Supplementary Information (SI) for RSC Sustainability.
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ProFA - Valorization of macroalgae biomass as source of proteins and formic acid

Supporting Information

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Table of Content

Synthesis and brief characterization of the OxFA catalysts

Heteropoly acids can be produced from their respective metalates by condensation in an acidic environment. For the synthesis of HPA-x ($H_{3+x}[PV_xMo_{12-x}O_{40}]\cdot y\,H_2O$), the catalysts are usually prepared from solutions of their salts (V_2O_5 and MoO_3). The catalyst was synthesized in two steps and the procedure is explained below. For HPA-2 and HPA-5 the stoichiometry has been adjusted accordingly, but the synthesis recipe remains the same. These are shown in Table S1.

Table S1: Components for the synthesis of HPA-x ($H_{3+x}[PV_xMo_{12-x}O_{40}]\cdot y\,H_2O$) catalysts as example HPA-2 and HPA-5.

Step	Material	m or V for HPA-2	m or V for HPA-5
A	Dest. H_20	350 mL	750 mL
	V_2O_5	8 g	20.0 g
	H_2O_2 (30 % in H_2O)	66 mL	165 mL
	H_3PO_4 (25 % in H_2O)	1.2	3.0 g
B	Dest. H_20	500 mL	500 mL
	MoO_3	63.38 g	44.3 g
	H_3PO_4 (25 % in H_2O)	23.6 g	16.9 g

Step A - Synthesis of Vanadium (V) solution

V_2O_5 and H_2O_2 (30 % in H_2O) were added to destillated water at 4 – 7 °C and stirred at 420 rpm. The mixture was stirred till it turned dark red due to the formation of Vanadium(V) peroxy compounds. The solution was then allowed to warm up to room temperature but was maintained below the temperature of 35 °C. With an increase in temperature, the color of the solution changed from dark red to brown orange along with gas evolution (in both cases). Once the gas evolution was completed, H_3PO_4 (25 % in H_2O) was added to the mixture turning the mixture into dark brown in color and was stirred for 45 minutes.

Step B - Synthesis of HPA-2 solution

MoO_3 was added along with H_3PO_4 (25 % in H_2O) to distilled water. The solution was stirred and heated to boiling in a round bottom flask with a reflux condenser. After some time, the solution turned yellow in colour to which the solution from step A was added gradually. The solution thus formed was (dark) cherry-red in color. The final solution was evaporated in a rotary evaporator at 85 °C and reduced pressure (180 mbar to 40 mbar). The solid was dried overnight under the same conditions (85 °C, 180 mbar to 40 mbar).

Characterization of HPA-5 and HPA-2

HPA-2

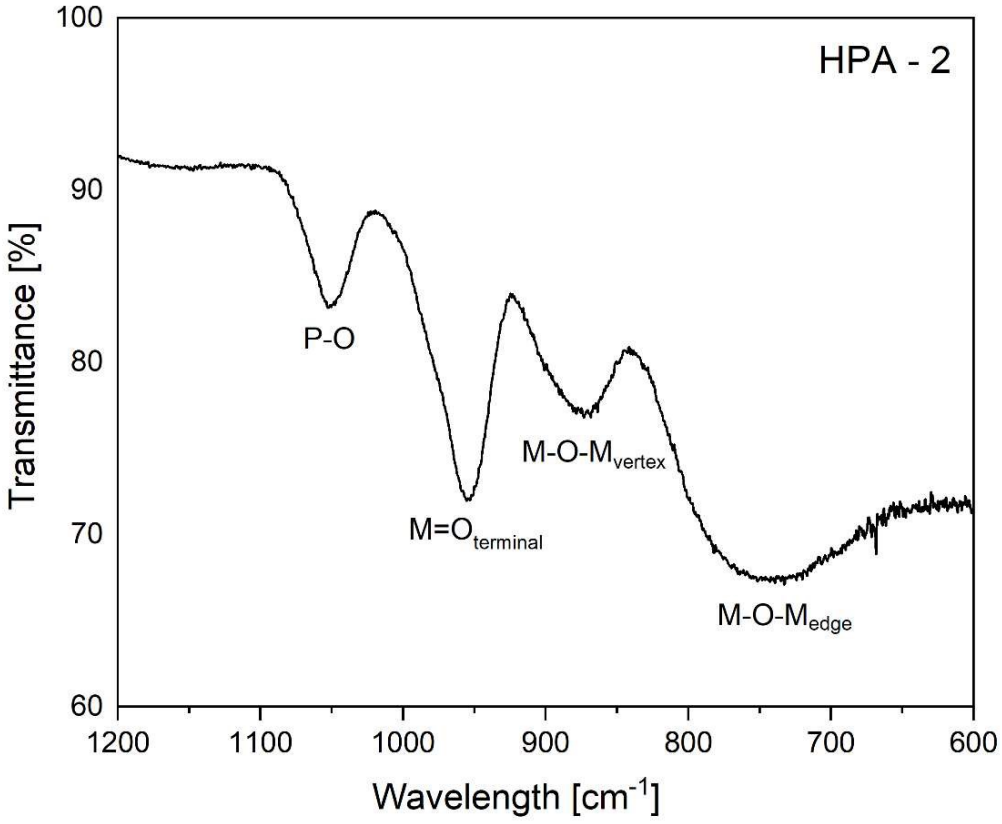

Figure S1: FT-IR spectra of a HPA-2.

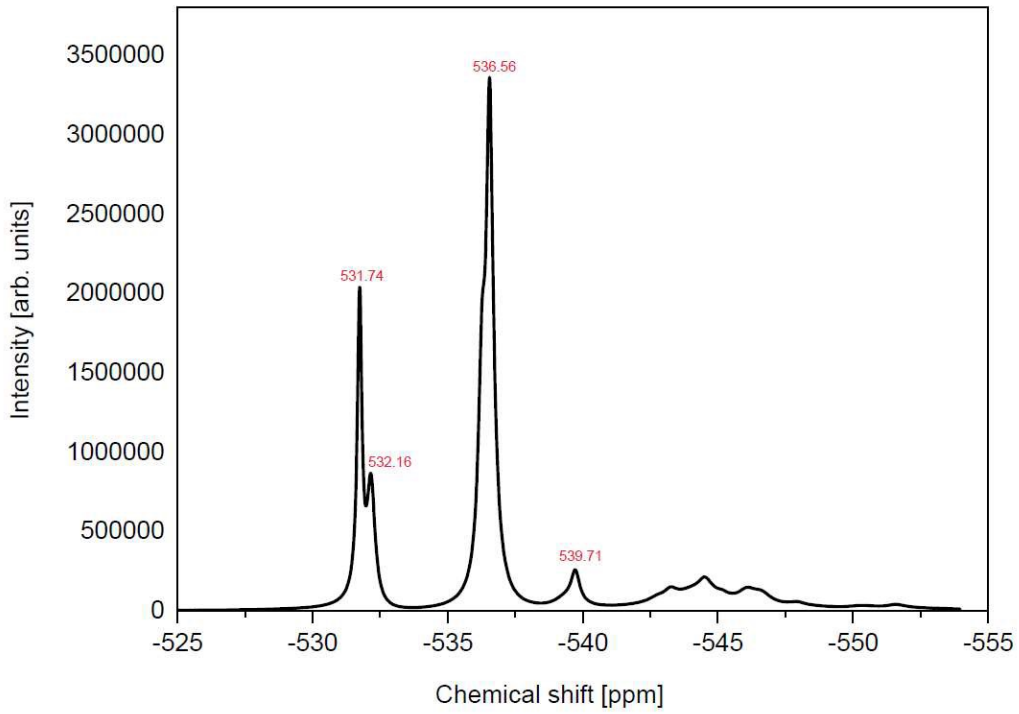

Figure S2: ^{51}V-NMR spectra of HPA-2.

