

Valorization of sea waste: a one-step synthesis route to biomimetic calcite and aragonite nanoparticles from *Sargassum* spp. ash

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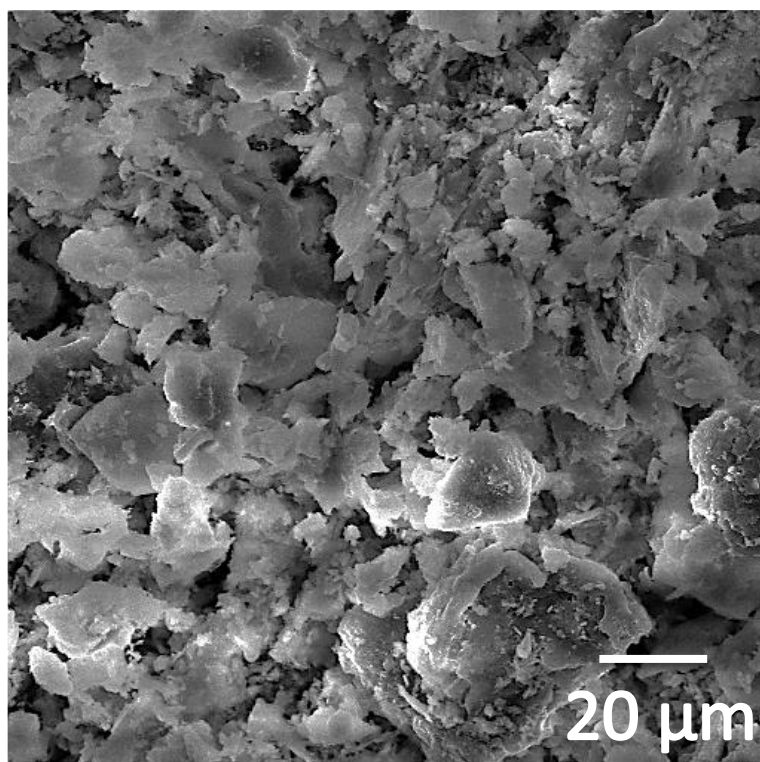


Figure S1 *Sargassum* ash using SEM, irregular, flake-like agglomerated particles with an average pore size of $4.57 \pm 2.19 \mu\text{m}$, using ImageJ.

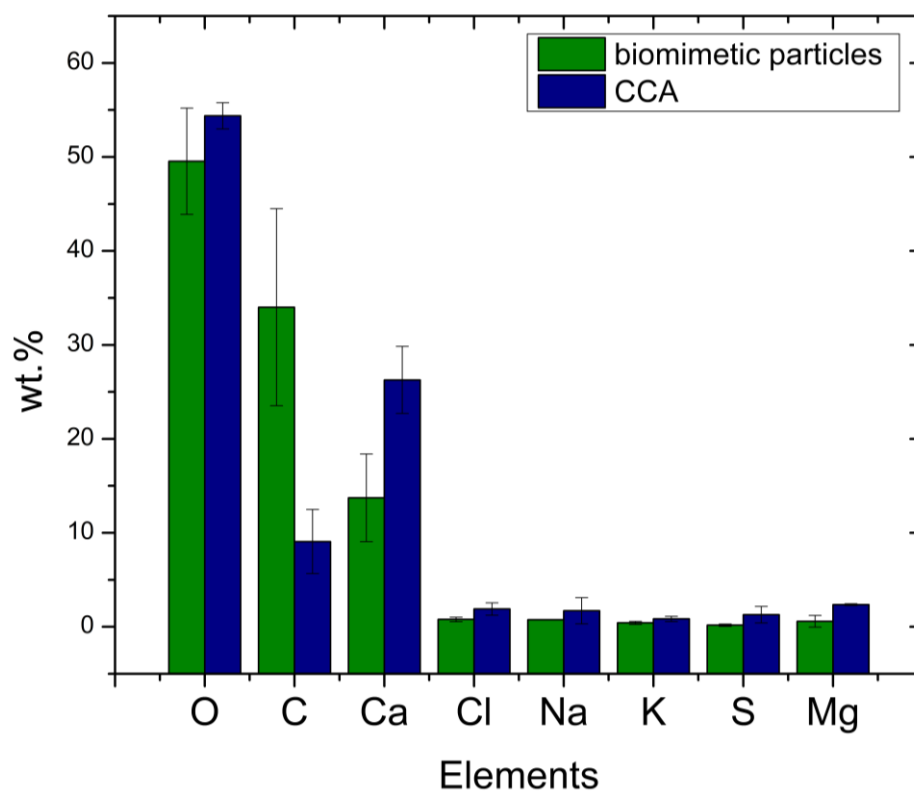


Figure S2 EDS results of biomimetic particles (in green) and the natural CCA (in blue), specifically *Neogoniolithon* rhodolith. Elements like oxygen, carbon, calcium, chlorine, sodium, potassium, sulfur and magnesium were found.

