

Fermentation of *Ulva*-derived sugars is attractive in theory but should be assessed against sugar concentration, inhibitor formation and the need to use non-glucose sugars. A hydrolysate that requires extensive detoxification or specialised strains may be less

attractive than an oligosaccharide or biostimulant route. For SMEs, lower-complexity applications may offer faster uptake than advanced fermentation products.

Residue products may have lower unit value but contribute strongly to circularity and waste minimisation. Compost, biochar, NPK-rich products or process-water nutrient recovery can improve the overall environmental profile and reduce disposal costs. They should therefore be included in the business model even if they are not the primary revenue driver.

15. Pilot implementation strategy

The pilot implementation strategy should begin with a limited number of robust experiments rather than a large unstructured screening. For each core pathway, the project should define a reference conventional extraction and two enhanced variants. For *Gracilaria*, the reference is cold aqueous pigment extraction followed by hot-water agar extraction. Enhanced variants can include ultrasound-assisted pigment extraction and microwave-assisted agar extraction. For *Ulva*, the reference is hot-water *Ulva*n extraction followed by enzymatic saccharification, with ultrasound and microwave variants tested for polymer recovery.

Experiments should use the same biomass batch for all comparisons within a pathway. This is essential because seaweed composition varies. If different batches are used, extraction differences may reflect biomass variability rather than technology performance. Each batch should be documented with harvest location, date, species identification, cultivation or collection conditions, pretreatment, moisture and storage conditions.

The pilot design should separate screening from optimisation. Screening identifies which technology family is promising. Optimisation then refines solid/solvent ratio, time, temperature and pH. Attempting to optimise every technology before screening can consume resources without improving decision quality. A two-stage design is therefore recommended: first rank conditions broadly, then optimise the top two or three.

For each condition, the project should collect a complete mass balance. This includes dry biomass input, extract volume, extract dry matter, target concentration, residue mass, water use and energy-relevant data. A condition that gives a high extract concentration but leaves little recoverable downstream material should be judged differently from one that supports a stronger full cascade.

Replicates and controls are necessary for credible selection. At minimum, analytical replicates should be used for key assays, and extraction replicates should be performed for the final selected conditions. Blank controls, calibration standards and reference materials

should be documented. Process variability is expected, but the pathway decision must be based on reproducible trends.

The pilot outputs should include samples, data tables and decision notes. Samples allow partners and potential end users to assess colour, gel performance, viscosity, odour and handling. Data tables support technical comparison. Decision notes explain why a route is advanced, modified or rejected. This combination is more useful for SMEs than a purely academic dataset.

Figure 16. IMTA system where Gracilaria feedstock is prepared, included from the draft report.

Table 7. Estimated agar and phycobiliprotein production from IMTA biomass feedstock.

Culture type	Density	Season	Estimated dry biomass per meter (kg)	Estimated phycobiliproteins (g)	Estimated agar (kg)
Mussel	Density 1	Summer	10.13	143.55	7.44
Mussel	Density 1	Autumn	6.63	94.01	4.87
Mussel	Density 3	Summer	6.06	85.90	4.45
Mussel	Density 3	Autumn	3.58	50.77	2.63
Mussel	Density 5	Summer	4.04	57.26	2.97
Mussel	Density 5	Autumn	2.38	33.85	1.75
Oyster	Density 1	Summer	4.01	5.69	0.29
Oyster	Density 1	Autumn	2.23	3.17	0.16

16. Integration with SMEs and cross-border value chains

Activity 2.1 is not isolated laboratory work. The application form links the activity to SME dissemination and to the broader goal of strengthening innovation capacities in the blue economy. The selected pathways should therefore be described in terms that SMEs can evaluate: required feedstock, equipment, process complexity, product form, storage stability, expected applications and residue handling.

For aquaculture producers, the key question is whether seaweed and side-streams can create additional value without disrupting the core business. The selected pathways support this because they can be modular. A producer may supply wet or dried biomass; a processor may perform extraction; a technology provider may supply ultrasound, microwave, drying or filtration equipment; an end user may formulate the extract into food, feed, agricultural or material products.

For SMEs, a graded product strategy is particularly important. Producing analytical-grade pigments or biomedical-grade *Ulva*n may be unrealistic initially. Producing crude or semi-purified extracts for testing in agriculture, cosmetics, feed or materials may be more feasible.

The pathway design should therefore avoid a single high-purity bottleneck and instead create several entry points into the value chain.

Cross-border cooperation between Italy and Croatia can improve robustness by testing biomass from different sites and systems. If a pathway works only for one batch under one laboratory condition, it has limited transferability. If it works across multiple Adriatic contexts with parameter adjustments, it becomes more relevant for the programme area. Shared methods and comparable data formats are therefore essential.

Living labs and stakeholder meetings can use simplified process maps derived from this deliverable. The graphical abstract, pathway tables and product-class summaries can support communication with non-specialists. This is important because the value of a biorefinery approach is not obvious to all stakeholders; it must be shown as a practical sequence that reduces waste and creates products.

17. Environmental and circularity considerations

The environmental value of a seaweed biorefinery depends on more than the renewable origin of the biomass. Drying energy, water demand, extraction temperature, solvent choice, chemical use, waste streams and product substitution all influence the overall profile. The selected pathways should therefore be evaluated using a circularity checklist before scale-up.

Water is a central issue because aqueous extraction is preferred for green processing. The process should record water use per kilogram dry biomass and evaluate opportunities for water recirculation. Recirculation must be balanced against salt accumulation, microbial risk and product quality. The most sustainable process may require partial recirculation combined with treatment or nutrient recovery.

Energy demand is strongly affected by drying and heating. Freeze-drying is excellent for preserving labile compounds during laboratory analysis but may not be feasible at scale.

Controlled low-temperature drying, solar-assisted drying or direct wet processing should be evaluated for pilot scenarios. For agar and *Ulva*n extraction, heat integration and insulation can reduce energy intensity.

Solvent choice affects safety, regulatory acceptance and residue use. Water and food-compatible solvents are preferred. Analytical solvent methods are acceptable for characterisation but should not be confused with production routes. If hydroethanolic extraction is used for phenolics or lipophilic compounds, solvent recovery and residual solvent limits should be considered.

Residue valorisation is essential for circularity. If residues are disposed of, the environmental benefits of extracting high-value compounds may be reduced. The selected pathways therefore include compost, digestion, biochar, nutrient recovery and biostimulant options. The best outlet should be chosen locally based on composition, regulation and market demand.

A future life-cycle assessment can be developed after pilot data are available. The first step is to collect mass and energy data consistently. The second step is to define functional units, such as one kilogram of agar, one gram of pigment, one kilogram of *Ulva*n or one ton of wet biomass processed. The third step is to compare single-product extraction with the cascade approach. This report provides the process boundaries needed for that later assessment.

18. Risk assessment and mitigation

The first risk is feedstock variability. Seasonal and site-specific changes can alter yield and product quality. Mitigation requires batch characterisation, flexible operating windows and seasonal planning. Rather than expecting identical biomass, BIOBASED should use variability as a design input and define processing rules for different composition profiles.