HPA-5

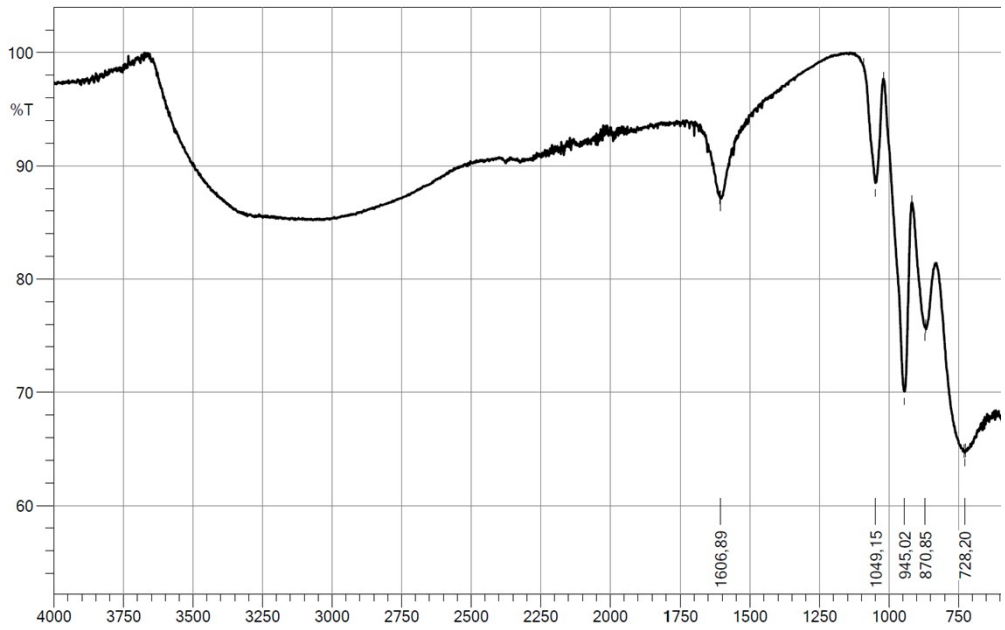

Figure S3: FT-IR spectra of HPA-5.

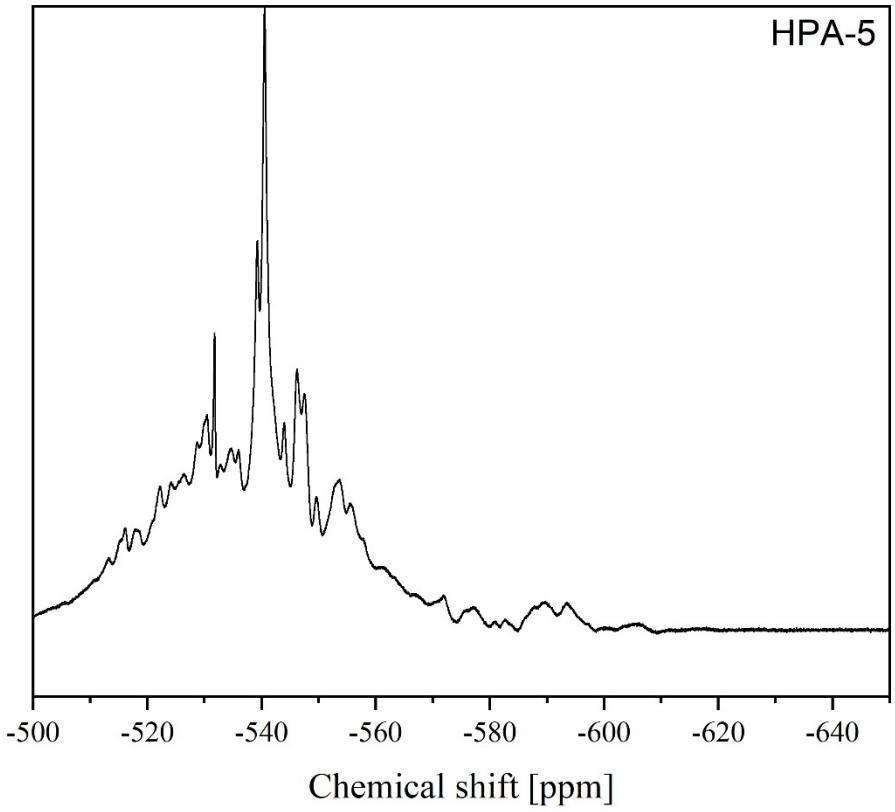

Figure S4: ^{51}V-NMR spectra of HPA-5.

Formulas for calculation

For the calculation of the molar mass of the different algal substrates the following set of equations (1) – (3) was used.

To calculate the molar amount of each element found with the CHNS-elemental analysis, equation (1) was used.

$$x_i = \frac{\frac{w_i}{M_i}}{\sum_{i=1}^{z} \frac{w_i}{M_i}} \quad (1)$$

Where,

x_i = molar amount for each element (C, H, N, S)

w_i = mass fraction for each element determined via CHNS-elemental analysis

M_i = molar mass for each element

The empirical formula was normalized with respect to elemental oxygen by using equation (2).

$$N_i = \frac{x_i}{x_0} \quad (2)$$

Where,

N_i = normalized molar amount for each element

x_i = molar amount for each element (C, H, N, S)

x_o = molar amount of oxygen

To determine the empirical molar mass of the macroalgae the following equation (3) was used.

$$M_{algae} = N_C * M_C + N_H * M_H + N_N * M_n + N_s * M_S + N_O * M_O \quad (3)$$

To determine the yields of the reaction products formic acid (liquid phase, measured via HPLC) and CO_2 as well as CO (gas phase, measured via GC) of the OxFA process, equation (4) was applied.

$$Y_i = \frac{n_i}{n_{i,\,max}} \quad (4)$$

Where,

Y_i = yield of the product with i = formic acid, CO_2, CO

$n_{i,}$= amount of product in mole

$n_{i,max}$ = maximum amount of moles formed assuming that all carbon present in the biomass reacts to the component I with no further side reactions

The maximum amount of substance that can be produced was determined by equation (5) and the amount of substance produced was calculated using equation (6).

$$n_{i,max} = N_c * n_{algae} \tag{5}$$

$$n_{i,product} = c_{i,product} * V_{reaction\ solution} \tag{6}$$

Where,

c_i = concentration of product

$V_{reaction\ solution}$ = volume of the reaction solution

For the calculation of the overall yield equation (7) was used.

$$Y_{summed} = \sum Y_i \tag{7}$$

The conversion of macroalgae biomass and the selectivity can be determined by equations (8) and (9).

$$X_{algae,\ C-content} = 1 - \frac{n_{solid\ redisue,\ C-content}}{n_{algae,\ C-content,\ initial}} \tag{8}$$

Where,

$X_{algae,\ C\text{-}content}$ = Conversion of algae in terms of the carbon content

$$S_i = \frac{Y_i}{X_{algae,\ C-content}} \tag{9}$$

Where,

S_i = selectivity of products

The protein content for each tested extraction approach was quantified. To compare and conclude the extraction approaches, an absolute value for the protein content is needed. For this purpose, the protein recovery was calculated via equation (10).

$$Protein\ Recovery\ (\%) = \frac{A_{protein}\left(\frac{g}{kg}\right) * m_{extract}(kg)}{A_{protein,initial}\left(\frac{g}{kg}\right) * m_{initial}(kg)} * 100\ \% \quad (10)$$

Where,

$A_{protein}$ = amount of protein in the extract (g/kg)

$m_{extract}$ = mass of the extract (kg)

$A_{protein,\ initial}$ = amount of protein in the initial biomass (g/kg)

$m_{initial}$ = mass of the initial biomass (kg)

The amino acid distribution was estimated to determine and compare the amino acid composition as calculated by equation (11).

$$Amino\ acid\ (AA)\ distribution\ (\%) = \frac{c_{AA}}{\sum c_{AA}} * 100\ \% \quad (11)$$

