

SUPPLEMENTARY MATERIAL

**Bio-based solvents as a sustainable alternative to develop a multiproduct biorefinery
process from archaeobacteria *Halobacterium salinarum* R1**

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Tables

Table S1. Real and coded values and experimental values of the CCRD (2³) for total carotenoids content (yield - $\mu\text{g}_{\text{carotenoids}} \cdot \text{g}_{\text{biomass}}^{-1}$) extracted by gamma-valerolactone (GVL) aqueous solution.

Assay	[GVL] – mM	RSL – $\text{g}_{\text{biomass}} \cdot \text{mL}_{\text{solvent}}^{-1}$	pH	Yield ($\mu\text{g}_{\text{carotenoids}} \cdot \text{g}_{\text{biomass}}^{-1}$)
	X_1	X_2	X_3	Y
1	80 (-1)	0.05 (-1)	4 (-1)	223.9
2	220 (1)	0.05 (-1)	4 (-1)	56.0
3	80 (-1)	0.15 (1)	4 (-1)	130.6
4	220 (1)	0.15 (1)	4 (-1)	31.1
5	80 (-1)	0.05 (-1)	10 (1)	246.3
6	220 (1)	0.05 (-1)	10 (1)	28.0
7	80 (-1)	0.15 (1)	10 (1)	82.1
8	220 (1)	0.15 (1)	10 (1)	20.5
9	32.4 (-1.68)	0.1 (0)	7 (0)	28.0
10	267.6 (1.68)	0.1 (0)	7 (0)	114.8
11	150 (0)	0.016 (-1.68)	7 (0)	291.6
12	150 (0)	0.184 (1.68)	7 (0)	735.3
13	150 (0)	0.1 (0)	1.96 (-1.68)	14.9
14	150 (0)	0.1 (0)	12.04 (1.68)	18.7
15	150 (0)	0.1 (0)	7 (0)	550.5
16	150 (0)	0.1 (0)	7 (0)	541.1
17	150 (0)	0.1 (0)	7 (0)	550.5
18	150 (0)	0.1 (0)	7 (0)	549.5
19	150 (0)	0.1 (0)	7 (0)	547.7
20	150 (0)	0.1 (0)	7 (0)	550.5

Table S2. Price of the materials used in the biorefinery approach developed used on the calculation of production costs *per* batch of biomass (8.4 g).

Steps	Required reagents/ batch ^a	Cost/batch (US\$)
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Step I (Cultivation of <i>H. salinarum</i> R1)	NaCl	12.99
	MgSO ₄ ·7H ₂ O	4.71
	KCl	0.65
	Na citrate	6.22
	CaCl ₂	0.03
	Tryptone	6.05
	Yeast extract	0.25
	Glycerol	0.01
	Energy (autoclave + incubators + centrifugation)	32.59
	Total cost step I (reagents + energy)	63.49
Step II (Solid-liquid extraction)	Water	0.30
	GVL	0.43
	Energy (trayster + centrifugation)	0.06
	Total cost step II (reagents + energy)	0.78
Step III (Precipitation/purification)	EtOH	8.69
	PBS	11.60
	Energy (centrifugation + refrigeration)	0.96
	Total cost step III (reagents + energy)	21.25
Step IV (Polishing)	Ultrafiltration tubes ^b	16.5
	Energy (centrifugation + rotatory evaporation)	0.36
	Total cost step IV (reagents + energy)	16.86
TOTAL COST OF THE BIOREFINERY APPROACH		102.39

^aprices of each reagent were sourced from the Sigma-Aldrich website.; ^brecycled 10-times; GVL: gamma-valerolactone; PBS: phosphate buffer solution (pH 7); EtOH: ethanol.