The second risk is degradation of labile compounds. Phycobiliproteins, PUFA-rich fractions and some pigments can degrade during drying, storage and extraction. Mitigation includes

rapid post-harvest processing, cold handling, limited light exposure, antioxidant control where appropriate and extraction before high-temperature steps.

The third risk is excessive process complexity. A cascade with too many steps can become difficult for SMEs to implement. Mitigation requires modular design, clear go/no-go criteria and staged purification. A route should only add a new step if the incremental value exceeds the added cost, time and operational burden.

The fourth risk is product-market mismatch. A technically successful extract may lack a buyer if specifications are unclear. Mitigation requires early end-user dialogue, sample generation, graded quality definitions and application-oriented testing. Product dossiers should be developed for pigments, agar, *Ulva*n, hydrolysates and residue products.

The fifth risk is residue unsuitability. Marine biomass can contain salts and metals that limit agricultural or feed uses. Mitigation requires residue analysis and multiple fallback routes. If a residue is unsuitable for one application, it may still be suitable for digestion, biochar or controlled energy recovery.

The sixth risk is scale-up performance. Laboratory extraction may not translate directly to pilot scale because mixing, heating, filtration and mass transfer change with equipment size. Mitigation requires pilot-relevant measurements, conservative scale-up assumptions and equipment trials with realistic biomass loading.

Table 8. Risk and mitigation matrix for Activity 2.1 pathway development.

Risk	Potential effect	Mitigation
Feedstock variability	Variable yield and inconsistent product quality	Batch characterisation, seasonal sampling and adaptive process windows
Pigment degradation	Loss of colour and value	Cold handling, dark processing, rapid clarification and stability testing
Polymer depolymerisation	*Ulva*n or agar quality loss	Mild extraction, molecular-weight monitoring and controlled temperature
High salt/ash	Limits feed, fermentation and agricultural uses	Washing optimisation, conductivity control and residue testing
Process complexity	Low SME uptake	Modular design, graded products and go/no-go criteria
Weak market fit	Products have no buyer or unsuitable grade	End-user testing, product dossiers and staged purity strategy

19. Final pathway selection and recommendations

The final selection is a portfolio of four routes. The first two are the required core pathways: *Gracilaria* pigment-to-agar and *Ulva* *Ulva*n-to-sugar. The third and fourth routes are supporting pathways: recovery of proteins, lipids, PUFA, phenolics and other minor fractions when justified, and conversion of residues and IMTA side-streams into nutrient, biostimulant, biochar, compost, energy or digestion products. Together they provide a coherent cascade for whole-biomass use.

For *Gracilaria*, the recommended starting point is cold aqueous extraction of phycobiliproteins followed by hot-water agar extraction. The main optimisation variables are solid/solvent ratio, pH, extraction time, temperature, clarification method and storage stability for pigments; and time, temperature, pretreatment and gel quality for agar. The residue should be characterised after each stage.

For *Ulva*, the recommended starting point is controlled aqueous extraction of *Ulva*n followed by enzymatic or mild chemical hydrolysis of the residual carbohydrate fraction. The main optimisation variables are washing intensity, extraction temperature, pH, time, pretreatment intensity, molecular weight and sulfate preservation for *Ulva*n; and enzyme loading, hydrolysis time, inhibitor formation and sugar profile for hydrolysates.

For minor fractions, the recommendation is screening rather than immediate full process integration. Proteins, phenolics, lipophilic pigments and PUFA-rich fractions should be measured in each relevant stream. If a fraction is consistently valuable, a targeted recovery step can be added. If not, the fraction should remain in the residue for lower-complexity valorisation.

For residues and IMTA biowaste, the recommendation is to develop a residue decision tree based on ash, salinity, C/N ratio, NPK, metals and organic matter. Potential outlets should include biostimulants, compost, anaerobic digestion, biochar and nutrient recovery. The selected outlet should depend on local feasibility and safety, not only on theoretical value.

The immediate next step for BIOBASED is to run comparative extraction trials under controlled conditions and populate a shared data template. This should include yield, product quality, mass balance, water use, energy-relevant observations and residue composition. The data should then be used to refine the pathway selection into a pilot-scale process scheme for each species and site.

Table 9. Pathway screening and selection matrix.

Candidate route	Technical fit	Green fit	Complexity	Residue fit	Overall priority	Decision
Gracilaria pigment-first	Very high	High	Medium	High	High	Selected core pathway
Gracilaria agar-first	Medium	Low for pigments	Medium	High	Medium	Not preferred; may be benchmark only
Ulva Ulvan-first	High	High	Medium	High	High	Selected core pathway
Ulva direct fermentation	Medium	Medium	Medium-high	Medium	Medium	Supporting/secondary; depends on sugar profile
Direct lipid/biodiesel route	Low	Medium	Medium	Low	Low	Rejected as primary route
Residue to biochar/digestion	Medium	High	Medium	Medium	High	Selected residue route

Appendix B. Suggested operating procedures for pilot screening

This appendix converts the selected pathways into practical operating procedures. The procedures are not final industrial protocols; they are structured screening methods to generate comparable data across partners and biomass batches. Each procedure should be adapted to available equipment and safety rules while preserving the same core variables and output measurements.

For every experiment, the biomass record should include species, harvest date, site, cultivation system, wet mass, dry matter, pretreatment, storage conditions and visual observations. Photographic documentation is recommended because colour, epiphytes, sediment and tissue condition can explain analytical variation. The biomass record should be linked to every extract and residue sample using a unique sample code.

A general washing protocol should begin with removal of visible debris and epiphytes, followed by filtered seawater rinsing and controlled freshwater desalting. Conductivity of the wash water should be recorded before and after washing. A subsample should be dried to determine dry matter loss during washing. This is important because aggressive washing can improve salt removal while losing soluble target compounds.

For *Gracilaria* phycobiliprotein extraction, the recommended screening condition is cold aqueous extraction at controlled pH using a defined solid/solvent ratio. Biomass should be protected from direct light and processed rapidly. After extraction, the suspension should be clarified by centrifugation or filtration. The extract should be analysed immediately and again after storage to assess stability.

For *Gracilaria* agar extraction, pigment-extracted residue should be transferred to hot-water extraction. The test matrix should vary extraction temperature and time while holding biomass loading constant. After extraction, the hot suspension should be filtered, the filtrate cooled to form a gel or recovered polymer, and the product dried under controlled

conditions. Gel performance should be measured before any strong claims about agar quality are made.

For *Ulva Ulva*n extraction, washed biomass should be extracted in water under controlled temperature and pH. Ultrasound or microwave assistance can be tested as a parallel treatment. The extract should be clarified and concentrated or precipitated according to the available laboratory method. The recovered material should be analysed for sugar composition, ash, sulfate indicators and molecular-weight-related properties where available.

For *Ulva* residue hydrolysis, the *Ulva*n-depleted residue should be washed if needed, then treated enzymatically or with a mild acid benchmark. The hydrolysate should be analysed for glucose, xylose, rhamnose, uronic acids, pH, salts and inhibitors. A fermentation test should not be started until the sugar profile and inhibitor risk are known.