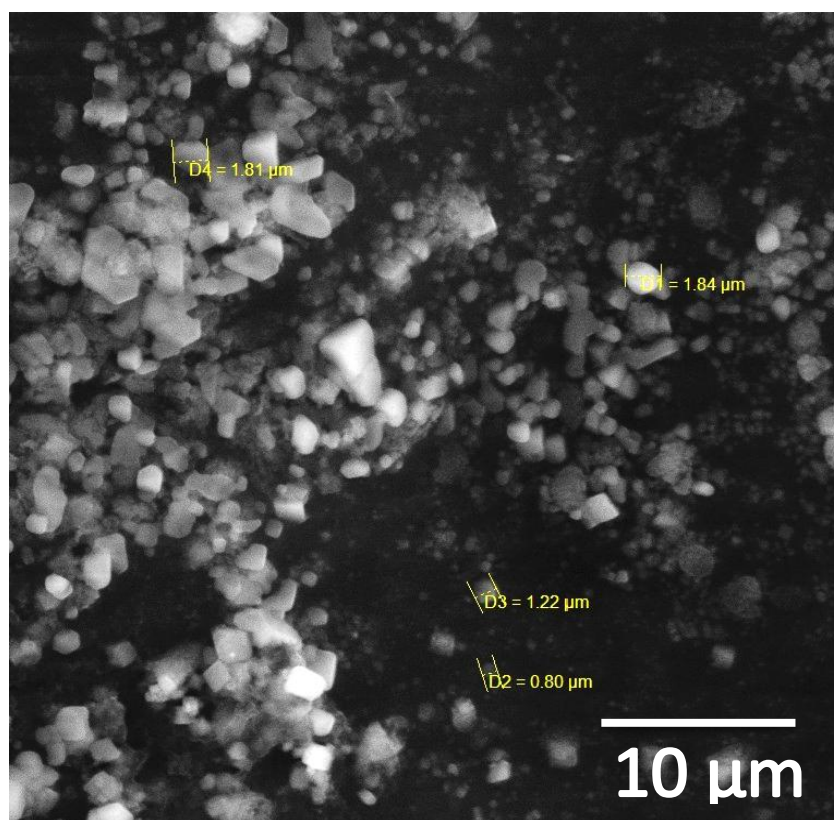


Figure S3 SEM image of 100% formation of Mg-calcite using 50% ethanol, and vigorous stirring time of 24 hours. No aragonite was observed.