Table S3. Comprehensive cost breakdown for the biorefinery approach concerning three possible scenarios

Scenarios	Steps performed in each scenario	Products obtained	Production cost (US\$)	Return (US\$)
1	I and II	Mixture of carotenoids, proteins and polysaccharides	64.28	546.01
2	I, II, and III	Purified carotenoids, proteins, and polysaccharides	85.53	802.79
3 cycle 1	I, III, III, and IV	Purified carotenoids, proteins, and polysaccharides	87.54	785.93
3 cycle 2	I, III, III, and IV	Purified carotenoids, proteins, and polysaccharides	78.85	1571.79
3 cycle 3	I, III, III, and IV	Purified carotenoids, proteins, and polysaccharides	78.18	2357.79
3 cycle 4	I, III, III, and IV	Purified carotenoids, proteins, and polysaccharides	78.13	3143.72
3 cycle 5	I, III, III, and IV	Purified carotenoids, proteins, and polysaccharides	78.13	3929.65
3 cycle 6	I, III, III, and IV	Purified carotenoids, proteins, and polysaccharides	78.13	4715.58
3 cycle 7	I, III, III, and IV	Purified carotenoids, proteins, and polysaccharides	78.13	5501.52

3 cycle 8	I, III, III, and IV	Purified carotenoids, proteins, and polysaccharides	78.13	6287.45
3 cycle 9	I, III, III, and IV	Purified carotenoids, proteins, and polysaccharides	78.13	7073.38
3 cycle 10	I, III, III, and IV	Purified carotenoids, proteins, and polysaccharides	78.13	7859.31

Figures

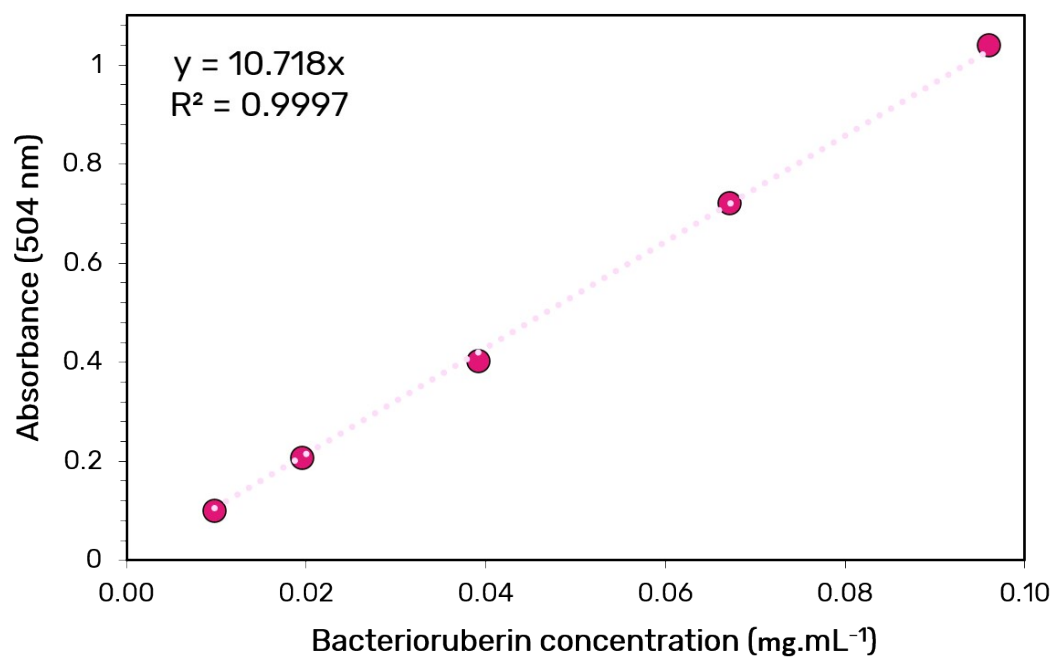


Figure S1. Calibration curve of bacterioruberin performed in UV-vis (204 nm), solvent aqueous solution of Tween 20 (250 mM).