Residue management tests should start with characterisation. Residue moisture, ash, C/N, NPK, salinity and metals should be measured before selecting compost, digestion, biochar or biostimulant options. A single residue can be split into multiple small tests to determine the most promising outlet. The residue route should be included in the mass balance, not treated as an afterthought.

Table 10. Practical comparison of screening procedures for the two core pathways.

Step	***Gracilaria* pigment-to-agar route**	***Ulva Ulva*n-to-sugar route**
1. Receive biomass	Record wet mass, site, date and condition	Record wet mass, site, date and condition
2. Wash/desalt	Gentle rinse to remove salts without pigment loss	Conductivity-controlled rinse to reduce ash
3. Mild extraction	Cold aqueous pigment extraction	Controlled aqueous *Ulva*n extraction
4. Clarify	Centrifuge/filter pigment extract	Centrifuge/filter *Ulva*n extract
5. Main polymer step	Hot-water agar extraction from residue	Polymer recovery and residue washing
6. Residue conversion	Characterise residue for nutrient/biochar/digestion route	Hydrolyse residual glucans or route to biostimulants

7. Quality control	R-PE/APC/PC, agar yield and gel quality	*Ulva*n sugar profile, sulfate/ash, hydrolysate sugars

Appendix C. Product dossiers for selected output classes

The product dossiers provide concise descriptions of the most relevant output classes. They are designed for communication with SMEs and end users. Each dossier identifies the feedstock, process stage, main quality indicators, possible applications and technical cautions. They should be updated after pilot trials with actual data and sample photographs.

Phycobiliprotein-rich extracts originate from the first *Gracilaria* extraction. Their value is linked to colour intensity, purity, fluorescence, protein integrity and stability. Potential applications include natural colourants, analytical reagents, cosmetic ingredients and antioxidant formulations. The main cautions are heat sensitivity, light sensitivity, microbial stability and the need for rapid clarification.

Agar fractions originate from the hot-water extraction of *Gracilaria* residue. Their value depends on gel strength, clarity, ash, moisture and thermal behaviour. Potential applications include food gels, microbiology media, encapsulation, packaging materials and biotechnology gels. The main cautions are quality variability, energy demand and the need to remove insoluble material efficiently.

*Ulva*n fractions originate from controlled aqueous extraction of *Ulva*. Their value depends on molecular weight, sulfate content, uronic acid content, ash and rheological properties. Potential applications include biostimulants, films, hydrogels, biomedical materials, nutraceutical research and functional ingredients. The main cautions are lack of universal market specifications and sensitivity of properties to extraction conditions.

Sugar hydrolysates originate from *Ulva*n-depleted *Ulva* residues or other carbohydrate-rich residues. Their value depends on sugar concentration, fermentability, inhibitor content and salt load. Potential applications include fermentation to organic acids, microbial biomass, biostimulant formulations or chemical building blocks. The main cautions are mixed sugar composition and the need for robust microorganisms.

Protein and peptide fractions can be obtained from selected extracts or residues. Their value depends on amino-acid profile, digestibility, solubility, taste, odour and salt. Potential applications include feed ingredients, hydrolysates, biostimulants and functional materials. The main cautions are extraction selectivity, regulatory status and competition with cheaper protein sources.

Residue-based products include compost, biochar, digestion substrates, NPK-rich amendments and process-water-derived nutrient products. Their value depends on safety, nutrient profile, stability and local demand. Potential applications are closely linked to agriculture and circular resource management. The main cautions are salinity, metals, pathogen control and consistent product claims.

Table 11. Minimum evidence required before advancing product classes.

Product	Minimum evidence before pilot scale	Recommended next test
Phycobiliprotein extract	Stable colour and reproducible absorbance-based yield	Shelf-life screening under light/dark and cold/ambient storage
Agar	Reproducible yield and measurable gel performance	Gel strength and ash reduction trials
*Ulva*n	Reproducible polymer recovery and sugar/uronic acid profile	Molecular-weight and viscosity screening
Sugar hydrolysate	Sufficient glucose/total sugar and low inhibitor profile	Small fermentation or biostimulant bioassay
Residue product	Acceptable salinity, NPK and safety profile	Compost, biochar or plant-growth screening

Appendix D. Data management and reporting template

A shared data template is recommended for all Activity 2.1 trials. The template should have separate tabs or sections for biomass metadata, pretreatment, extraction conditions, extract analysis, residue analysis, mass balance, energy observations, water use, product notes and decision outcome. Consistency is more important than complexity. A simple template completed reliably is better than an advanced template with missing fields.

Biomass metadata should include sample code, species, site, country, date, cultivation or collection method, wet mass, dry matter, storage time and visible quality. The sample code should appear on every extract, residue, photograph and analytical file. This prevents loss of traceability when multiple partners and laboratories are involved.

Extraction-condition fields should include biomass dry weight, solvent type, solvent volume, solid/solvent ratio, pH, temperature, time, equipment, agitation, ultrasound or microwave settings, enzyme type and enzyme loading where applicable. Any deviation from the planned method should be recorded as a note rather than corrected silently.

Extract-analysis fields should include extract volume, dry matter, target concentration, purity indicators, colour, pH, conductivity, ash, relevant sugar or protein assays and stability observations. For polymer products, molecular-weight or viscosity-related data should be included where available. For pigments, absorbance and stability are essential.

Residue-analysis fields should include wet mass, dry mass, moisture, ash, protein, carbohydrate, elemental data, NPK, salinity, metals where available and intended residue outlet. The residue should be coded as a new feedstock for the next cascade stage. This reinforces the biorefinery logic and supports complete mass balance.

The decision-outcome field should classify each condition as advance, modify, hold or reject. The reason should be written in a short sentence. Examples include low target yield, good yield but poor residue quality, high water use, polymer degradation, promising product

quality or operational simplicity. These decision notes will help convert laboratory data into pilot recommendations.

Table 12. Suggested data-management template for Activity 2.1 trials.

Template block	Mandatory fields	Purpose
Biomass metadata	Species, site, date, wet mass, dry matter, storage	Traceability and comparison across batches
Pretreatment	Washing, drying, milling, pH, temperature	Interpretation of yield and product quality
Extraction conditions	S/L ratio, solvent, time, temperature, equipment	Process optimisation and reproducibility
Extract analysis	Volume, target concentration, purity indicators, stability	Product assessment
Residue analysis	Dry mass, ash, protein, carbohydrates, NPK, salinity	Cascade design and residue outlet selection
Decision	Advance, modify, hold or reject, with reason	Translation of data into pathway decisions

Appendix E. Communication notes for non-specialist stakeholders

Seaweed biorefinery can be explained simply as using the whole seaweed rather than taking one product and throwing the rest away. The first step recovers the most delicate or valuable molecules. The second step recovers the bulk polymers. The final steps turn remaining material into nutrients, soil products, energy or other useful outputs. This is similar to using different parts of a crop for food, feed, fibre and compost.