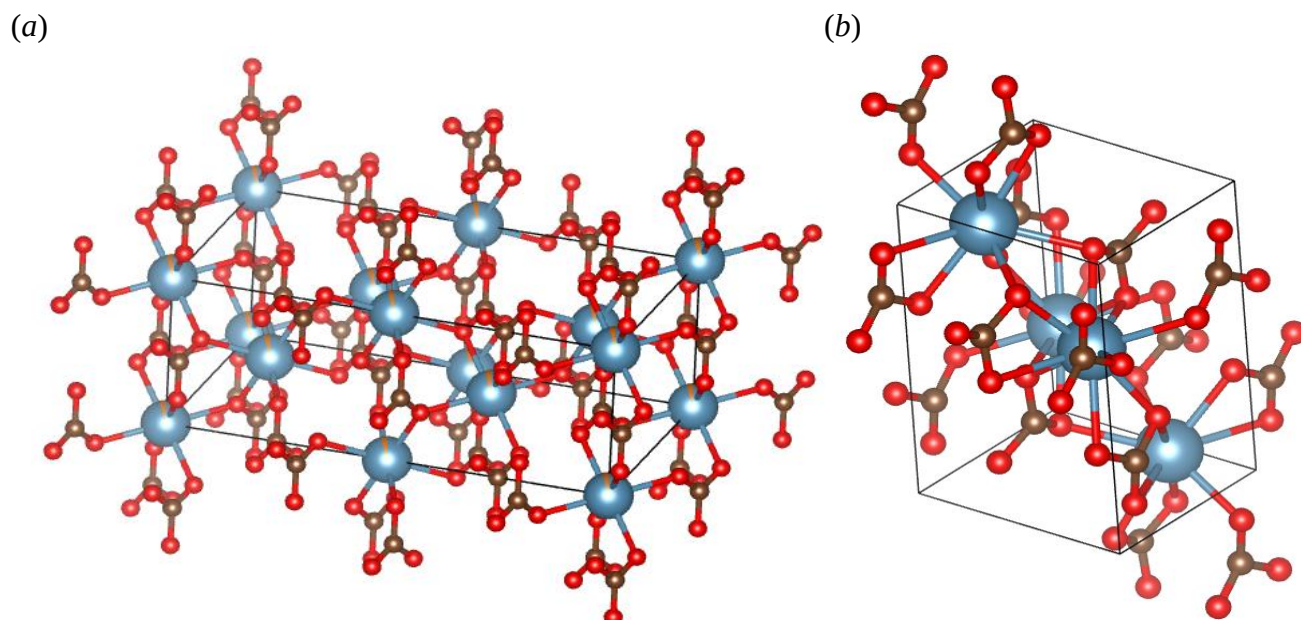


Figure S4 Schemes done in Vesta software for crystal structures of (a) calcite ($\text{Ca}_{0.94}\text{Mg}_{0.06}\text{CO}_3$), showing rhombohedral symmetry, where the framework of some Ca^{2+} ions (blue) allows limited Mg^{2+} substitution (b) aragonite (CaCO_3) displaying orthorhombic symmetry, where Ca^{2+} ions are blue, carbon is brown, and red spheres are oxygen.

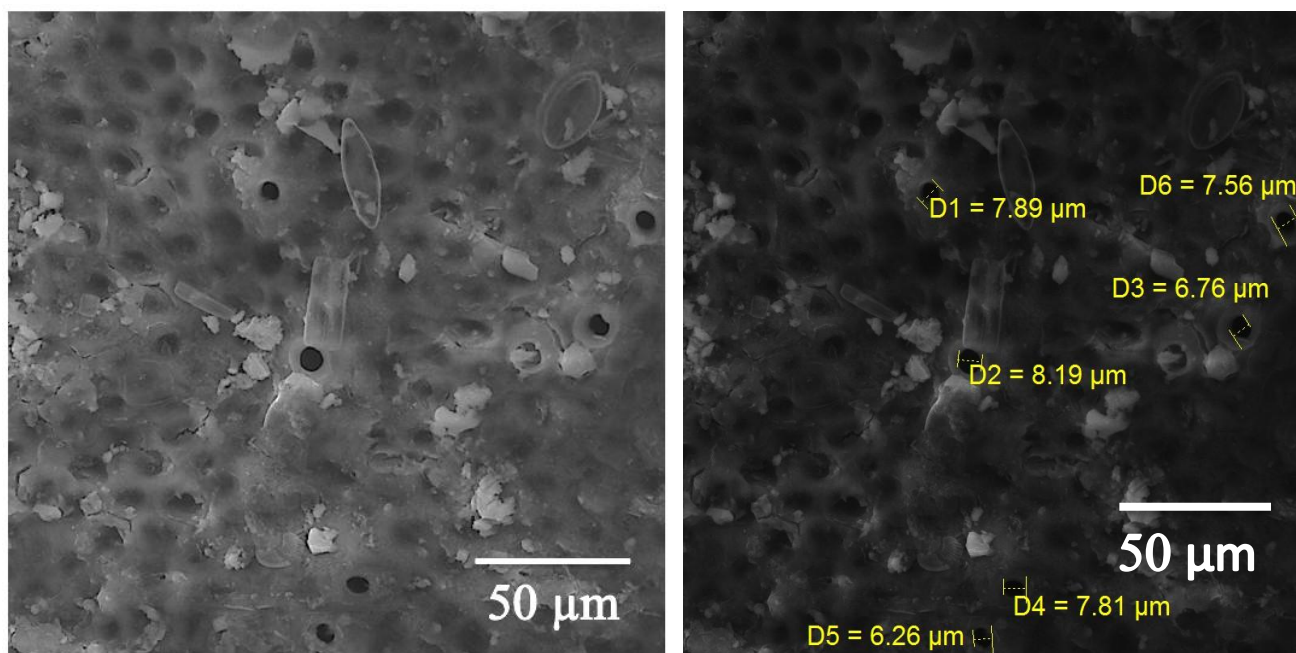


Figure S5 SEM morphology of natural *Neogoniolithon* rhodolith or CCA found in Puerto Morelos, Mexico with an average pore dimension of $7.41 \pm 1.52 \mu\text{m}$ using ImageJ.