Amino acid profiles of the macroalgae species

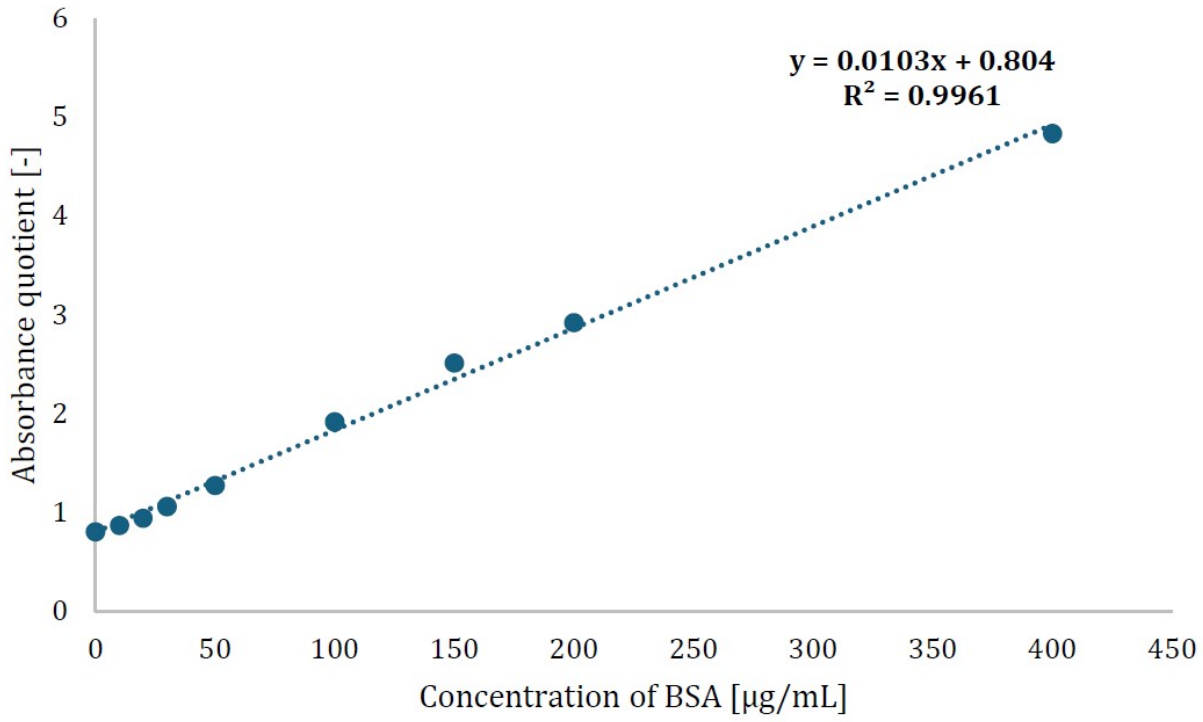

Figure S5: Bradford Assay calibration curve.

The most abundant amino acid in the porphyra dioica sample are alanine (14.6 %) and aspatic acid (12.7 %) (Supporting Information, Figure S2), so a hydrophobic and an hydrophilic amino acid. Ulva fenestrata and fucus vesiculosus, on the contrary, has high amino acid values of the amino acids glutamic acid (12.2 % and 25.2 %) and aspartic acid (14.5 % and 16.2 %), i.e. a high proportion (approx. 27 % and 42 %) of hydrophilic acids (Supporting Information, Figure S3 and Figure S4). The other amino acids present in the fucus vesiculosus are below 8 %. This means that this brown algae is not a favored substrate for the production of biopolymer films for the current stage.

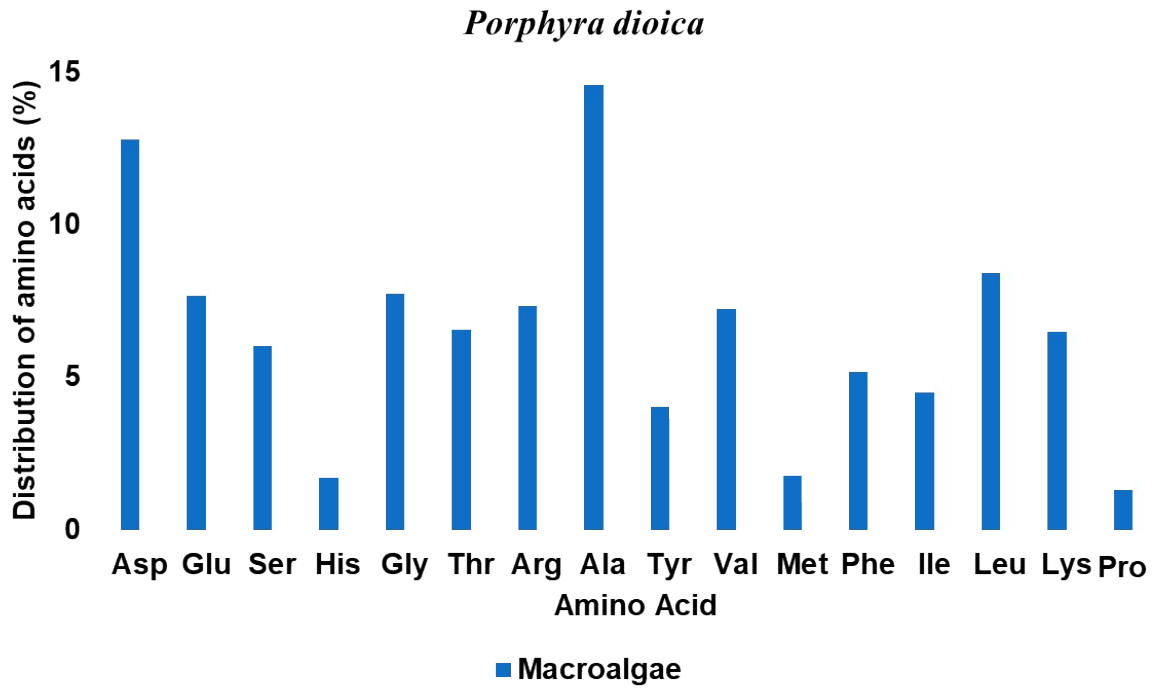

Figure S6: Amino acid profile of Porphyra dioica.

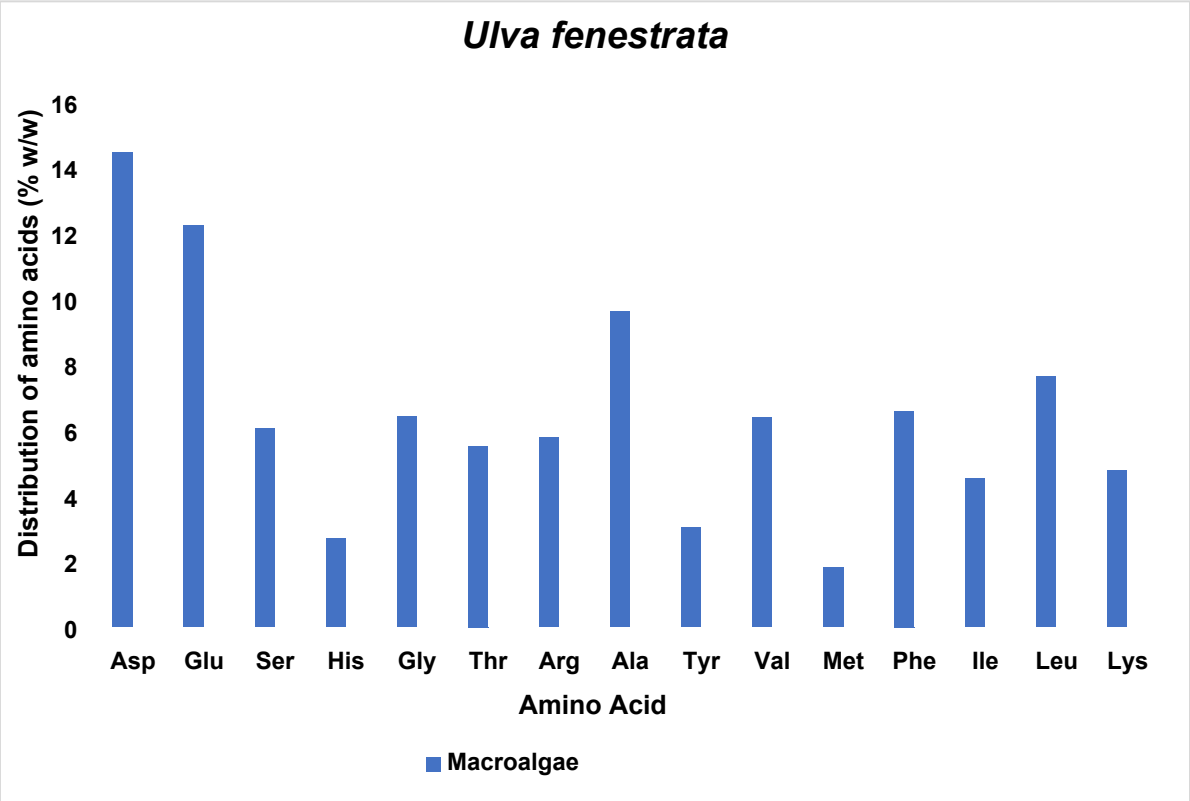

Figure S7: Amino acid profile of ulva fenestrata.