Gracilaria is useful because it contains red pigments and agar. The red pigments can be recovered gently in water. The leftover seaweed can then be heated in water to recover agar, the gelling material used in several food and laboratory applications. The remaining residues can still contain nutrients and organic matter that should be valorised.

Ulva is useful because it contains *Ulva*n and sugars. *Ulva*n is a special seaweed polymer with potential uses in materials, agriculture and bioactive products. After *Ulva*n is extracted, the remaining material can be broken down into sugars or used for biostimulants and nutrient products. This means *Ulva* should not be seen only as waste or bloom biomass but as a resource.

IMTA systems can strengthen this approach by producing seaweed in connection with aquaculture. Seaweeds can take up nutrients from the surrounding water and create biomass that can be processed. The biorefinery then converts that biomass into product classes. This closes a loop between aquaculture, environmental management and local economic diversification.

For SMEs, the opportunity is not necessarily to produce every product in-house. One company may cultivate or collect biomass, another may dry or preprocess it, another may extract polymers, and another may formulate products. BIOBASED can help by identifying which routes are technically realistic and which product classes are worth testing with end users.

The key message is that seaweed and aquaculture side-streams can be part of a circular blue economy. The selected pathways show how a material that might otherwise be underused can become pigments, gels, polymers, sugars, nutrients and soil-related products. This supports innovation while reducing waste.

Appendix A. Consolidated experimental data tables

This appendix consolidates the numerical data used in this deliverable. Values were extracted from the draft report charts and tables and are presented here to support transparent pathway selection. The tables should be updated when new Activity 2.1 experiments are completed.

Table A1. Macromolecular composition data.

Sample	Sugars (% dw)	Protein (% dw)	Total lipids (% dw)
G. gracilis A	41.85	20.26	4.87
G. gracilis B	40.34	18.43	4.69
U. laetevirens A	64.24	6.38	0.52
Ulva sp.	45.66	24.04	3.06
U. laetevirens B	43.38	19.04	4.44

Table A2. Elemental composition and total phenolic content.

Sample	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Total phenols (mg GAE/g dw)
G. gracilis A	33.91	5.46	3.97	0.10
G. gracilis B	33.30	5.22	3.61	0.08
U. laetevirens A	32.89	4.79	1.25	0.15
Ulva sp.	38.90	6.61	4.71	0.30
U. laetevirens B	32.09	6.32	3.73	0.22

Table A3. Amino acid composition (% dry weight).

Amino acid	G. gracilis A	G. gracilis B	U. laetevirens A	*Ulva* sp.	U. laetevirens B
Phenylalanine	0.643	0.726	1.015	1.110	0.208
Leucine	0.381	0.401	1.073	1.488	0.126
Isoleucine	0.509	0.557	0.763	0.887	0.171
Methionine	0.123	0.139	0.193	0.223	0.075

Tyrosine	0.101	0.104	0.646	0.724	0.034
Valine	0.839	0.897	1.236	1.649	0.212
Proline	0.434	0.444	0.852	0.098	0.166
Alanine	0.548	0.601	0.993	1.582	0.298
Threonine	0.197	0.156	0.543	0.805	0.049
Glycine	0.496	0.540	0.644	0.520	0.181
Serine	0.290	0.285	0.622	1.198	0.092
Glutamic acid	0.280	0.222	2.410	2.989	0.036
Aspartic acid	0.279	0.372	3.508	3.601	0.000
Histidine	0.009	0.000	0.034	0.054	0.000
Arginine	0.824	0.645	0.640	1.008	0.098
Lysine	0.110	0.092	0.000	0.147	0.025
Cystine	0.000	0.623	0.150	0.505	0.000

Table A4. Lipid, unsaponifiable fraction and FAME data.

Sample	Total lipids (% dw)	UNSAP (% of lipids)	FAMEs (% dw)
G. gracilis A	4.873	1.619	0.178
G. gracilis B	4.692	21.277	0.160
U. laetevirens A	0.519	84.615	0.080
Ulva sp.	3.058	0.000	0.000
U. laetevirens B	4.443	9.091	1.595

Table A5. Phycobiliprotein composition in Gracilaria gracilis.

Pigment	G. gracilis A (mg/g dw)	G. gracilis B (mg/g dw)
R-PE	10.57	5.51
APC	2.15	0.66
PC	1.45	0.29

Technology fiche 1 - Ultrasound-assisted extraction

Ultrasound-assisted extraction is relevant to Activity 2.1 because it can intensify mass transfer while using water or other green solvents. Cavitation can disrupt cell walls and improve release of intracellular or wall-associated compounds. The method is particularly interesting for reducing extraction time and temperature, but its intensity must be controlled to protect sensitive proteins and polymers.

For *Gracilaria* pigment extraction, ultrasound should be tested at low to moderate intensity and under cooling. The objective is to enhance pigment release without denaturing phycobiliproteins. The evaluation should include pigment yield, purity index, absorbance profile and stability. Excessive sonication may increase turbidity or release impurities that complicate clarification.

For *Ulva Ulva*n extraction, ultrasound can increase polymer release but may alter molecular weight. Because *Ulva*n functionality depends partly on polymer size and sulfation pattern, yield must be interpreted together with structural indicators. A high-mass crude yield is not automatically positive if the polymer becomes too degraded or ash-rich.

The main scale-up issues are equipment geometry, energy input, heat generation and reproducibility. Pilot tests should report actual power, treatment time, biomass loading and temperature control. Ultrasound is selected as an enhancement candidate rather than a mandatory step because its benefit will depend on species, particle size and target product.

Technology fiche 2 - Microwave-assisted extraction

Microwave-assisted extraction can accelerate heating of aqueous biomass suspensions and improve extraction kinetics. It is most relevant for thermal extraction steps such as agar and *Ulva*n recovery. It is less suitable for unprotected phycobiliproteins unless applied very gently and with rigorous temperature control.

For *Gracilaria*, microwave assistance should be evaluated after pigment removal, during the agar extraction stage. The key question is whether it can reduce time or energy while maintaining gel quality. Evaluation should include agar yield, colour, ash, gel strength and thermal properties. The residue should be checked for suitability for final valorisation.

For *Ulva*, microwave extraction may improve *Ulva*n release but can also affect polymer properties. Tests should compare microwave extraction with conventional hot-water extraction at similar final temperatures. This prevents attributing improvements to microwaves when they may simply result from higher thermal exposure.

The main scale-up issues are penetration depth, vessel design, safety, continuous operation and energy balance. Microwave technology should be advanced only if it improves the complete cascade rather than one isolated yield value.

Technology fiche 3 - Enzyme-assisted processing

Enzyme-assisted processing can support mild extraction and controlled depolymerisation. Enzymes may help release proteins and pigments from cell walls or convert residual polysaccharides into oligosaccharides and sugars. This is attractive for green chemistry because enzymes can operate under mild pH and temperature, reducing harsh chemicals.

For *Gracilaria*, enzyme assistance may improve pigment liberation before agar extraction, but it must be assessed carefully because enzymes can change the residue and potentially influence agar quality. A screening test should compare enzyme-treated and untreated biomass using the same extraction conditions and mass balance.

