

Electronic Supplementary Information

Fabrication of living soft matter by symbiotic growth of unicellular microorganisms

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Immobilisation of *C. reinhardtii* cells in bacterial cellulose

The microalgae cells were successfully integrated into the cellulose gel produced by the acetobacter cells. Both the microorganisms were grown in the same culture vessel containing the mixed GY+TAP media which supports the growth and proliferation of both *C.reinhardtii* and *A.aceti*. A symbiotic food chain was established with the microalgae supplying the oxygen to the acetobacter and in return using the acetic acid produced by the acetobacter. The acetobacter cells prefer to populate around the areas in the culture vessel with the microalgae cells and vice versa.

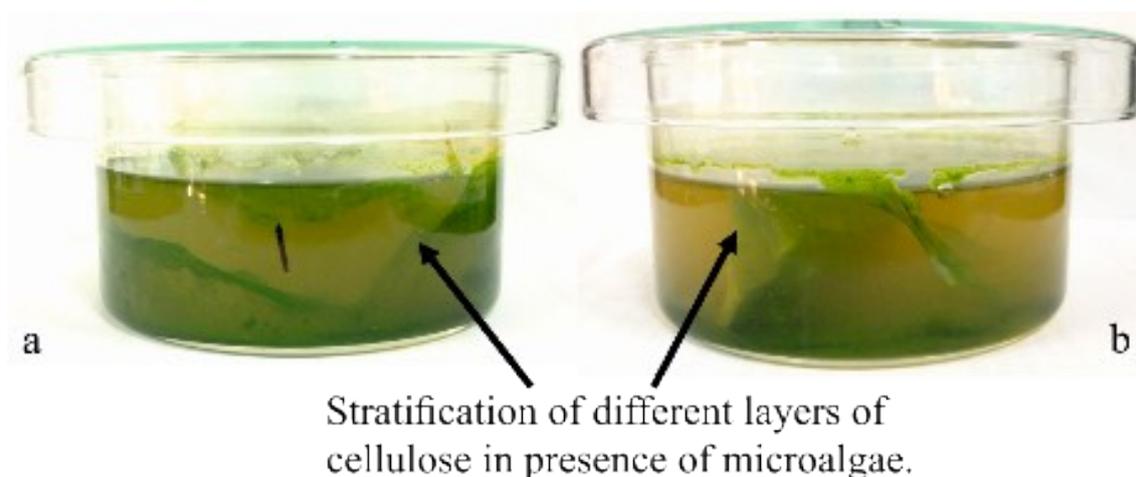


Figure S1 Photographs of the culture vessel showing the immobilisation of *C. reinhardtii* cells in cellulose produced by *A. acetii*, where both the microorganisms grow and proliferate in a symbiotic relationship. The culture vessel contains 70:30 GY: TAP media (v/v) type containing similar amount of *C. reinhardtii* and different amount of *A. acetii* cells culture: (a) 2 mL and (b) 4 mL.

A. aceti tends to produce cellulose on the surface of the media due to the availability of ample oxygen, but in case of symbiotic cultures with the photosynthetic microalgae in the system, the acetobacter tend to populate the whole culture vessel and not only the air-water surface of the media due the supply of oxygen from the *C. reinhardtii* cells (Figure S1).

Immobilisation of *C. reinhardtii* cells using beads of pre-chopped *A. aceti* cellulose

The other method used to make the living biomaterial by immobilisation of microalgae was the use of chopped cellulose as a source for the *A. aceti* cells. The cellulose in specific media was chopped using the Tefal Minipro Blender. The chopped cellulose was added to the culture vessel along with the microalgae cells according to the protocol shown in Table 1.

Table S1 The protocol of the culture and inoculums in the culture vessel for making the living biomaterial using pro-chopped cellulose beads.