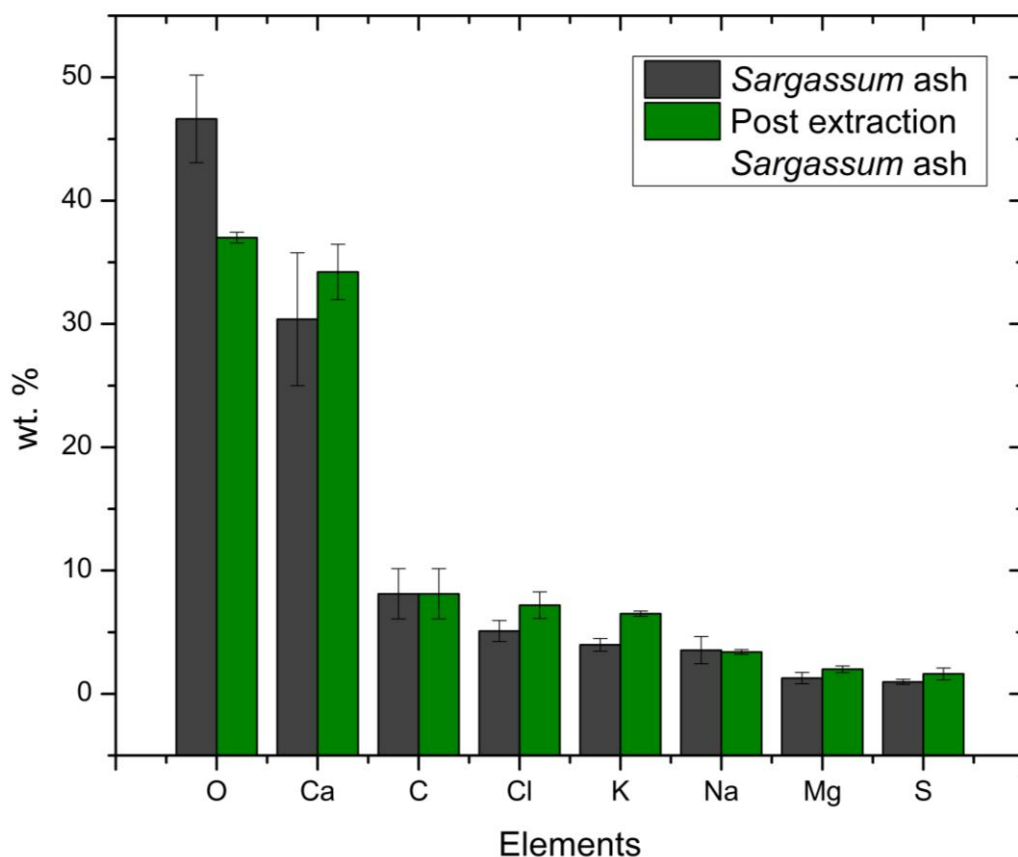


Figure S6 Comparative elemental analysis of *Sargassum* ash by EDS. Weight percentage of major elements (gray bars) versus post-extraction with chloroform:methanol 1:1 (green bars) *Sargassum* ash, proving the chemical composition of the ash will not change significantly if the *Sargassum* extract is obtained before calcination.

