Semi-continuous cultivation of EPS-producing marine cyanobacteria: a green biotechnology to remove dissolved heavy metals obtaining metal-organic materials

Supplementary material

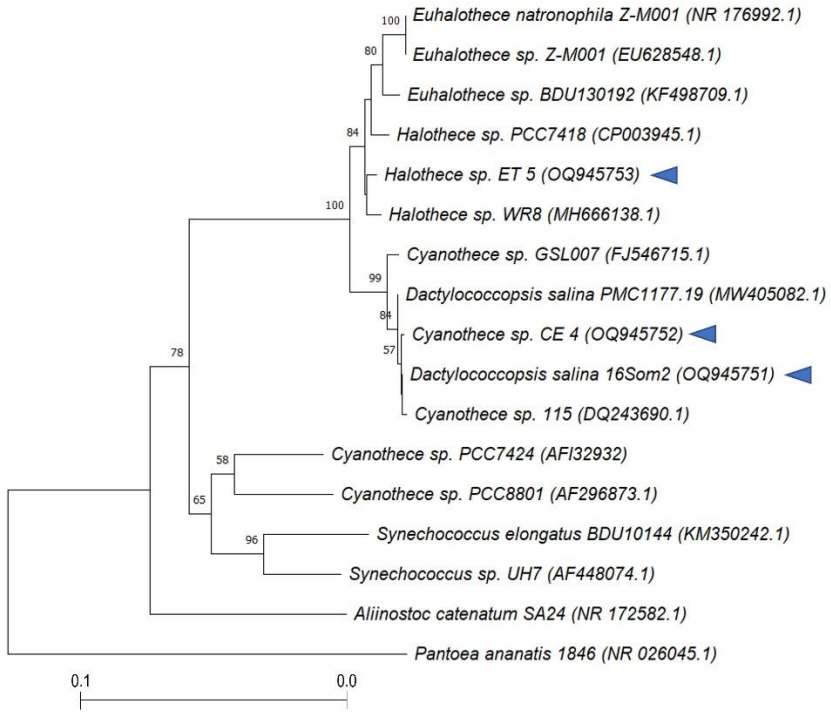

Fig. S1. Phylogenetic tree of partial 16S rRNA gene sequences of ET 5, CE 4, 16Som2, and related cyanobacteria. The evolutionary history was inferred using the neighbour-joining method, performed by MEGA11. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown below the branches. The percentages of bootstrap support of branches (>50%) are indicated at each node. *Pantoea ananatis* 1846 was used as an outgroup. The corresponding GenBank accession numbers for 16S rRNA genes of each strain are indicated in brackets.

The cultures were managed in semi-continuous mode. Every week half culture (0.4 L) for each cyanobacterium was collected and replaced with fresh growth medium before being pre-treated and adopted for bio-removal assays of a selected metal (Cu, Ni and Zn). Chlorophyll a concentration during cyanobacteria cultivation is shown in Fig. S2.

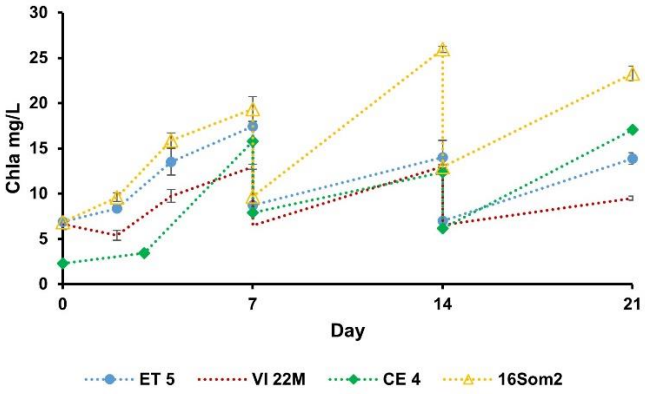

Fig. S2. Chlorophyll a (mg/L) concentration during semi-continuous cultivation of cyanobacteria.

In the table below (table S1), cellular and soluble carbohydrate concentration, quantified before the pre-treatment, as well as dry weight and total carbohydrate content, quantified after the pre-treatment, are shown per each harvest. Dry weight content before the pre-treatment was not included because the high salt content of the cultivation medium and the high exopolysaccharides content of the cultures made the measures inaccurate.

Table S1. Carbohydrate content and dry weight concentration of biosorbent at different harvests

Metal		**Harvested culture**		**Pre-treated biosorbent**	
		cCH_2O mg/L	sCH_2O mg/L	DW g/L	tCH_2O mg/L
Cu	**ET 5**	1162.5	426.0	1.65	1143.7
	VI 22M	1566.5	1110.0	0.96	1478.0
	CE 4	191.0	338.0	0.80	306.3
	16Som2	1384.5	628.8	1.43	1831.2
Ni	**ET 5**	940.0	560.0	1.13	1080.0
	VI 22M	820.0	479.8	1.01	935.9
	CE 4	408.8	437.3	0.98	599.5
	16Som2	1606.5	705.8	1.70	2068.8
Zn	**ET 5**	590.5	709.0	0.70	930.0
	VI 22M	1566.5	1110.0	0.96	1478.0
	CE 4	534.0	496.8	1.46	864.0
	16Som2	1469.8	720.0	1.90	1959.3
Multi-metal	**VI 22M**	748.8	855.0	0.60	1188.3
	CE 4	248.3	363.3	0.45	412.2
	16Som2	918.3	644.0	0.87	1374.7

cCH_2O cellular carbohydrate; sCH_2O soluble carbohydrate; DW dry weight; tCH_2O total carbohydrate.