Sample	Cellulose (g)	<i>C. reinhardtii</i> (g)	Media Composition
A	21	0.4	70:30 GY: TAP (v/v)
B	25	0.4	TAP media
C	21	0.4	TAP media+20 g/L Glucose



Figure S2 Photographs of the culture vessel showing the immobilisation of *C. reinhardtii* cells in cellulose produced by *A. aceti* (chopped cellulose), where both the microorganisms grow and proliferate in a symbiotic relationship in media containing (a) 70:30 GY: TAP (v/v), (b) TAP media and (c) TAP media+20 g/L Glucose.

The culture vessel containing 70:30 GY: TAP (v/v) and TAP media showing the produced living material is presented in Figures S2a and S2b. The sample with added glucose in the TAP media to promote cellulose production showed no produced biomaterial as shown in Figure S2c. This shows that only the presence of glucose which is a substrate for cellulose production does not promote cellulose generation by *A. aceti*.

Beads of living biomaterial

We produced living biomaterials of different shapes and sizes (Please refer to the main paper). The small beads of algae immobilised cellulose are shown in Figure S3.

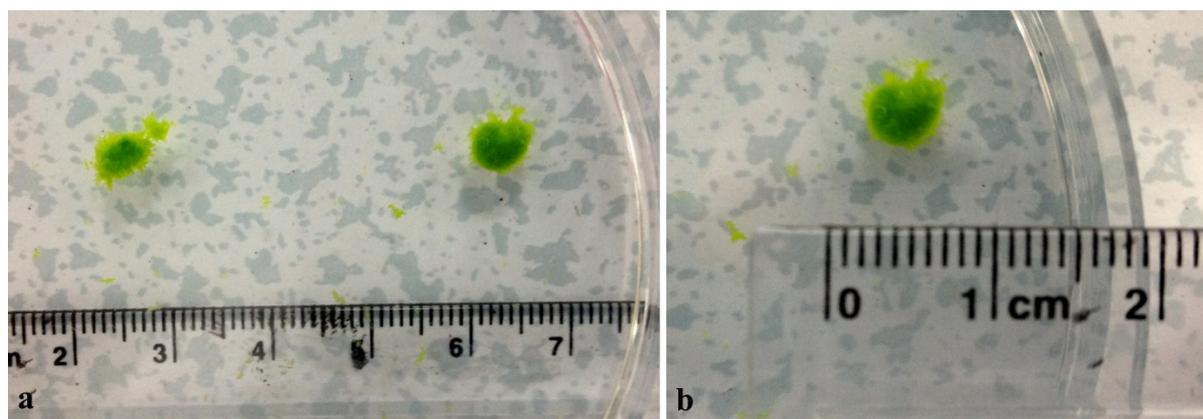


Figure S3 The photographs of *C.reinhardtii* immobilised cellulose beads produced in the agitated culture vessel, where both the microorganisms grow and proliferate in a symbiotic relationship. The culture vessel contains 70:30 GY: TAP media (v/v) with 12 ml and 0.5g of *A. aceti* and *C. reinhardtii* cells respectively.

Scanning electron micrographs of differently shaped bacterial cellulose gels with integrated *C. reinhardtii* cells

This section shows all the residual SEM images of the cells and the living materials produced by the symbiotic growth of *C. reinhardtii* (Figure S4) and *A. aceti* (Figure S5) in different kinds of culture vessels (Figure S6,S7,S8 and S9).