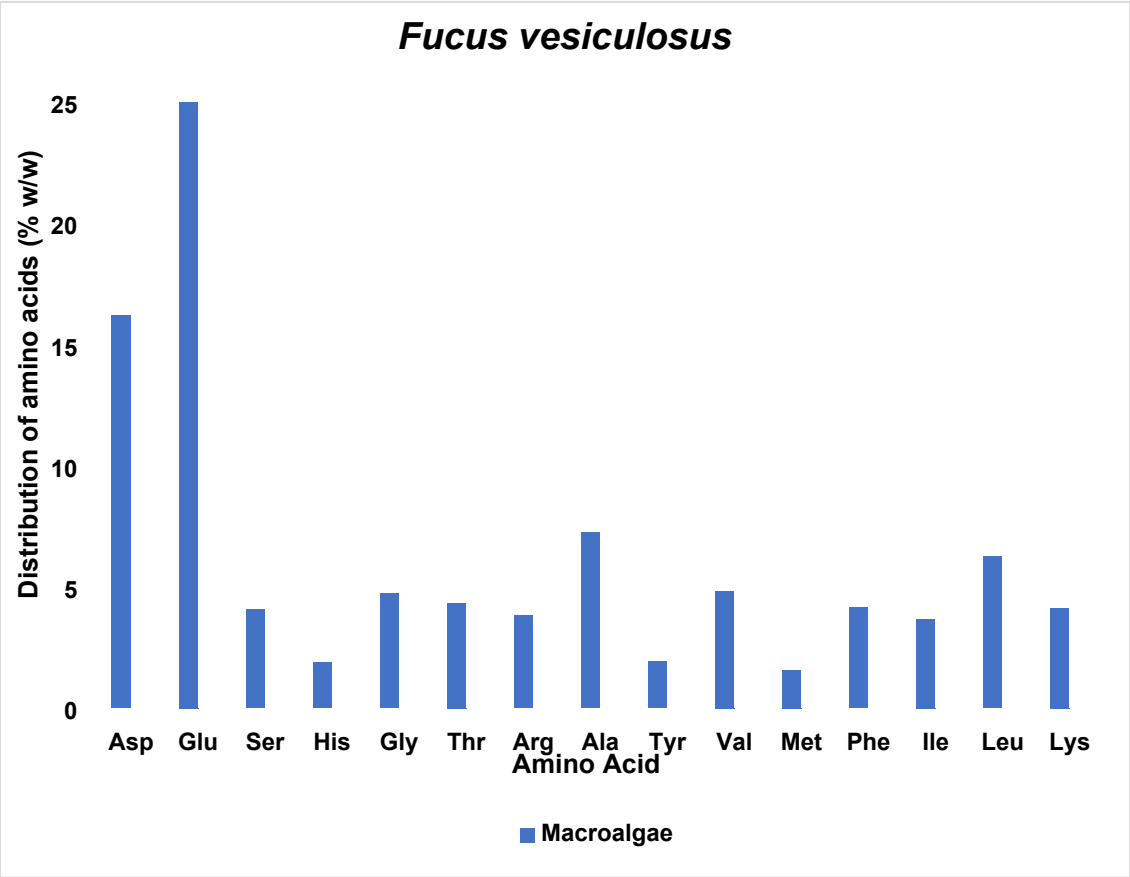

Figure S8: Amino acid profile of fucus vesiculosus.

Elemental analysis of the macroalgae species

Table S2: Elemental analysis of the macroalgae samples.

Entry	Substrate	C (wt.%)	N (wt.%)	H (wt.%)	S (wt.%)	O (wt.%)
1	Porphyra dioica	33	4.7	4.8	1.7	55.8
2	Fucus vesiculosus	32	1.4	3.6	2.1	60.9
3	Ulva fenestrata	37	2.7	4.4	5.3	60.6

Table S3: Elemental analysis of the solid residues after the OxFA process.

Enry	Solid residue of	C (wt.%)	N (wt.%)	H (wt.%)	S (wt.%)	O (wt.%)
1	Ulva fenestrata	29	4.3	4.6	0.23	61,9
2	Fucus vesiculosus	43	5.4	3.4	0.32	47,9
3	Porphyra dioica	29	3.7	6.7	0.36	60,2

Electrochemical data from the two screened catalysts

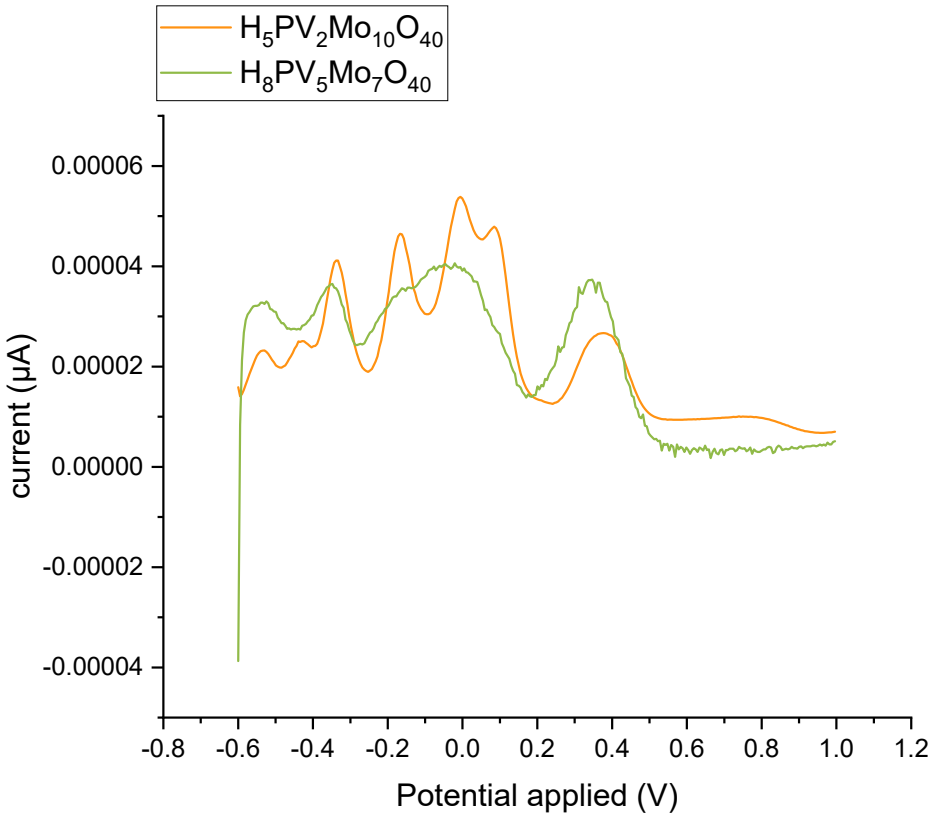

Figure S9: SWV data of the POMs HPA-2 ($H_5PV_2Mo_{10}O_{40}$, orange) and HPA-5 ($H_8PV_5Mo_7O_{40}$, green). The signals between -200 mV and 200 mV merge with increasing V(V) content. [1,2]

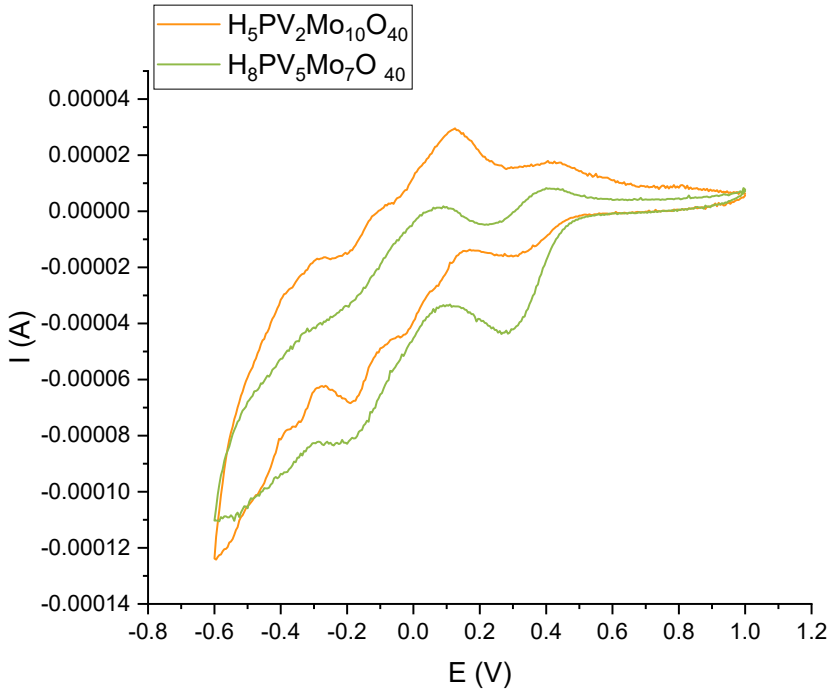

Figure S10: CV data of the POMs HPA-2 ($H_5PV_2Mo_{10}O_{40}$,organge) and HPA-5 ($H_8PV_5Mo_7O_{40}$, green). The signals between -200 mV and 200 mV merge with increasing V(V) content.[1,2]

