Supplementary information

1. Presence of cellulose and chitin in *Chlorella vulgaris*

Evidence for the presence of cellulose and chitin in *C. vulgaris* cells was obtained by using a double staining approach with confocal microscopy imaging where wheat germ agglutinin (WGA) was used to specifically stain chitin (red), and calcofluor white (CW) to label both cellulose and chitin (blue) (**Figure S1**). In the cell wall surrounding the cell the two signals overlap indicating the presence of both biopolymers. Intracellular compartments stained only with CW and not WGA indicate the presence of cellulose without chitin.

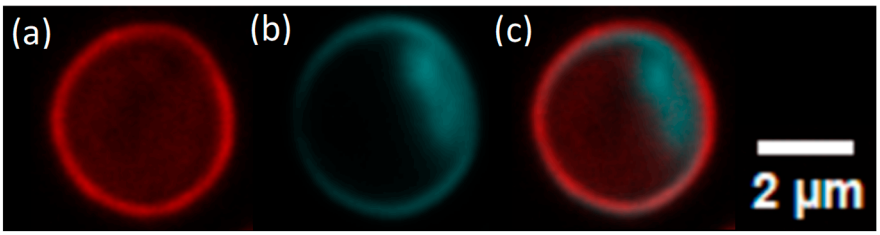


Figure S1: *Confocal microscopy images of C. vulgaris cells stained with: (a) WGA (red), (b) CW (blue), and (c) WGA (red) + CW (blue) show distinct compartments of chitin (red) and cellulose (blue but no red) in the cell. WGA: Wheat germ agglutinin; CW: Calcofluor white*

1.1 Staining with Calcofluor white and fluorescent wheat germ agglutinin (WGA):

Cells were fixed in 4% paraformaldehyde for 15 min at room temperature, washed once with phosphate-buffered saline (PBS), resuspended in 1% Triton X-100 for 20 min at room temperature for cell permeabilization, then washed twice with PBS.

Staining of cellulose and chitin biopolymers in of *C. vulgaris* was performed, using a commercial kit (Remel Calcofluor White Stain Kit, Thermo Fisher Scientific) and by following the instructions of the commercial supplier. Chitin was labelled using a CF®568-conjugated wheat germ agglutinin (WGA) probe (Biotium, Fremont, CA, USA). WGA labelling was performed by resuspending permeabilized and fixed algae cells in PBS containing 0.1 g/L of WGA-CF®568, then incubated at 30

°C in the dark for 45 min at 650 rpm, and washed three times with PBS before confocal microscopic imaging.

1.2 Laser-scanning confocal microscopy

Fluorescently labelled *C. vulgaris* cells were recorded using laser-scanning confocal microscopy (SP8, Leica Microsystems) with a 63x (oil) objective for single cell imaging. CW fluorescence signal used a 405 nm laser (2% intensity) for excitation, PMT detector at 415-500 nm detection range and 370% gain. WGA-CF®568 signal was recorded with a HyD detector (560-581 nm detection range, 50% gain) after excitation with a 552 nm laser excitation (9% intensity. For all cases a small pinhole of 0.5 was used for sharp z-sectioning, and pixel resolution in x-y was pushed to the Nyquist limit to obtain high optical resolution images.

2. Biopolymer fibrillar network obtained after purification

Scanning electron microscopy images obtained at higher magnification are showing a cellulose-chitin biopolymer fibrillar network mixed with a certain quantity of impurities after purification of *Chlorella vulgaris* biomass (**Figure S2**).