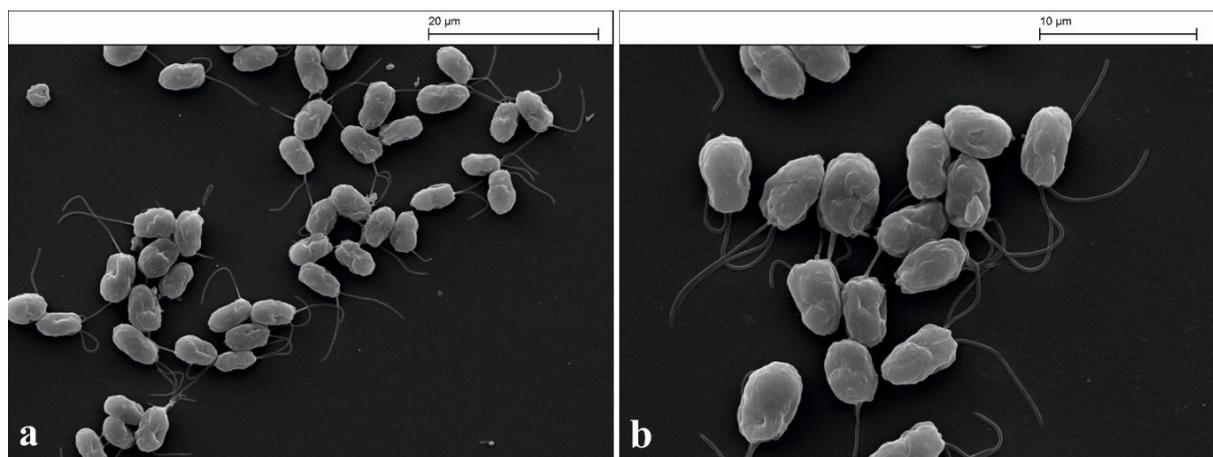


Figure S4 Scanning electron micrograph of (a) the microalgae *C. reinhardtii* and (b) higher magnification image the microalgae *C. reinhardtii* showing the two anterior flagella and the cell body.

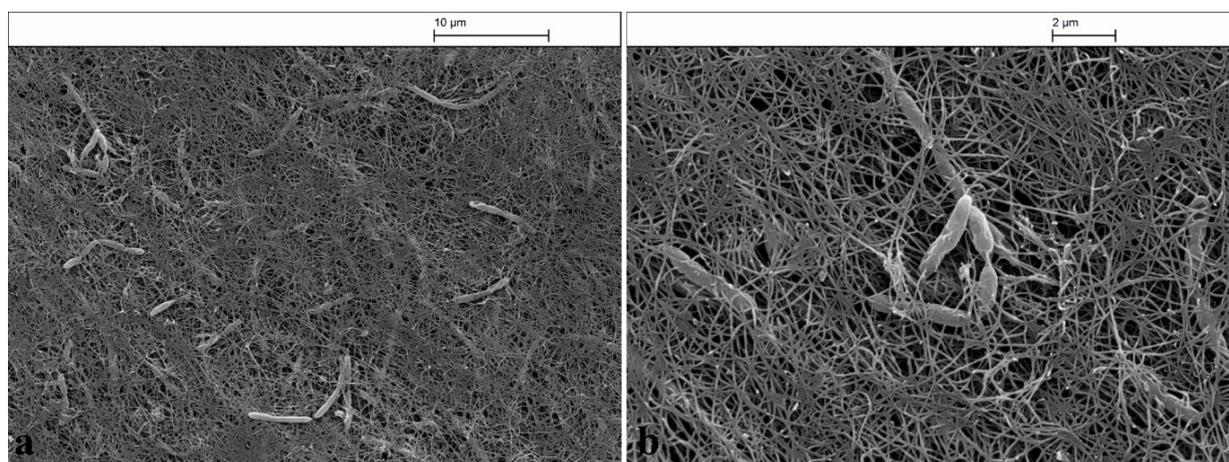


Figure S5 Scanning electron micrograph of (a) the cellulose producing bacteria *A. aceti* and (b) higher magnification image of bacterial cellulose produced by *A. aceti* showing the lateral division into daughter cells in the form of a long ribbon.