Amino acid profiles of macroalgae and solid residue

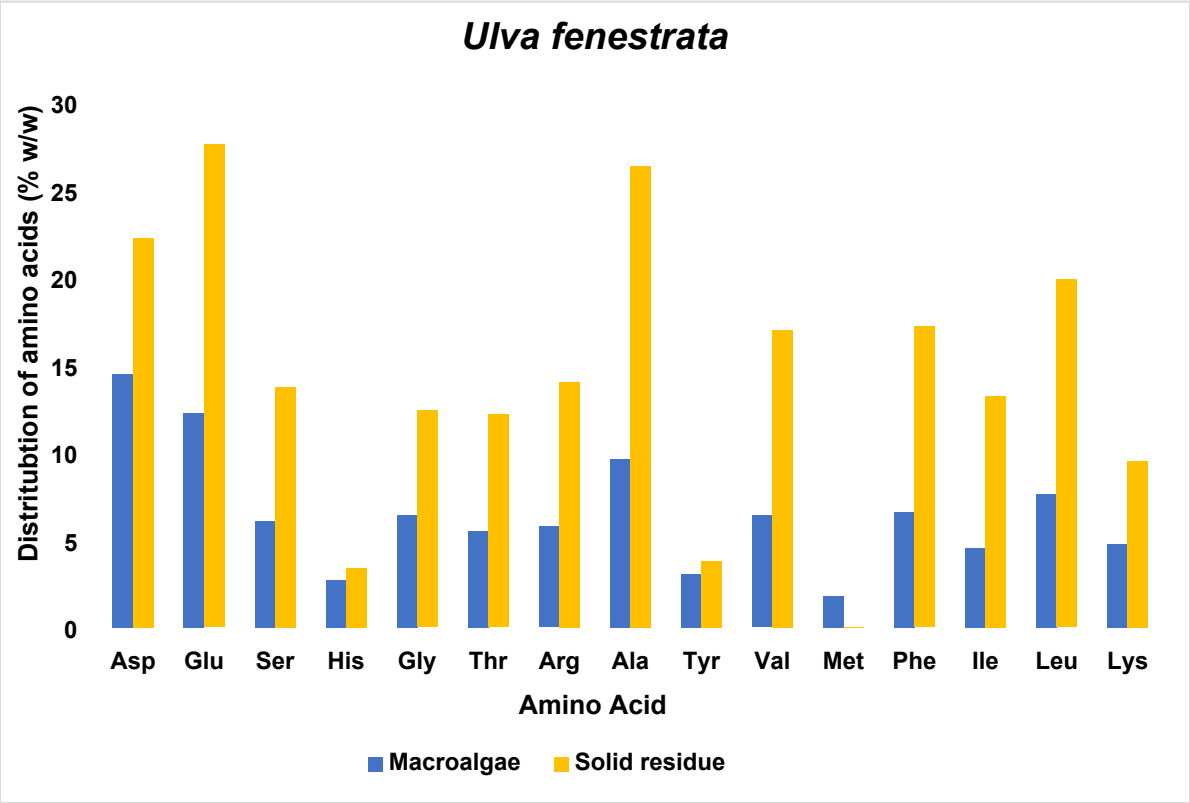

Figure S11: The amino acid profile for the macroalgae ulva fenestrata and its solid residue after the OxFA process. *Reaction conditions: $H_5PV_2Mo_{10}O_{40}$ at initial temperature 90 °C, non-isothermal reaction, 30 bar oxygen pressure, 1000 rpm stirrer speed, 24 h reaction time. The solvent was 200 g of water with 10 g suspended macroalgae and 1.952 g of dissolved catalyst (1 mmol, $H_5PV_2Mo_{10}O_{40}$).*

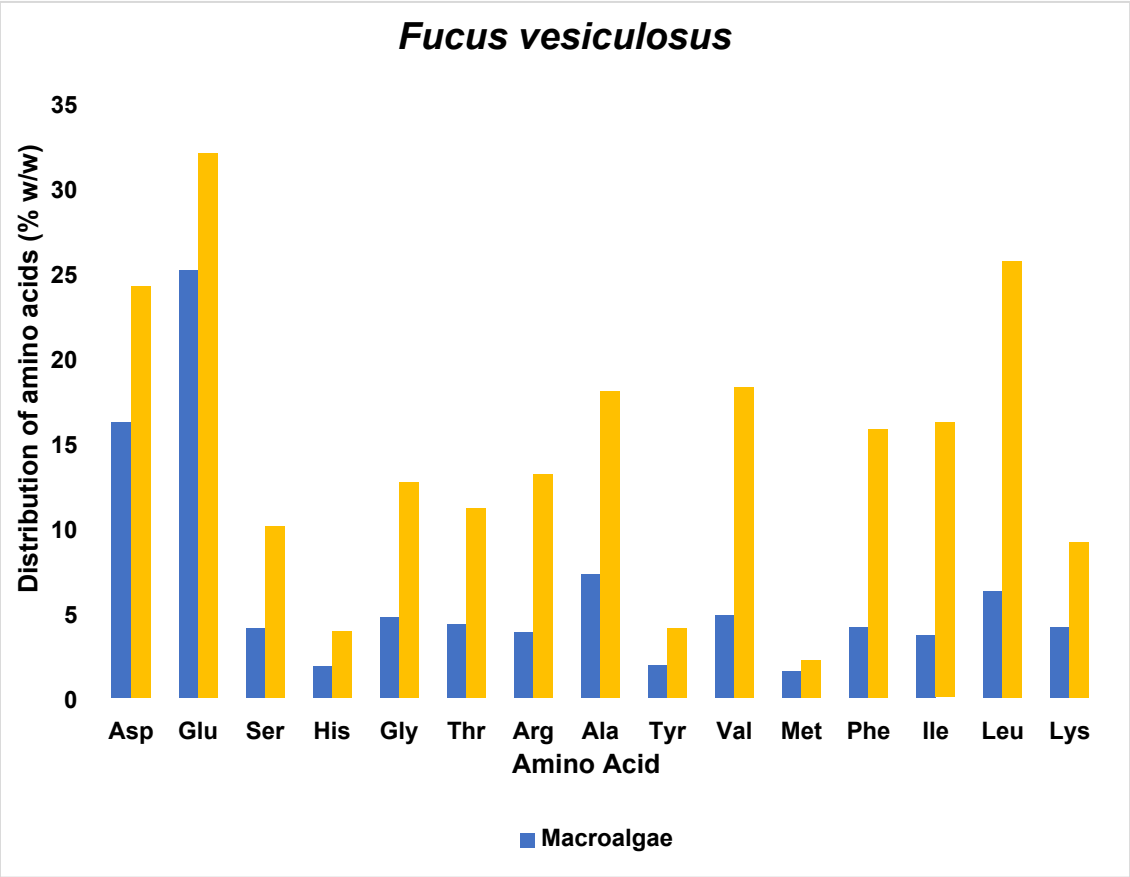

Figure S12: The amino acid profile for the macroalgae focus vesiculosus and its solid residue after the OxFA process. *Reaction conditions: H5PV2Mo10O40 at initial temperature 90 °C, non-isothermal reaction, 30 bar oxygen pressure, 1000 rpm stirrer speed, 24 h reaction time. The solvent was 200 g of water with 10 g suspended macroalgae and 1.952 g of dissolved catalyst.*

Characterization of 2nd batch of porphyra dioica in comparison with the 1st batch