For *Ulva*, enzymes are particularly relevant after *Ulva*n extraction. Cellulases, glucanases or other carbohydrate-active enzymes can convert residual glucans into glucose-rich hydrolysates. *Ulva*n lyases may be considered if the objective is to produce *Ulva*n oligosaccharides. Enzyme cost and salt tolerance are important constraints.

The main scale-up issues are enzyme cost, residence time, inhibition by salts, downstream deactivation and product specification. Enzyme assistance should be used where selectivity creates value, not merely as an additional process step.

Technology fiche 4 - Water-based extraction and process-water management

Water-based extraction is the foundation of the selected BIOBASED pathways. It is compatible with pigments, proteins, agar, *Ulva*n and many agricultural applications. It also aligns with green-solvent principles. However, water extraction can generate large volumes of dilute streams, so process-water management must be designed from the beginning.

For pigments, water quality, pH, salts and microbial control influence extract stability. For agar and *Ulva*n, water volume affects polymer concentration and downstream drying costs. For residues, washing water can carry nutrients and salts that may be recovered or recirculated. Water is therefore both a solvent and a process stream requiring management.

The recommended approach is to measure water input and output for every trial. Conductivity, pH, dry matter, nitrogen and chemical oxygen demand can be used to decide whether a stream is suitable for recirculation, nutrient recovery or treatment. This transforms water management from an afterthought into a core part of process optimisation.

At pilot scale, water reduction may be achieved by counter-current washing, partial recirculation, membrane concentration, heat integration and direct wet processing. These options should be evaluated after laboratory trials identify the most promising extraction conditions.

Appendix F. Decision trees for routing biomass and residues

A practical biorefinery needs routing rules. These rules translate analytical data into processing choices, so that a batch of biomass can be directed to the most suitable pathway without redesigning the process every time. The decision trees below are intended as working tools for Activity 2.1. They can be converted into laboratory worksheets, pilot decision forms or SME-facing process guides.

The first decision is feedstock identity. If the batch is *Gracilaria gracilis*, the default route is pigment-first extraction followed by agar extraction. If the batch is *Ulva*, the default route is *Ulva*n-first extraction followed by carbohydrate-residue valorisation. If the batch is mixed seaweed or IMTA biowaste, it should not be forced into either route without characterisation; it should first be screened for moisture, ash, proteins, total sugars, salts and visible contamination.

The second decision is biomass condition. Fresh biomass may preserve pigments and reduce drying energy, but it is more variable in water content and more perishable. Dried biomass is easier to transport and store, but drying can damage heat-sensitive compounds. Freeze-drying is useful for analytical preservation but usually too costly for commercial-scale processing. Therefore, fresh or low-temperature dried biomass should be compared when the target is pigments, while controlled drying may be acceptable for polysaccharides.

The third decision is whether to desalt. Marine biomass often contains salts that influence ash content, extraction behaviour and product quality. Desalting is recommended when the product is a purified polymer, protein extract, fermentation feedstock or agricultural input sensitive to salinity. However, desalting should be limited when the target compound is water-soluble and easily leached. Conductivity and dry-matter loss should be measured to find a balanced washing intensity.

The fourth decision is whether to apply an intensification technology. Ultrasound, microwave and enzyme assistance should be used when they solve a defined problem: slow extraction,

low yield, high temperature or limited release from the matrix. They should not be added as a default because they can increase complexity and damage product quality. The decision should be based on a comparison with conventional extraction under the same biomass batch.

The fifth decision concerns residue outlet. Residue should be routed after analysis, not by assumption. A protein-rich, low-salt residue may be suitable for feed or biostimulant development. A carbon-rich residue with balanced C/N may be suitable for digestion or composting. A mineral-rich or salt-rich residue may be better suited to controlled thermochemical conversion or nutrient recovery. A residue that contains inhibitory compounds may require blending or detoxification.

The sixth decision is product grade. The same molecule class can generate different products depending on purity and application. A crude pigment extract, semi-purified colourant and analytical fluorophore are different products. Crude agar, food-grade agar and molecular biology-grade agarose are different products. Crude *Ulva*n, agricultural *Ulva*n and biomedical-grade *Ulva*n are different products. The grade decision must be made before designing purification.

The final decision is whether to advance the pathway. A route should advance when it offers reproducible yield, acceptable product quality, manageable water and energy demand, and useful residue outlets. It should be modified when only one of these dimensions is weak and can be improved. It should be held when market specification is unclear. It should be rejected when it is technically weak and would consume resources better used in a stronger cascade.

Table F1. Decision tree checkpoints for Activity 2.1 processing.

Decision point	Question	Recommended action	Evidence required
Feedstock identity	Is the batch *Gracilaria*, *Ulva* or mixed biowaste?	Apply species-specific default route or characterise mixed stream	Species ID, visual inspection, composition data
Condition	Fresh, wet, dried or lyophilised?	Choose processing route that protects target compounds	Moisture, storage time, colour, odour

Desalting	Is salt likely to limit product quality?	Optimise washing without excessive soluble-product loss	Conductivity, ash, dry-matter loss
Intensification	Does UAE/MAE/enzyme solve a defined bottleneck?	Test against conventional benchmark	Yield, quality, energy, residue condition
Residue outlet	What valuable components remain?	Route to feed, biostimulant, digestion, compost, biochar or nutrient recovery	C/N, NPK, salts, metals, organic matter
Product grade	Which market grade is realistic?	Define crude, semi-purified or refined product specification	Purity, functionality, stability, end-user feedback

Appendix G. Scale-up and equipment considerations

Laboratory extraction gives chemical evidence, but scale-up determines whether a pathway can become a usable process. The main scale-up issues for seaweed biorefineries are biomass logistics, water handling, mixing, heating, solid-liquid separation, drying, extract stabilisation and residue management. The selected BIOBASED pathways should therefore be evaluated using equipment assumptions from the beginning, even if experiments are performed at bench scale.

Biomass logistics begin at harvest. Seaweed is bulky and wet, so transport cost is strongly influenced by water content. Wet processing can reduce drying costs but requires rapid handling and nearby processing capacity. Dried processing improves storage but requires energy and can degrade sensitive compounds. For *Gracilaria* pigments, wet or gently dried biomass may be preferable; for agar and *Ulva*n, controlled drying may be acceptable if quality is preserved.

Size reduction is often underestimated. Milling improves surface area and extraction rate, but excessive milling can increase fines, complicate filtration and accelerate oxidation. A practical pilot should define particle-size ranges rather than a vague instruction to homogenise. Wet chopping, coarse milling and fine powdering should be compared where relevant to extraction performance and separation time.

Mixing becomes more difficult at scale because seaweed slurries can be fibrous and viscous. Solid/solvent ratio must therefore be chosen with agitation limits in mind. A ratio that works in a beaker may fail in a pilot vessel because of poor suspension or dead zones. Mixing observations should be recorded during laboratory trials, especially for high biomass loading.