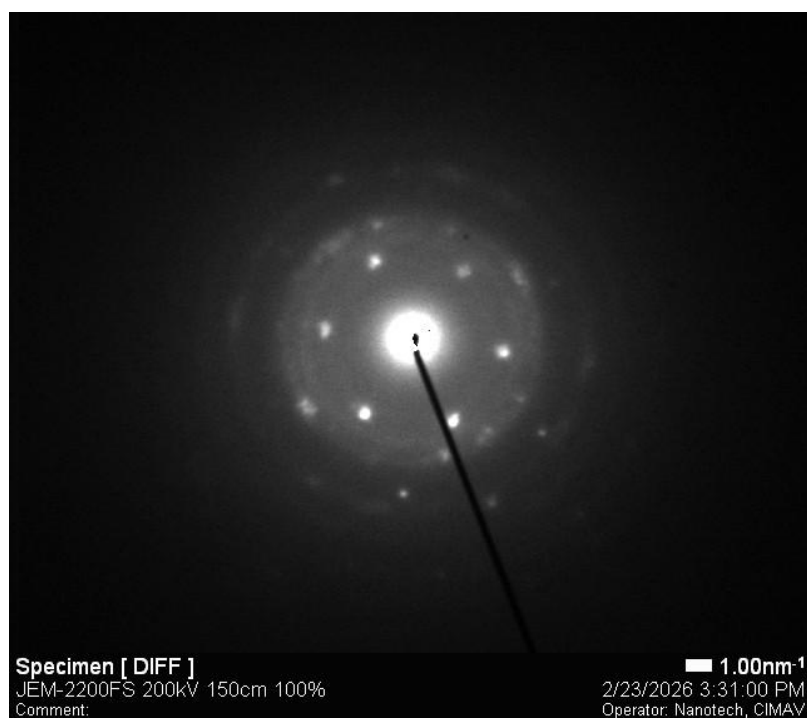


Figure S7 Selected Area Electron Diffraction (SAED) pattern of the synthesized nanoparticles confirming their crystalline nature, with diffraction rings consistent with calcium carbonate.

MATERIALS AND METHODS

S1. Sargassum Collection and Ash Preparation

Sargassum spp. (*S. natans* and *S. acinarium*) were collected on March 10, 2025, from pelagic waters near Puerto Morelos, Mexico (20.8131°N, 86.8662°W) via boat. Two biomass fractions were evaluated: (1) whole *Sargassum* and (2) post-extraction biomass residue using 3 extractions of chloroform:methanol (1:1 v/v), with maceration time of 72 h between cycles. No significant difference was found between whole *Sargassum* and post-extracted biomass (S6), validating both biomass types for this approach. Both were washed three times with tap water (10 min per rinse) to remove excess sea salts and surface debris prior to drying and calcination. All subsequent synthesis steps were carried out using distilled water. After drying at 80 °C to constant weight for 24 h and pulverizing to powder using a mechanical blender, the powder was washed with distilled water and filtered. Dried biomass was calcined in a muffle furnace (Thermolyne F6000) at 850°C for 5 hours under ambient atmosphere and no heating ramp (direct insertion). Cooled ash was manually ground using a porcelain mortar.

S2. Sargassum Ash Characterization and Synthesis of Biomimetic Particles

Micro- and nanoparticle preparation (one method): *Sargassum* ash, 0.125 g, was adjusted to Mg/Ca = 0.15 by addition of 0.010 g MgCl₂·6H₂O based on EDS-determined ash composition. The adjusted ash was suspended in 3.75 mL ethanol and followed immediately by dropwise addition of 12.5 mL of 25 mM Na₂CO₃ solution (0.0331 g Na₂CO₃ in 8.75 mL distilled water) to achieve a final volume of 25 mL with 30% (v/v) ethanol concentration. The mixture was stirred at 140 rpm for 1 hour at ambient temperature. The methodology was based on the phase diagram reported by Sand *et al.* for CaCO₃ crystallization in water-alcohol mixtures for calcite-aragonite, with one-month aging time to promote crystallographic stabilization and reduce metastable phases in calcium carbonate. Subsequently, three coats were applied by spray deposition using an aerosol system at 0.2 Pa and 5 cm distance from the sample, with 5 minutes drying time at 50°C between coats in carbon tape for X-Ray Diffraction (XRD) characterization, copper tape for Scanning Electron Microscope (SEM-EDS) and Transmission Electron Microscope (TEM). The mineral composition for the Mg-calcite/aragonite polymorph replicating the crustose coralline algae (CCA), *Neogoniolithon* rhodolith, was verified by SEM-EDS and XRD.

For the characterization, a diffractometer (D8 Advance, Bruker AXS Company) was used, with CuK α (λ = 1.5418 Å, 40 kV, 40 mA) radiation, and with the diffraction angles of 2 θ from 10° to 80° at 0.02 degrees/step for 1 second per step for DRX. The determination of the phase and proportions of the calcium carbonate polymorphs was completed using Rietveld refinement and MAUD software.

A Scanning Electron Microscope TESCAN VEGA 3 microscope was used to study the microparticles obtained via the synthesis and the *Neogoniolithon* rhodolith.

A JEM-2200FS Transmission Electron Microscope (TEM) was used to determine the particle size of the nanoparticles. The nanoparticle suspension was prepared by passing it through a 0.22 μ m PTFE membrane filter (Whatman) to eliminate large particles and/or other possible contaminants. One drop (approximately 10 μ L) of the filtered suspension was placed on the carbon-coated copper transmission electron microscope (TEM) grid (300 mesh) and allowed to dry (at ambient temperature) prior to analysis. The particle size distribution was calculated by measuring a statistically meaningful number of particles from the resultant micrographs using ImageJ.