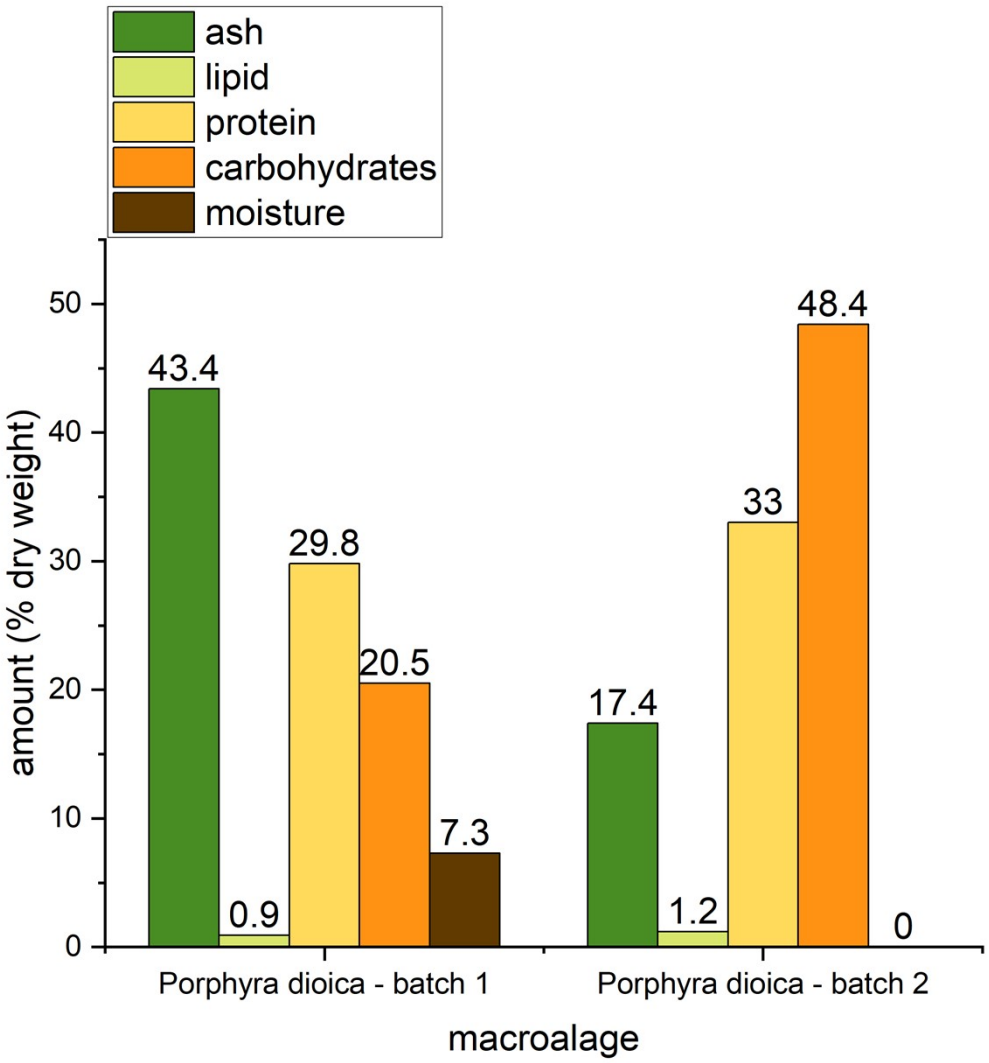

Figure S13: Comparison of the compostition of the two different batches of porphyra dioica used.

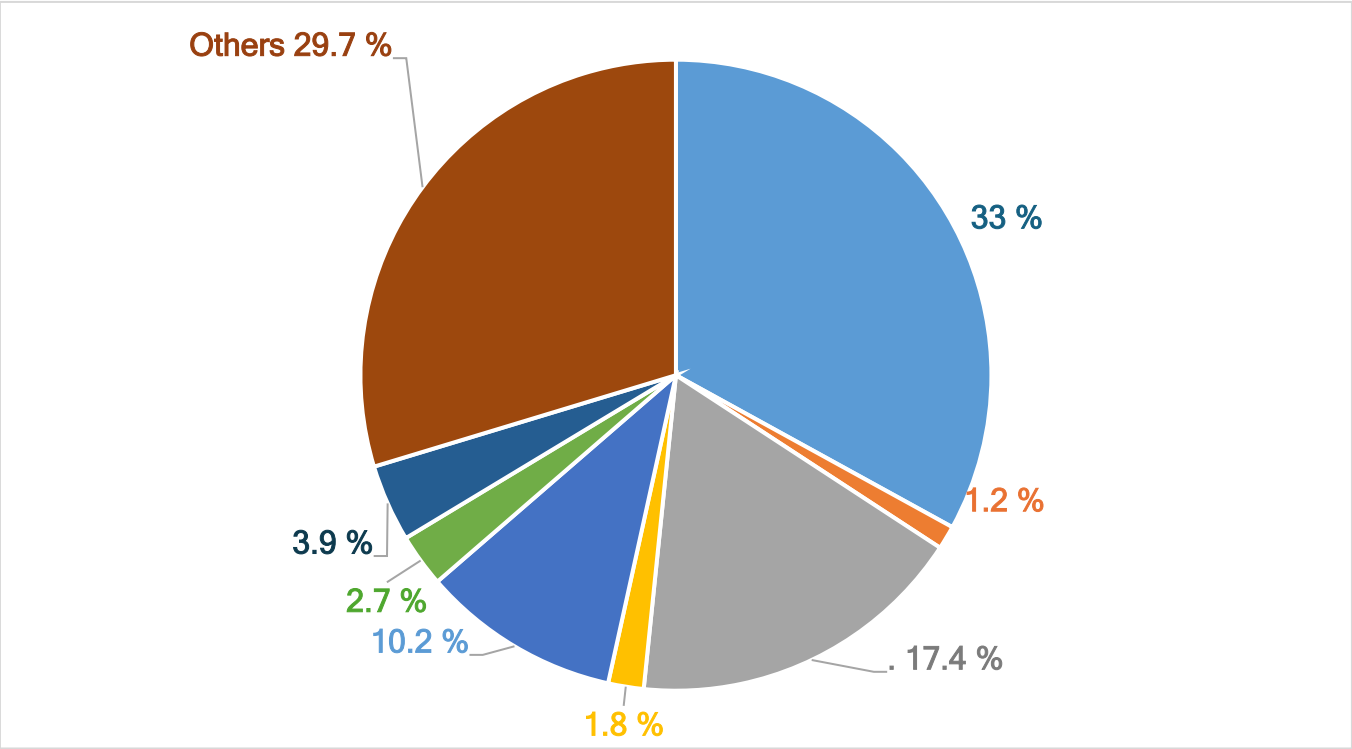

Figure S14: Composition of the porphyra dioica batch 2 with the detailed information about the carbohydrate composition determined via the NREl TP 510-42618.[3]

Table S4: Comparison of the two batches porphyra dioica via CHNS-elemental analysis.

Entry	Substrate	C (wt.%)	N (wt.%)	H (wt.%)	S (wt.%)	O (wt.%)
1	Porphyra dioica batch 1	33	4.7	4.8	1.7	55.8
2	Porphyra dioica batch 2	36	5	6	1	52

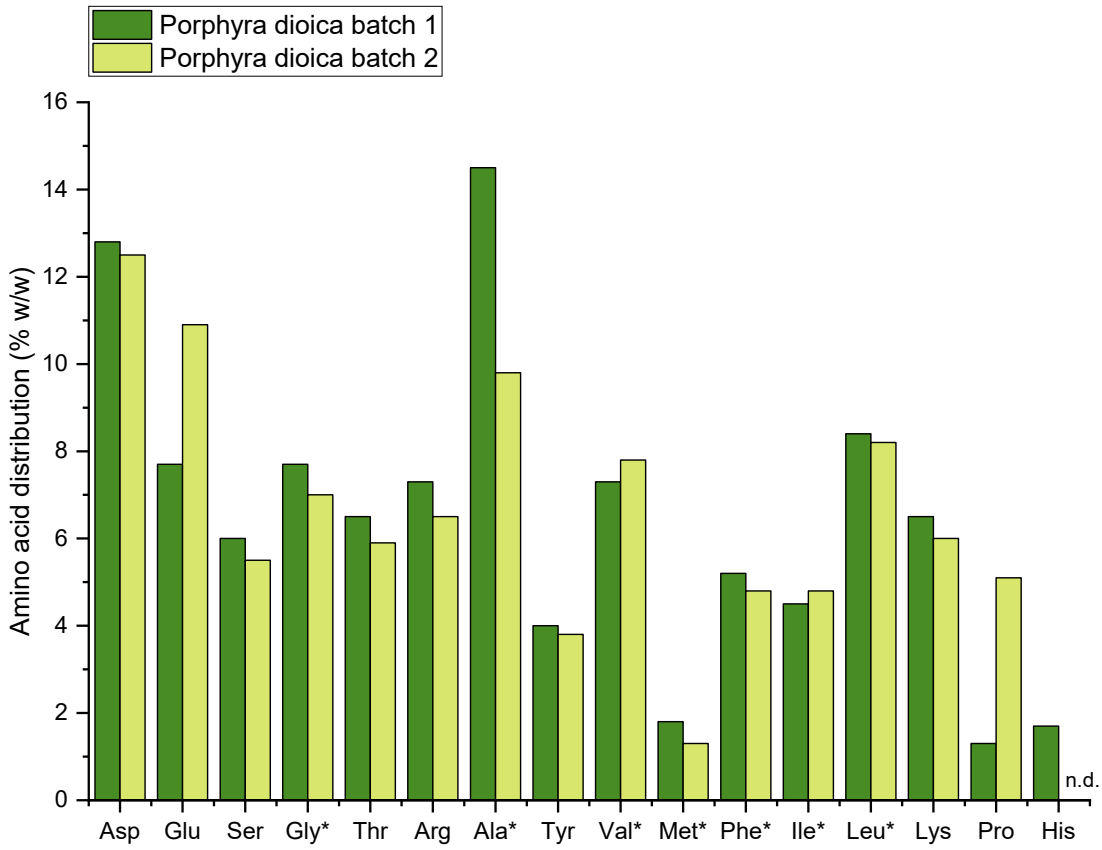

Figure S15: Amino acid distribution for the two used porphyra dioica batches. Hydophilic acids are labelled with *.