Heating strategy differs by product. Pigment extraction requires cold or ambient conditions, while agar and some *Ulva*n extractions require heat. Heat-up time, not only final temperature, matters because the biomass experiences a temperature history. Microwave-

assisted extraction may reduce heat-up time but requires specific equipment and safety control. Conventional jacketed heating may be simpler and easier to scale.

Solid-liquid separation is a likely bottleneck. Seaweed particles, gels and colloids can clog filters or slow centrifugation. For pigments, clarification must be gentle and rapid. For agar, hot filtration may be needed before gelation. For *Ulva*n, filtration and precipitation or concentration can influence ash and polymer quality. Pilot planning should include separation equipment, not only extraction vessels.

Concentration and drying of extracts can dominate cost and energy use. Dilute pigment extracts may require membrane concentration or precipitation. Agar and *Ulva*n may require drying to stable powders. Hydrolysates may need concentration before fermentation. The project should record extract solids and volume because these determine downstream costs.

Residue storage is another practical issue. Wet residues can degrade quickly and may develop odours or microbial growth. If residues are intended for digestion, composting or biochar, storage and transport conditions should be defined. A residue that is chemically valuable but logistically unstable may be difficult to use. Stabilisation options include dewatering, ensiling, controlled drying, immediate digestion or blending.

Equipment availability should guide pathway selection for SMEs. A route requiring specialised chromatography may be appropriate for research but difficult for local uptake. A route based on washing, aqueous extraction, filtration, heating and drying is more accessible. Advanced purification can be added later for selected high-value fractions, but the first demonstration should prioritise robust unit operations.

The scale-up recommendation is to define a modular pilot line: receiving and washing, size reduction, mild aqueous extraction, solid-liquid separation, thermal polymer extraction or hydrolysis, product recovery, residue treatment and analytical sampling. Each module can

be improved independently while preserving the cascade structure. This makes the process easier to communicate to equipment suppliers and SMEs.

Table G1. Equipment-oriented scale-up considerations for selected pathways.

Unit operation	Main equipment options	Critical scale-up issue	Suggested pilot observation
Receiving/washing	Tanks, screens, spray washers	Water use and soluble losses	Conductivity, dry matter, visual cleanliness
Size reduction	Chopper, wet mill, dry mill	Particle size and filtration behaviour	Slurry viscosity and settling/filtering time
Cold extraction	Stirred tank, cooled vessel	Protein stability and mixing	Temperature profile and colour stability
Hot extraction	Jacketed vessel, microwave unit	Heating rate and polymer quality	Time-temperature curve and gel/viscosity
Separation	Filter press, centrifuge, membrane	Clogging, solids capture, throughput	Filtration time and turbidity
Concentration/drying	Membrane, evaporator, dryer	Energy use and product stability	Dry solids, product moisture and colour
Residue handling	Dewatering, digester, composter, pyrolysis	Storage stability and outlet quality	Moisture, odour, C/N, salinity

Appendix H. Quality assurance, quality control and acceptance criteria

Quality assurance for Activity 2.1 has two purposes. The first is scientific reliability: results must be reproducible and comparable across partners. The second is product relevance: extracts and residues must be described using indicators that matter for end users. A high analytical value is not enough if the sample is unstable, contaminated, too salty or unsuitable for the intended application.

Sample coding should be standardised. A recommended code includes project acronym, partner, species, site, harvest date, batch number, process step and replicate. For example, a code can identify raw *Gracilaria* from a specific site and distinguish it from pigment extract, pigment residue, agar extract and final residue. This prevents confusion in a cascade where one starting biomass creates many samples.

Analytical calibration should be documented. Protein, sugar, phenol, pigment and elemental analyses should include calibration curves, blanks and quality-control samples. If different laboratories perform the same analysis, an inter-laboratory comparison should be considered for key methods. The objective is not to create administrative burden but to ensure that pathway decisions are based on comparable data.

Acceptance criteria should be defined for each pathway stage. For phycobiliproteins, criteria may include minimum absorbance signal, colour stability, absence of visible contamination and acceptable clarification. For agar, criteria may include gel formation, dry polymer yield and ash or colour limits. For *Ulva*n, criteria may include polymer recovery, uronic acid/rhamnose indicators and ash. For hydrolysates, criteria may include sugar concentration and inhibitor limits.

Residue acceptance criteria are equally important. A residue should not be advanced to agricultural testing if salinity, metals or hygiene are unsuitable. A residue should not be directed to fermentation if inhibitors or salts are excessive. A residue should not be sent to

digestion without considering C/N ratio and salt load. Acceptance criteria protect downstream work from avoidable failures.

Stability testing should be included early. Pigment extracts should be assessed over time under defined storage conditions. *Ulva*n and agar powders should be checked for moisture and microbial stability. Hydrolysates should be monitored for contamination if stored. Stability data help identify whether a product can be transported to SMEs or end users for testing.

Documentation should include photographs. Colour, gel formation, precipitation, phase separation, foaming and residue appearance can be easier to interpret visually than through numbers alone. The draft report already uses photographic evidence effectively; future trials should continue this practice with standardised captions and sample codes.

Quality control should culminate in a batch summary sheet. The sheet should report feedstock, process conditions, main yields, quality indicators, residue composition and recommended next action. This one-page summary is useful for project meetings and for communicating with stakeholders who do not need full analytical details.

Table H1. Quality-control indicators and acceptance logic.

Stream	Critical quality indicators	Suggested acceptance logic
Raw biomass	Species, moisture, ash, visual quality, storage time	Accept if traceable, uncontaminated and composition is measured
Pigment extract	R-PE/APC/PC signal, protein, colour, stability, turbidity	Advance if colour and absorbance are reproducible and stable
Agar product	Yield, gel formation, gel strength, ash, colour, moisture	Advance if gel performance matches target application
*Ulva*n product	Yield, rhamnose/uronic acids, sulfate/ash, viscosity/MW	Advance if polymer identity and quality are consistent
Sugar hydrolysate	Glucose, total sugars, inhibitors, pH, salinity	Advance if sugar profile is compatible with selected outlet
Residue	C/N, NPK, salinity, metals, stability	Route according to safety and composition, not by default

Appendix I. Experimental design for comparative pathway testing

A comparative experimental design should be simple enough to execute but structured enough to support decisions. The recommended approach is a two-phase design. Phase 1 screens a small number of technology options against a conventional benchmark. Phase 2 optimises the best-performing option by varying the most important process variables. This avoids a large experiment matrix with too many conditions and too little replication.

For *Gracilaria*, Phase 1 should compare conventional cold aqueous pigment extraction, ultrasound-assisted cold extraction and an enzyme-assisted mild extraction if suitable enzymes are available. The residue from the best pigment condition should then be used for agar extraction. A separate benchmark can test direct agar extraction without pigment recovery, but this should be treated as a comparison route rather than the preferred route.

For *Ulva*, Phase 1 should compare conventional hot-water *Ulva*n extraction, ultrasound-assisted extraction and microwave-assisted extraction. The comparison should use the same biomass batch and similar final extraction severity where possible. The residue should then undergo a standard hydrolysis test so that polymer extraction conditions can be evaluated against downstream sugar recovery.

