Supplementary information

Towards scalable production of bound extracellular polymeric substances (B-EPS): autoclave hydrothermal extraction coupled with solvent-free ultrafiltration

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Table S1 Chemical composition of ASP-12 growth medium

Component	Concentration
HEPES	0.71 g/L
NaCl	28.00 g/L
KCl	0.70 g/L
$MgSO_4 \cdot 7H_2O$	7.00 g/L
$MgCl_2 \cdot 6H_2O$	4.00 g/L
$CaCl_2 \cdot 2H_2O$	1.48 g/L
$NaNO_3$	0.10 g/L
$K_3PO_4 \cdot 3H_2O$	13.00 mg/L
Na_2-Glycerophosphate	6.90 mg/L
$Na_2SiO_3 \cdot 9H_2O$	0.15 g/L
Triplex I	0.10 g/L
Vitamin	**Concentration**
Vitamin B12	0.20 µg/L
Biotin	1.00 µg/L
Thiamine-HCl	0.10 mg/L
Niacinamide	0.10 µg/L
Trace Metal	**Concentration**
$Na_2EDTA \cdot 2H_2O$	4.36 g/L
$FeCl_3 \cdot 6H_2O$	3.15 g/L
K_2CrO_4	1.90 mg/L
$CoCl_2 \cdot 6H_2O$	0.012 g/L
$CuSO_4 \cdot 5H_2O$	2.50 mg/L
$MnCl_2 \cdot 4H_2O$	0.18 g/L
$Na_2MoO_4 \cdot 2H_2O$	0.020 g/L
$NiSO_4 \cdot 6H_2O$	2.63 mg/L
H_2SeO_3	1.29 mg/L
Na_3VO_4	1.84 mg/L
$ZnSO_4 \cdot 7H_2O$	0.023 g/L

Table S2 Face-centered central composite design (CCD) matrix with three levels and three variables for B-EPS extraction of C. *submarinus* and model predicted response

N	Variables			Response - B-EPS recovery (%)		
	Temperature (ºC)	Time (min)	Biomass-to-solvent ratio (g:mL)	Experimental values[#]	Predicted values	Relative deviation (%)[*]
1	134	25	1:20	83.88 ± 3.53	84.47	−0.70
2	134	5	1:20	83.60 ± 1.61	83.63	−0.04
3	134	15	1:15	82.53 ± 5.70	81.61	1.11
4	134	25	1:10	72.10 ± 1.42	71.94	0.22
5	134	5	1:10	75.48 ± 4.71	75.94	−0.61
6	108	25	1:20	71.86 ± 4.36	71.25	0.85
7	108	5	1:20	66.92 ± 4.95	63.84	4.60
8	108	15	1:15	38.93 ± 2.06	35.84	7.94
9	108	25	1:10	60.36 ± 3.39	60.19	0.28
10	108	5	1:10	18.38 ± 1.03	17.63	4.08
11	121	15	1:20	78.93 ± 2.67	86.17	−9.17
12	121	25	1:15	77.83 ± 4.40	78.18	−0.45
13	121	15	1:15	73.11 ± 3.28	75.72	−3.57
14	121	5	1:15	56.22 ± 2.88	56.48	−0.46
15	121	15	1:10	76.19 ± 4.31	76.81	−0.81

All values are reported as mean ± SD. * Relative deviation (%) = [(experimental value - predicted value)/experimental value] × 100. [#]Factorial and star points: n = 3; centre point: n = 6 (total = 48 runs).

Table S3 Model external validation runs (not included in the CCD).

N	Variables			Response - B-EPS recovery (%)		
	Temperature (ºC)	Time (min)	Biomass-to-solvent ratio (g:mL)	Experimental values[#]	Predicted values	Relative deviation (%)[*]
1	110	6	1:20	39.57 ± 2.79	36.70	-2.87
2	115	16	1:20	75.46 ± 6.67	77.30	-1.84
3	127	10	1:20	84.21 ± 6.98	87.05	-2.84
4	131	23	1:20	87.34 ± 2.44	90.10	2.76
5	130	16	1:20	91.59 ± 1.97	93.62	2.03

All values are reported as mean ± SD. * Relative deviation (%) = [(experimental value - predicted value)/experimental value] × 100. # n = 3.

Table S4 First-order energy and material-efficiency metrics for B-EPS extraction (batch basis: 10 g biomass, 150 mL water).

Method	Biomass (g)	Solvent (mL)	Purified B-EPS recovery (%)	Ash-free B-EPS per batch (g)	Rated power (W)	Treatment time	Estimated energy per batch (kWh)	Estimated energy per g ash-free B-EPS (kWh g^{-1})*
Reflux	10	150	40.48	3.505	270	15 min	0.0675	0.0193
Autoclave	10	150	99.50	8.835	2500	15 min	0.6250	0.0707
Ultrasonic bath	10	150	39.52	3.256	1200	15 min	0.3000	0.0921
Microwave	10	150	50.79	4.433	900	60 s	0.0150	0.0034

* Energy estimates were calculated from rated device power × effective treatment time and do not include ancillary operations (*e.g.*, cultivation, centrifugation, UF pumping, or freeze-drying). Values should be interpreted as comparative indicators rather than full process energy balances.