Additional information about the DoE

Table S5: Box-Behnken experimental design for Porphyra dioica.

Entry	Duration (h)	Temperature (°C)	Catalyst/substrate ratio ($\frac{g_{catalyst}}{g_{substrate}}$)
1	18	100	0.5
2	24	100	0.275
3	24	120	0.5
4	24	100	0.275
5	24	80	0.5
6	18	100	0.05
7	24	80	0.05
8	30	100	0.5
9	24	100	0.275
10	18	80	0.275
11	30	120	0.275
12	24	120	0.05
13	18	120	0.275
14	30	100	0.05
15	30	80	0.275

Fixed reaction conditions: 30 bar oxygen pressure, 1000 rpm stirrer speed, 10 g of macroalgae Porphyra dioica as substrate, 200 g water as solvent.

Protein recovery

Table S6: Detailed statistical evaluation from ANOVA for protein recovery.

Source	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**	significant?
Model	4629.46	4629.46	90.15	< 0.0001	yes
A-temp	4629.46	4629.46	90.15	< 0.0001	
Residual	667.55	51.35			
Lack of Fit	660.59	60.05	17.26	0.0560	no
Pure Error	6.96	3.48			
Cor Total	5297.01				

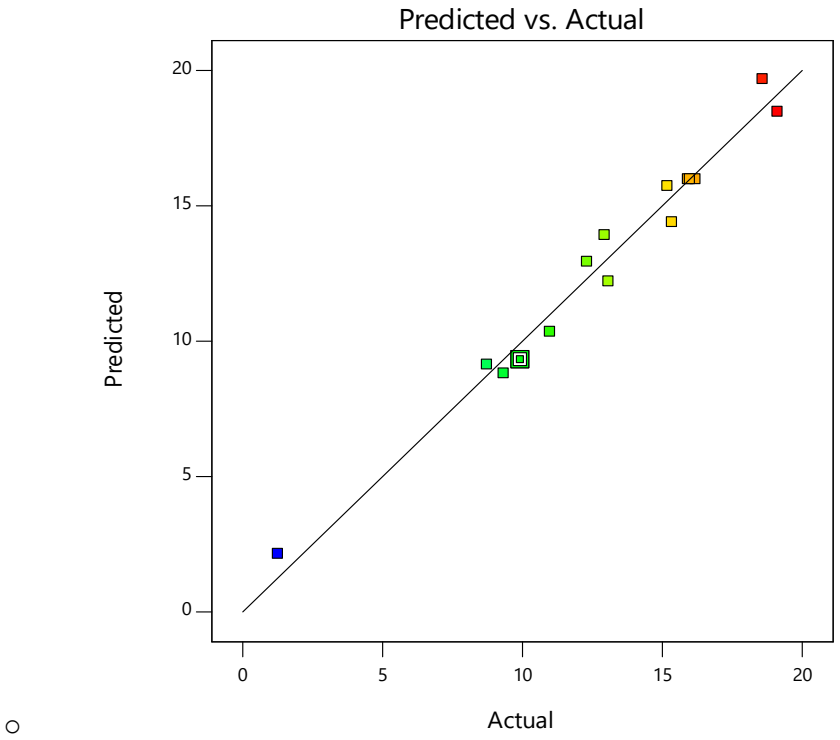

Figure S16: The fit comparison of the actual value to the predicted value for protein recovery. The values represented are protein recovery / %

Yield of formic acid

Table S7: Detailed statistical evaluation from ANOVA for the yield of formic acid.

Source	**Sum of Squares**	**Mean Square**	**F-value**	**p-value**	
Model	285.01	31.67	31.68	0.0007	significant
A-temp	17.04	17.04	17.05	0.0091	
B-time	2.92	2.92	2.92	0.1480	
C-ratio	174.17	174.17	174.26	< 0.0001	
AB	4.79	4.79	4.79	0.0803	
AC	18.09	18.09	18.10	0.0081	
BC	1.95	1.95	1.95	0.2216	
A^2	59.71	59.71	59.74	0.0006	
B^2	0.0343	0.0343	0.0344	0.8602	
C^2	9.21	9.21	9.21	0.0289	
Residual	5.00	0.9995			
Lack of Fit	4.96	1.65	87.25	0.0114	significant
Pure Error	0.0379	0.0189			
Cor Total	290.01				

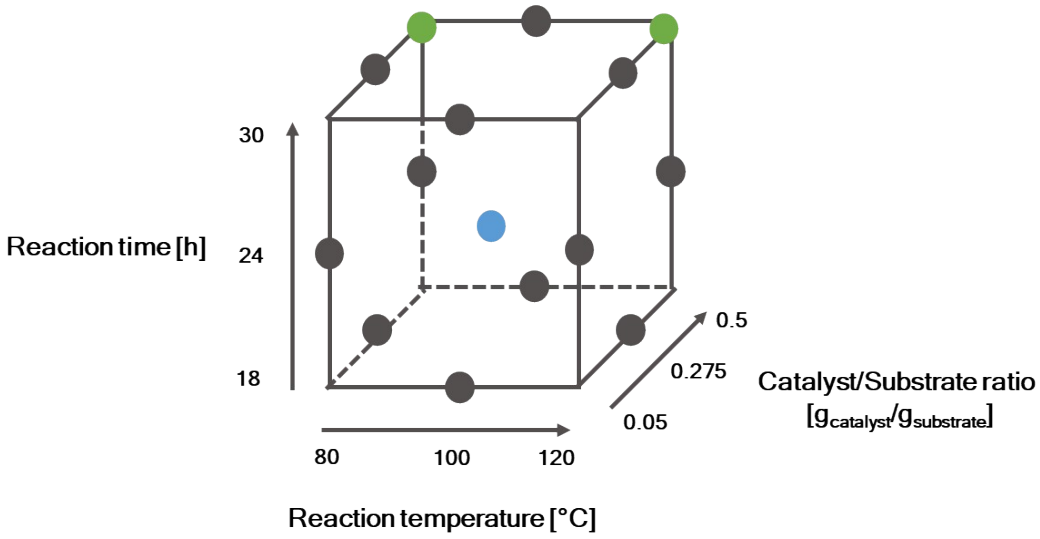

Figure S17: The Box-Behnken-Design including the verification runs which are represented in the edges of the cube in green colour.