Phase 2 optimisation should focus on solid/solvent ratio, time and temperature. For pigments, pH and storage stability should also be included. For agar and *Ulva*n, product functionality should be included. For hydrolysis, enzyme loading, time and inhibitor formation should be included. Each optimisation should have a clear response variable and a clear go/no-go criterion.

Replication should be strategic. Early screening can use analytical replicates and limited process replicates, but final selected conditions should be repeated on at least two biomass batches if material is available. This is important because a pathway selected from one batch may not be robust enough for pilot use. Biomass variability should be treated as a factor rather than a nuisance.

The design should include negative and positive controls where possible. A no-pretreatment control helps determine whether ultrasound, microwave or enzymes truly improve performance. A known extraction method can serve as a positive benchmark. For hydrolysis, a glucose standard or cellulose control can confirm enzyme performance. Controls make the results easier to interpret and defend.

Data analysis should report trade-offs. For example, a condition may increase *Ulva*n yield but lower molecular weight. Another may reduce agar extraction time but produce darker product. Another may improve pigment yield but create a dilute extract. The best condition is the one that fits the selected application and whole-cascade objectives, not necessarily the maximum numerical yield.

The recommended final output of the experimental design is a pathway decision table for each species. The table should list the conditions tested, the main results, the selected condition and the reason for selection. This will allow Deliverable D.2.1.1 to feed directly into subsequent project activities and pilot planning.

Table I1. Two-phase experimental design for selected pathways.

Phase	***Gracilaria* tests**	***Ulva* tests**	**Decision output**
Screening	Cold water, UAE, enzyme-assisted pigment extraction; direct agar benchmark	Hot water, UAE and MAE *Ulva*n extraction	Rank routes by yield, quality and residue condition
Cascade check	Agar extraction from pigment residues	Hydrolysis of *Ulva*n-depleted residues	Check downstream compatibility
Optimisation	S/L ratio, time, temperature, pH, clarification	S/L ratio, time, temperature, pH, pretreatment	Define operating window
Validation	Repeat selected condition on new batch	Repeat selected condition on new batch	Confirm robustness
Decision	Advance, modify or reject *Gracilaria* route	Advance, modify or reject *Ulva* route	Prepare pilot recommendation

Appendix J. Glossary and abbreviations

This glossary defines the main terms used in the report. It is included to support communication with non-specialist readers and SMEs. The definitions are written in practical language and are not intended to replace formal regulatory or scientific definitions.

The glossary also helps harmonise terminology across partners. This matters because the same material can be described differently by chemists, biologists, aquaculture producers and market actors. A shared vocabulary reduces ambiguity when discussing extracts, residues and product grades.

Table J1. Glossary and abbreviations.

Term	**Meaning in this deliverable**
Biorefinery	Integrated processing of biomass into multiple products and energy or residue outlets, with the aim of using as much of the feedstock as possible.
Cascade process	A sequence in which the residue from one extraction or conversion step becomes the feedstock for the next step.
IMTA	Integrated Multi-Trophic Aquaculture, where species from different trophic levels are cultivated together to improve resource use and environmental performance.
Gracilaria gracilis	Red seaweed selected here as a pigment and agar feedstock.
Ulva	Green seaweed selected here as an *Ulva*n and carbohydrate feedstock.
Phycobiliproteins	Water-soluble pigment proteins, including R-phycoerythrin, allophycocyanin and phycocyanin.
R-PE	R-phycoerythrin, the main red phycobiliprotein considered in the *Gracilaria* route.
Agar	Gel-forming red-seaweed polysaccharide fraction primarily associated with galactan structures.
*Ulva*n	Sulfated polysaccharide from *Ulva* containing rhamnose, uronic acids, xylose and sulfate groups.
UAE	Ultrasound-assisted extraction.
MAE	Microwave-assisted extraction.
S/L ratio	Solid-to-liquid or solid/solvent ratio used during extraction.
FAMEs	Fatty acid methyl esters, used analytically to characterise fatty acid composition.

UNSAP	Unsaponifiable fraction of lipids.
NPK	Nitrogen, phosphorus and potassium nutrients relevant to fertiliser or biostimulant products.
5-HMF	5-hydroxymethylfurfural, a sugar-degradation product that can indicate hydrolysis severity and possible fermentation inhibition.

Appendix K. Implementation worksheet for the selected *Gracilaria* cascade

This implementation worksheet converts the *Gracilaria* pigment-to-agar pathway into a field-to-product workflow. It is intended for project partners who must translate the deliverable into experiments. The worksheet deliberately separates operational notes from analytical decisions so that the same biomass batch can be compared across different extraction technologies without losing traceability.

The first block is biomass receipt. The operator records biomass origin, harvest date, wet mass, visible condition, temperature during transport, time since harvest and whether the material is fresh, chilled, frozen, dried or lyophilised. The record should include photographs. These details may appear administrative, but they explain a large part of the variability observed in phycobiliprotein yield and agar performance.

The second block is cleaning and desalting. *Gracilaria* should be rinsed to remove sediments, epiphytes and surface salts. Conductivity of washing water and dry-matter loss should be recorded. If the pigment pathway is prioritised, washing should be gentle and short. If agar is prioritised, stronger desalting may be acceptable. The chosen approach should be aligned with the product target before processing begins.

The third block is pigment extraction. A defined mass of dry-equivalent biomass is extracted with cold water or buffer. The worksheet should record solid/solvent ratio, pH, temperature, time, agitation and exposure to light. If ultrasound or enzymes are used, the exact settings are recorded. The extract is clarified and analysed immediately for R-PE, APC, PC, total protein, pH, conductivity and colour stability.

The fourth block is residue transfer. The pigment-depleted residue is weighed, sampled and either processed wet or dried under controlled conditions. This intermediate residue is a critical sample because it links the pigment step to the agar step. The worksheet should include residue moisture, visual colour, residual protein and an estimate of remaining carbohydrate. Without this intermediate record, the cascade mass balance is incomplete.

The fifth block is agar extraction. The pigment-depleted residue is treated by hot-water extraction under defined time and temperature. If alkaline conditioning or microwave assistance is tested, it is recorded as a separate condition. Filtration should be performed before gelation whenever possible. Agar recovery, gel formation, gel strength, colour and ash should be measured or at least recorded as qualitative observations in early tests.

The sixth block is final residue routing. After agar extraction, remaining solids and process waters are coded for residue evaluation. They can be assessed for composting, digestion, biochar or nutrient products. The decision depends on salts, ash, C/N ratio, NPK and remaining organic matter. This final step ensures that the route remains a biorefinery rather than a two-product extraction scheme with waste at the end.

The worksheet also includes an interpretation line. The operator writes whether the condition should advance, be modified, be repeated or be rejected. This short interpretation is valuable because it captures practical observations that may not appear in the numerical dataset, such as filtration difficulty, foaming, odour, gel weakness or excessive dilution.