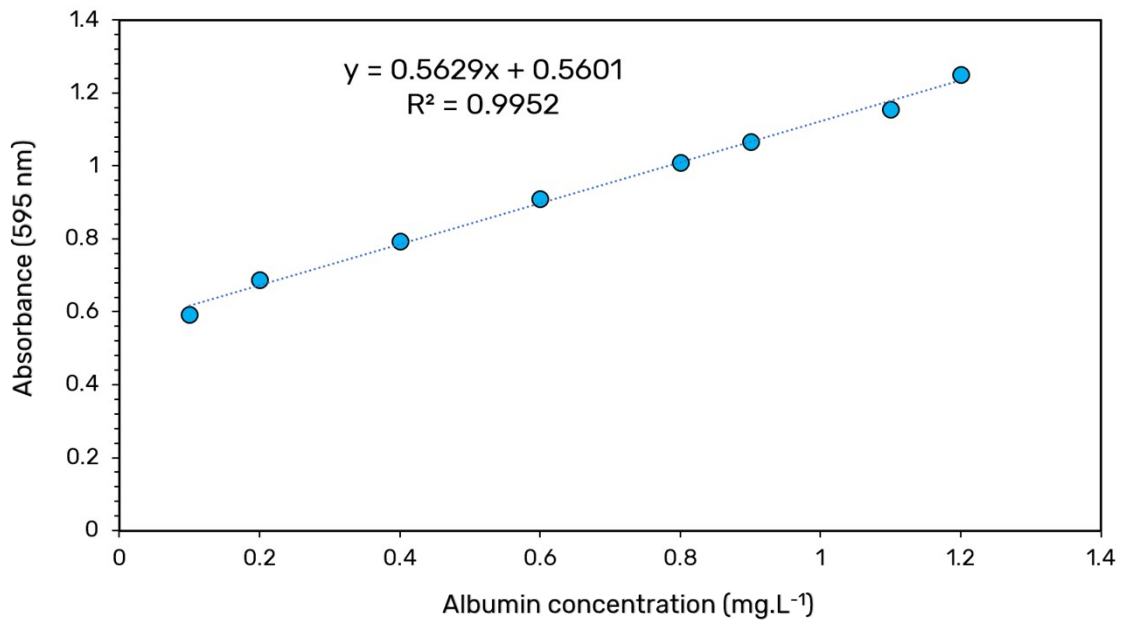


Figure S2. Calibration curve of bovine serum albumin performed in UV-vis (595 nm) for Bradford analysis.