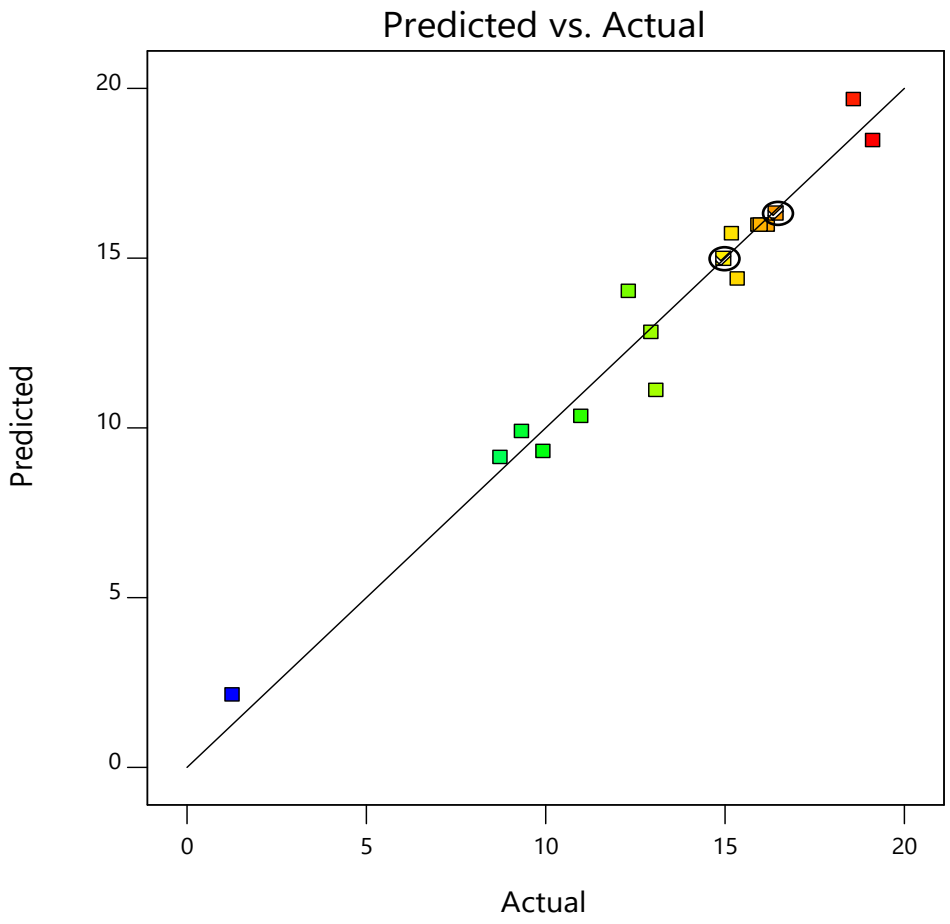

Figure S18: The fit comparison of the actual value to the predicted value for the verification experiments. The circled points indicate the verification experiments. The values presented are yield of formic acid / %.

Additional Information for the protein extraction

SDS-PAGE profiles

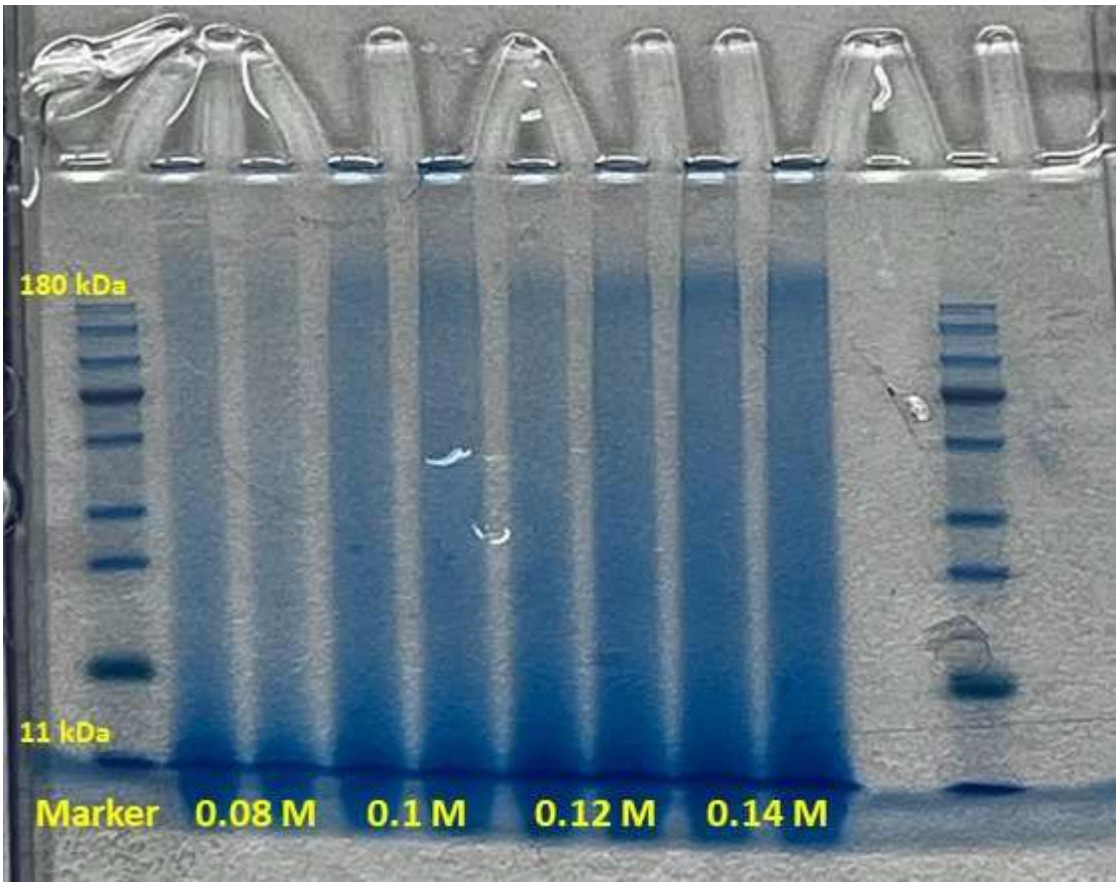

Figure S19: The SDS-PAGE profile for alkaline hydrolysis.

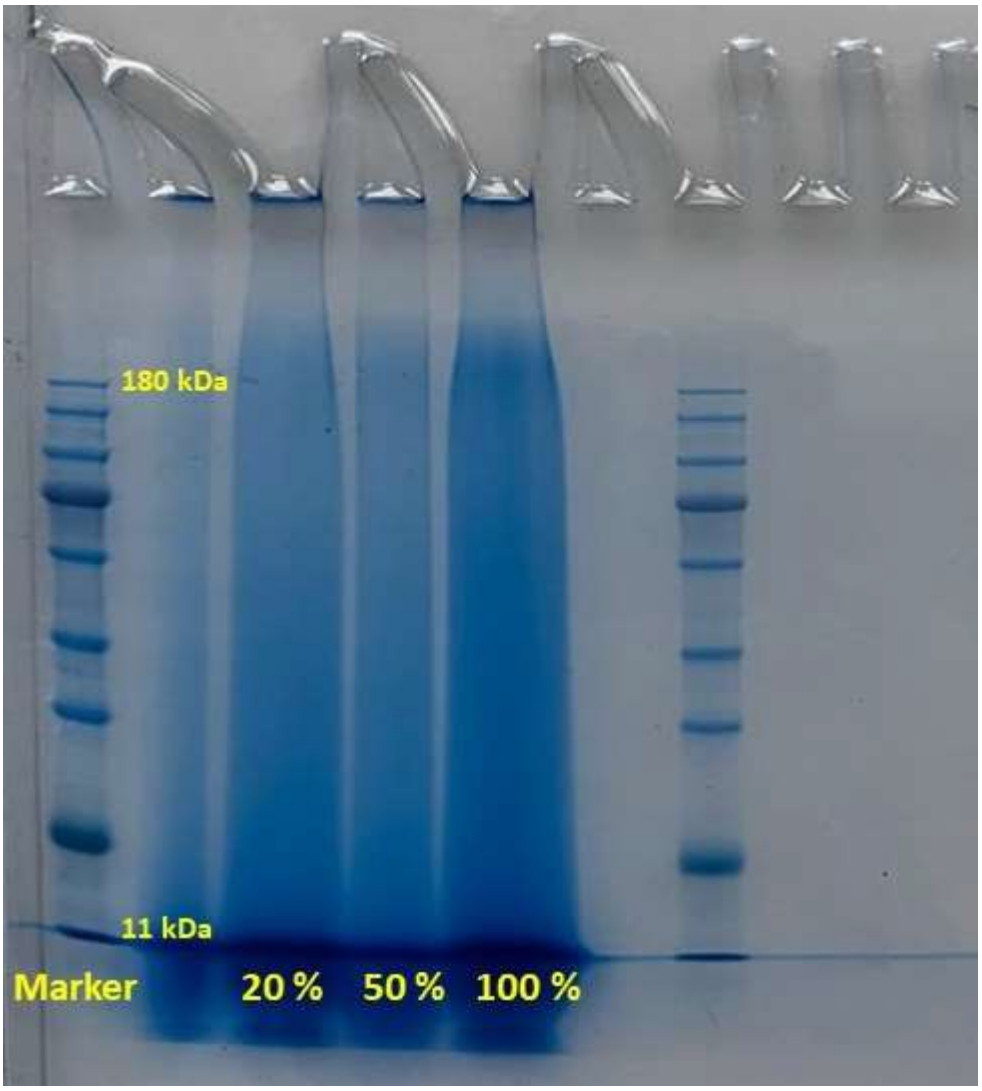

Figure S20: The SDS-PAGE gel electrophoresis profile for the ultrasonic alkaline extraction.

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