Table K1. Gracilaria cascade implementation worksheet.

Workflow block	Minimum record	Main decision supported
Biomass receipt	Species, site, wet mass, dry matter, storage, photographs	Batch acceptance and comparability
Cleaning/desalting	Water volume, conductivity, dry-matter loss	Salt reduction without excessive leaching
Pigment extraction	S/L ratio, pH, temperature, time, equipment settings	Pigment yield and stability
Residue transfer	Wet/dry mass, moisture, colour, sample code	Cascade mass balance
Agar extraction	Temperature, time, pretreatment, filtration, drying	Agar yield and gel quality
Final residue	C/N, NPK, salinity, ash, outlet choice	Residue valorisation decision

Appendix L. Implementation worksheet for the selected *Ulva* cascade

This implementation worksheet converts the *Ulva* *Ulva*n-to-sugar pathway into a practical processing sequence. It is designed to help partners compare conventional water extraction, ultrasound-assisted extraction and microwave-assisted extraction while preserving the information needed to evaluate downstream sugar and residue routes.

The first block is biomass description. *Ulva* samples can vary substantially in morphology, thickness, epiphyte load and water content. The operator should record whether the biomass is dominated by sheet-like *Ulva*, tubular forms or mixed green biomass. Photographs are recommended. Because *Ulva* can accumulate salts and nutrients, ash and conductivity data are particularly important.

The second block is controlled washing. Washing aims to reduce salts and sediments while limiting the loss of soluble organic matter. Conductivity should be measured after each wash. A small dried subsample should be used to estimate dry-matter loss. If the intended product is a low-ash *Ulva*n, stronger washing may be needed. If the intended route is a crude biostimulant, less intensive washing may be acceptable.

The third block is *Ulva*n extraction. The extraction condition records biomass dry equivalent, solvent volume, pH, temperature, time, agitation and any ultrasound or microwave setting. The extract is clarified and recovered as crude polymer, concentrated solution or precipitated fraction depending on the laboratory method. The key point is that yield must be interpreted together with ash, uronic acid, rhamnose, sulfate indicators and molecular-weight-related properties.

The fourth block is residue preparation. *Ulva*n-depleted residue should be weighed, sampled and characterised. Because the next step may be hydrolysis, the residue record should include moisture, ash, residual carbohydrates and signs of degradation. If the extraction was severe, inhibitors may already be present. A mild extraction that preserves residue quality may be better than a harsh extraction with slightly higher polymer mass.

The fifth block is hydrolysis or alternative residue conversion. Enzymatic hydrolysis is recommended as the green benchmark, with dilute acid hydrolysis used only as a comparative option where appropriate. The hydrolysate is analysed for glucose, xylose, rhamnose, uronic acids, pH, salts and possible inhibitors. If sugar concentration is too low, the stream may be better used as a biostimulant substrate rather than a fermentation feedstock.

The sixth block is product routing. The *Ulva*n fraction, hydrolysate and final residues should be assigned to potential applications. *Ulva*n can be tested in films, gels or biostimulant assays. Hydrolysates can be tested for microbial conversion or plant-growth stimulation. Residues can be tested for compost, digestion, biochar or nutrient recovery. This routing should be based on data rather than assumptions.

The worksheet ends with a pathway decision. The condition advances only if it gives acceptable *Ulva*n recovery and leaves a usable downstream residue. This prevents the process from maximising crude polymer yield while undermining the whole-cascade objective. In early trials, qualitative observations such as viscosity, colour, filtration difficulty and odour should be written into the decision note.

Table L1. Ulva cascade implementation worksheet.

Workflow block	Minimum record	Main decision supported
Biomass description	Species/morphotype, site, wet mass, dry matter, photographs	Batch acceptance and comparability
Washing	Water volume, conductivity, ash reduction, dry-matter loss	Desalting intensity
*Ulva*n extraction	S/L ratio, pH, temperature, time, UAE/MAE settings	Polymer yield and quality
Residue preparation	Mass, moisture, ash, residual carbohydrates	Hydrolysis or residue route
Hydrolysis	Enzyme or acid conditions, sugars, inhibitors, salts	Fermentation or biostimulant suitability
Product routing	*Ulva*n, hydrolysate and residue application tests	Whole-cascade decision

Appendix M. Final submission checklist for D.2.1.1

The following checklist is intended to support final internal review before submitting Deliverable D.2.1.1. It is written as a practical quality-control list rather than a scientific chapter. The checklist ensures that the report remains anchored to Activity 2.1, uses the available data transparently and communicates clearly to both technical and non-technical readers.

The first requirement is traceability to the signed application form. The report should explicitly mention the BIOBASED project, Work Package 2, Activity 2.1 and Deliverable D.2.1.1. It should state that Activity 2.1 focuses on green technologies, sequential extraction, comparison of conventional and unconventional techniques, green solvents, process parameters and characterisation of extracts and residues.

The second requirement is pathway selection. The report should identify at least two conversion pathways and justify them using the experimental dataset. This version identifies more than two: the *Gracilaria* pigment-to-agar cascade, the *Ulva Ulva*n-to-sugar cascade, the supporting minor-fraction route and the residue/IMTA biowaste valorisation route. The two core routes should remain prominent in the executive summary and graphical abstract.

The third requirement is use of data, figures and tables. The report integrates the draft figures, recreates the composition charts from the draft data, and includes tables for C/N ratio, *Ulva*n-related sugars, agar-related composition, pathway selection, product classes and biomass productivity estimates. Any future analytical updates should be inserted into the same structure.

The fourth requirement is writing quality. The report should avoid repeating the same rationale in every chapter. The introduction explains the project and public value; the technical chapters explain feedstocks and pathways; the appendices provide operational tools. This division helps maintain clarity and prevents unnecessary repetition.

The fifth requirement is graphical consistency. The document uses black text, Open Sans, 12-point body text, 1.15 line spacing, 16-point Heading 1 and 14-point Heading 2. Tables use smaller Open Sans text for readability. Figures have captions and are positioned close to the relevant sections.

The sixth requirement is scientific caution. Market and application statements should be framed as potential applications unless validated by product testing and end-user feedback. This is important for deliverable credibility. The report should show clear promise without overstating readiness or regulatory approval.

The seventh requirement is actionability. The report should end not only with conclusions but with operational recommendations: which pathways to test, which variables to optimise, which analytical indicators to measure and which residues to characterise. This ensures that D.2.1.1 can guide subsequent BIOBASED activities.

Table M1. Final submission checklist.

Checklist item	**Status to confirm before submission**
Project reference	BIOBASED, Interreg Italy-Croatia, WP2, Activity 2.1 and D.2.1.1 are explicitly identified
Pathway requirement	At least two pathways are selected and justified
Data use	Draft data, figures and tables are integrated and interpreted
Graphical abstract	*Gracilaria*, *Ulva* and IMTA biowaste pathways are shown
Formatting	Open Sans, black text, 12 pt body, 1.15 spacing, heading sizes applied
Residues	Extracts and residues are both addressed
Recommendations	Pilot variables, analytical plan and next steps are stated

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