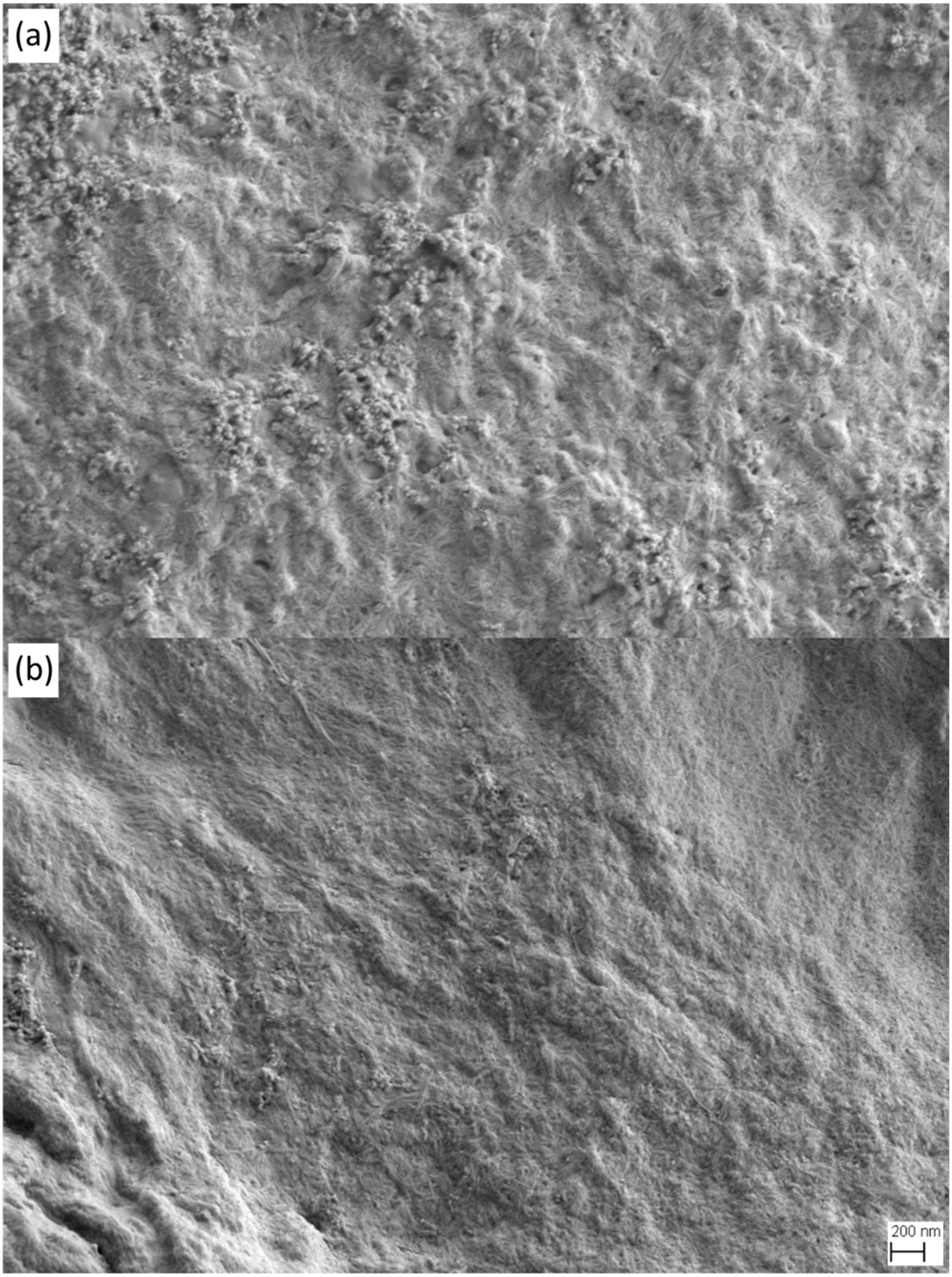


Figure S2: *Scanning electron microscopy images of the residues after the third extraction step (Magnification 20'000 x). A biopolymer fibrillar network is visible for all treatments assessed, example of images is provided for (a) the treatment*

leading to the lowest purity of the biopolymer (hot water), and (b) to the highest purity (nitric/acetic acid, HNO_3/CH_3COOH).