In the figure below (Fig. S3), absolute removal of biomass assay, expressed as µg of metal removed, is shown.

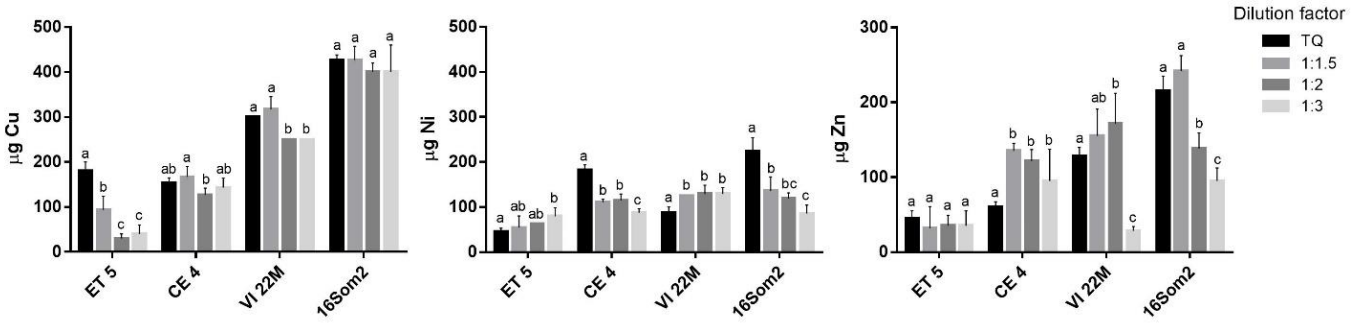

Fig. S3. Cu, Ni, and Zn absolute removal expressed as µg of metal removed at different biomass dilution (biomass assay).

Specific HM uptake (q) at different concentrations at the equilibrium were plotted according to Langmuir and Freundlich adsorption isotherms; graphs that have shown R^2 value ≥ 0.95 are represented in Fig. S4.

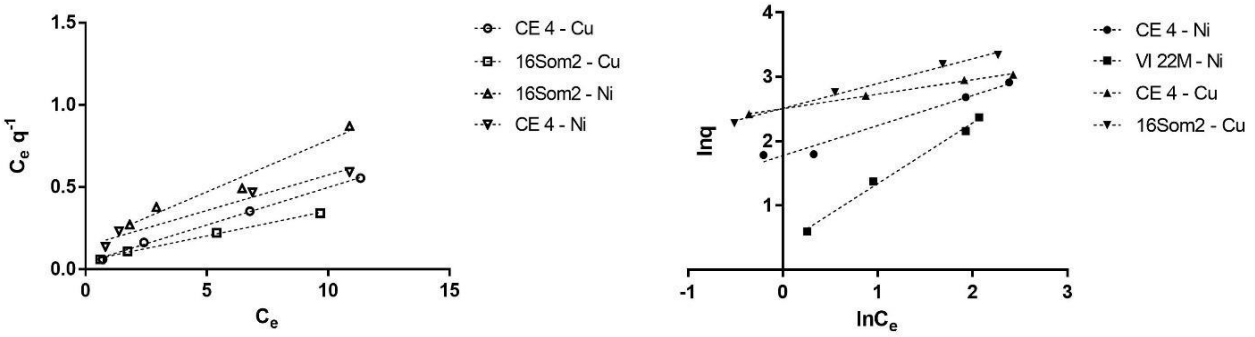

Fig. S4. Metal specific uptake (q) plotted following Langmuir (on the left) and Freundlich (on the right) adsorption isotherms. Only data with an R^2 value ≥ 0.95 are shown. For Langmuir isotherm (on the left): empty symbols corresponding to Cu uptake by CE 4 (circle) and 16Som2 (square), Ni uptake by 16Som2 (triangle pointing up) and CE 4 (triangle pointing down). For Freundlich isotherm (on the right): full symbols corresponding to Ni uptake by CE 4 (circle) and 16Som2 (square), Cu uptake by CE 4 (triangle pointing up) and 16Som2 (triangle pointing down).

The relationship between metal removal and dry weight or carbohydrate content of the biosorbents was observed by performing Pearson correlation coefficient (Table S2)

This table show Pearson product moment correlations between each pair of variables. These correlation coefficients range between -1 and +1 and measure the strength of the linear relationship between the variables. P-value which tests the statistical significance of the estimated correlations is also added for each coefficient. P-values below 0.05 indicate statistically significant non-zero correlations at the 95.0% confidence level.

Table S2. Pearson correlation coefficient between Cu, Ni, Zn absolute removal and dry weight or total carbohydrate content

		Cu removal	**Ni removal**	**Zn removal**	**Metal removal by ET 5**	**Metal removal by CE 4**	**Metal removal by VI 22M**	**Metal removal by 16Som2**
DW	Correlation	0.2349	0.5878	-0.253	0.8782	-0.8669	-0.6660	0.1942
	P-value	0.4623	0.0445	0.4276	0.0018	0.0025	0.0502	0.6166
tCH_2O	Correlation	0.8784	0.4602	0.9745	0.7186	-0.7095	0.6660	-0.8632
	P-value	0.0002	0.1322	0.0000	0.0292	0.0323	0.0502	0.0027

DW dry weight; tCH_2O total carbohydrate