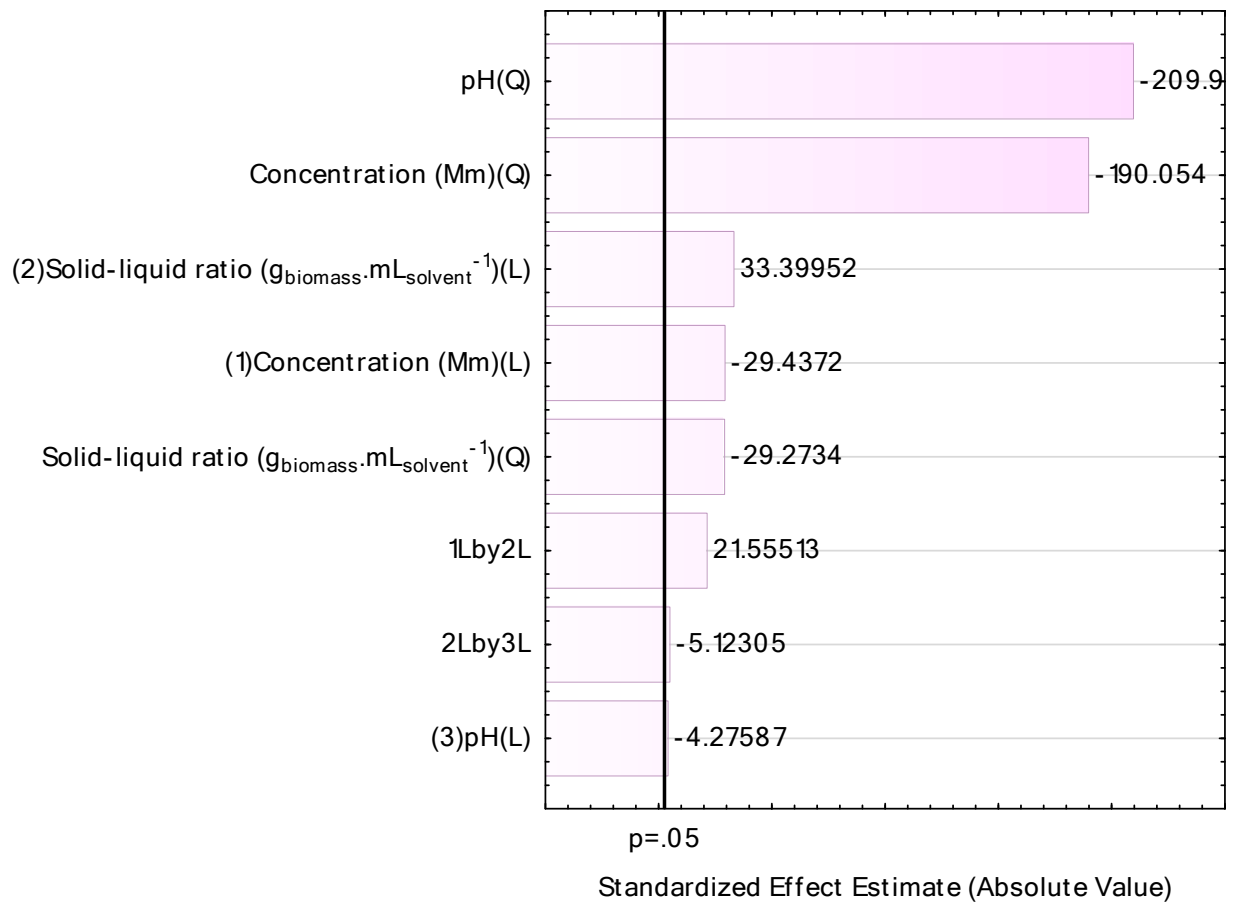


Fig. S3. Pareto Chart of the CCRD (2³ + axial points) regarding the yield of extraction of carotenoids from *H. salinarum* R1 using an aqueous solution of GVL.