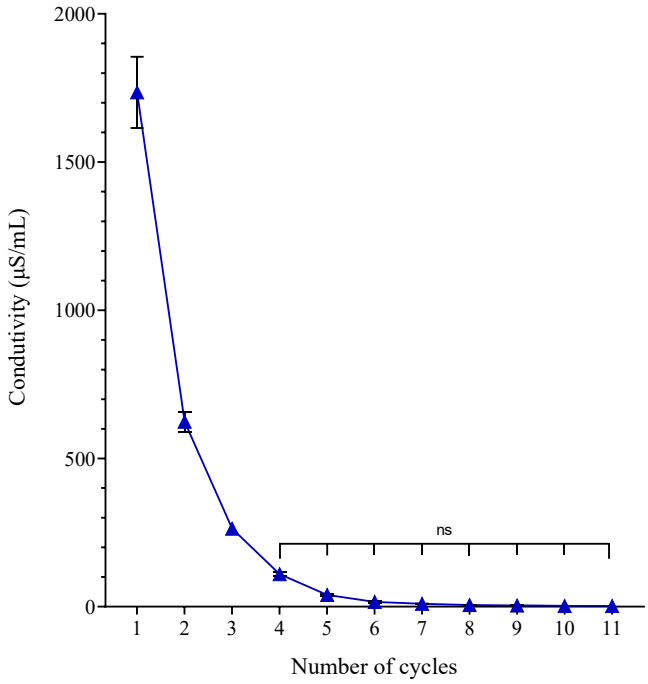

Fig. S1 Conductivity of the C. submarinus B-EPS solution measured over 11 ultrafiltration cycles using a Vivaflow 50R unit. Conductivity reached a plateau after successive cycles; differences between plateau cycles were not significant (ns, $p > 0.05$), indicating stable conductivity after desalting/purification.

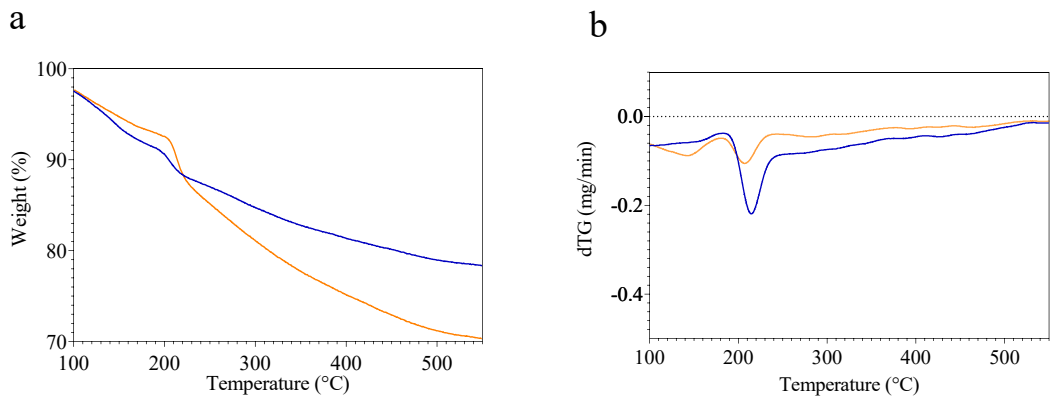

Fig. S2 TGA (a) and dTG (b) of *C. submarinus* biomass before (blue line) and after (orange line) three washing cycles.

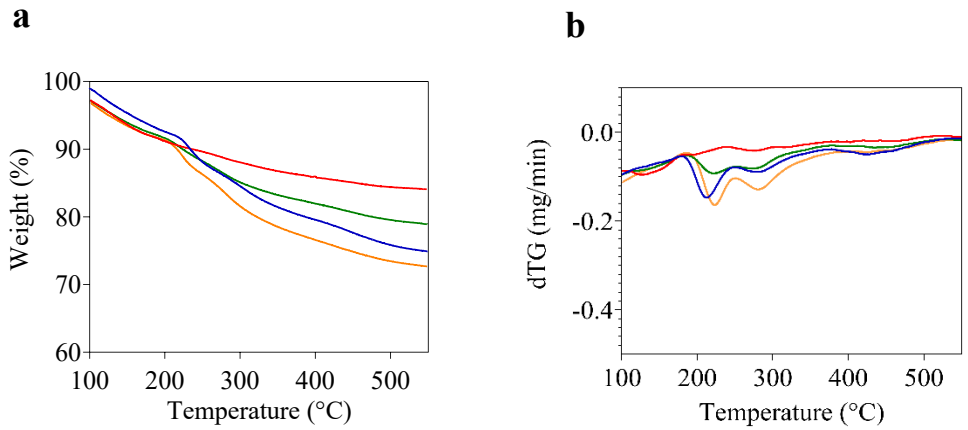

Fig. S3 TGA (a) and dTG (b) of *C. submarinus* B-EPS after various extraction methods before ultrafiltration: reflux (blue line), autoclave (orange line), ultrasonic (green line) and microwave (red line).