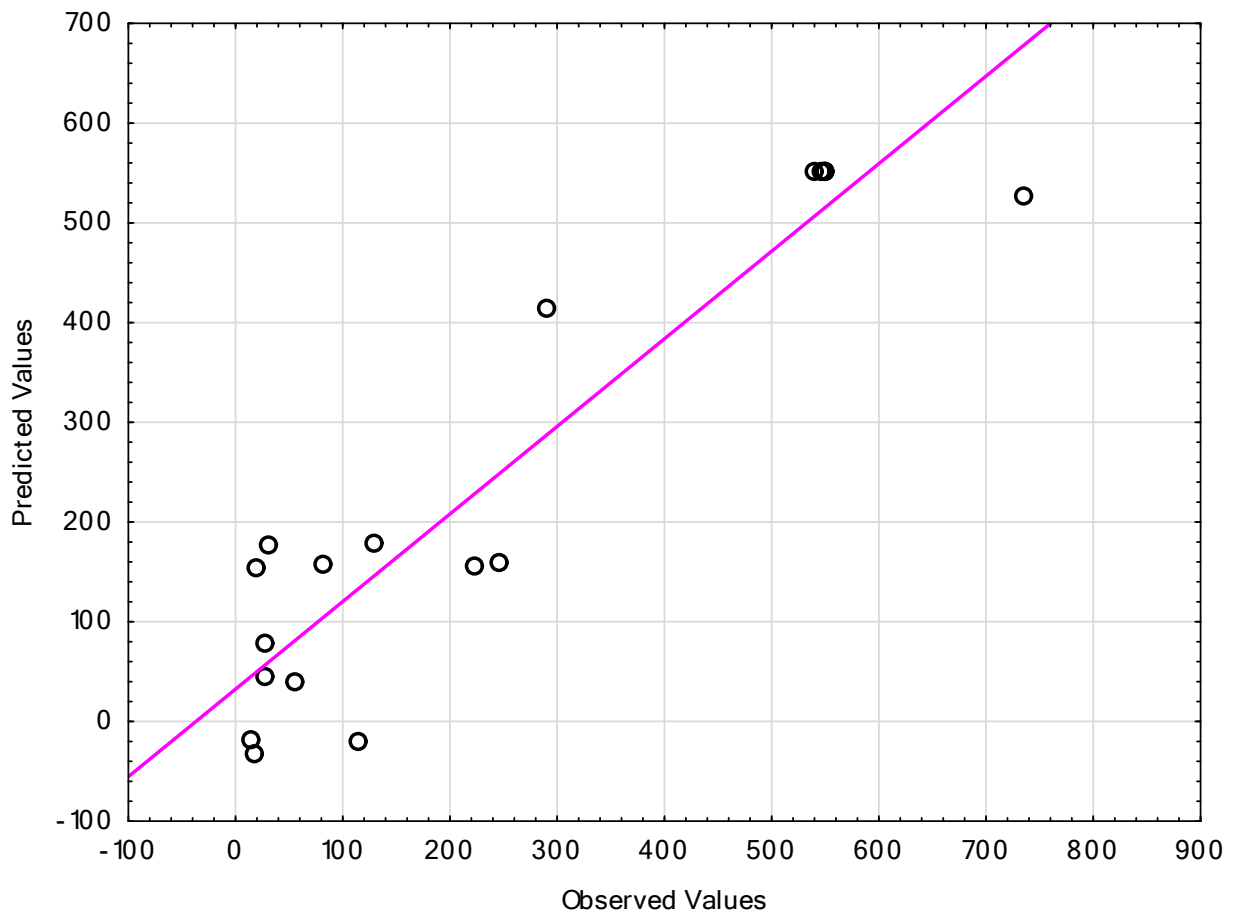


Fig. S4. Graphical representation of the predicted vs experimental values by the model when GVL was used as solvent in the extraction of carotenoids from *H. salinarum* R1.

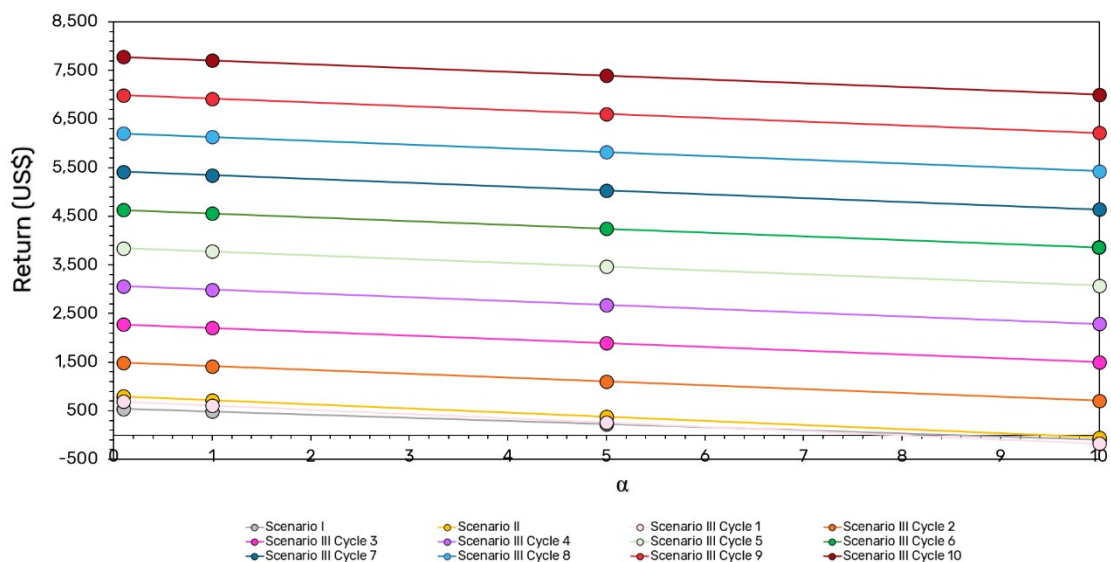


Fig. S5. Return analysis expressed as US\$/batch, of the extraction of carotenoids, proteins and polysaccharides considering different market prices and α scenarios ($\alpha = 0.1, 1, 5, \text{ or } 10$) along the different commercial scenarios.