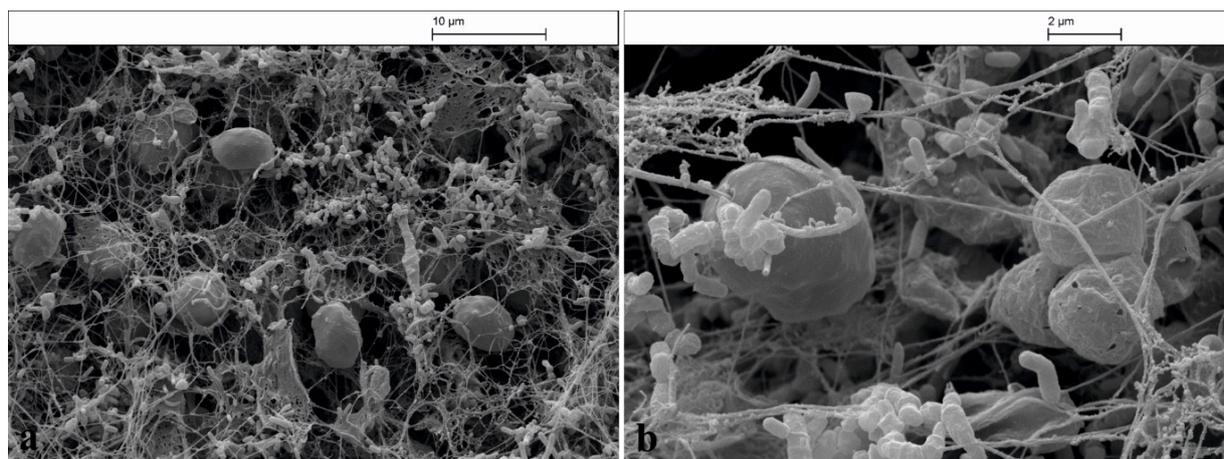


Figure S6 Scanning electron micrograph of (a) small cellulose bead (Figure S3) produced by *A. aceti* with *in-situ* immobilised *C. reinhardtii* cells, (b) higher magnification image of the bead showing the microalgae trapped in a mesh of cellulose fibre along with *A. aceti*.

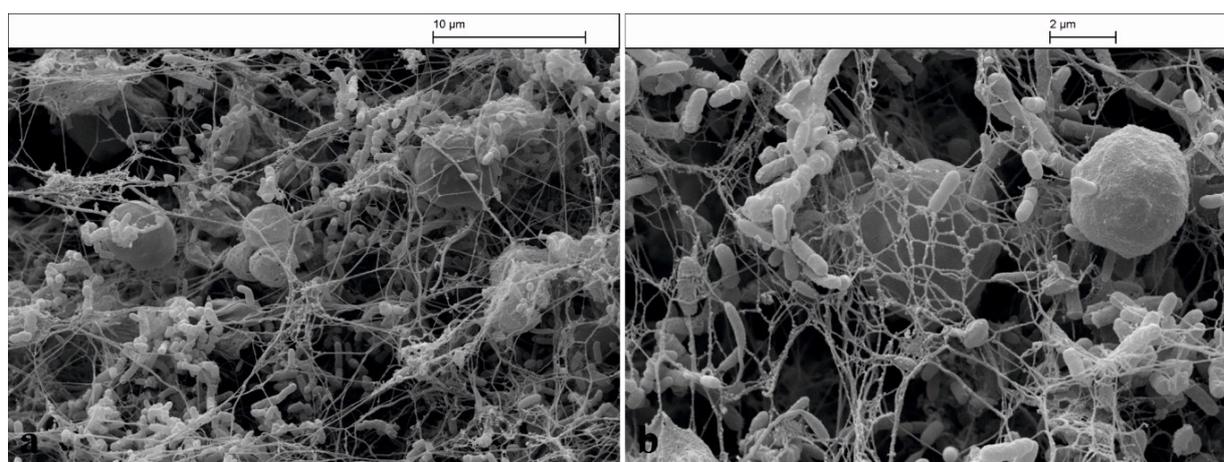


Figure S7 Scanning electron micrograph of (a) large cellulose bead (Figure 9b) produced by *A. aceti* with immobilised *C. reinhardtii* cells, (b) higher magnification image of the bead showing the microalgae trapped in the mesh of the bacterial cellulose nanofibres along with *A. aceti*.

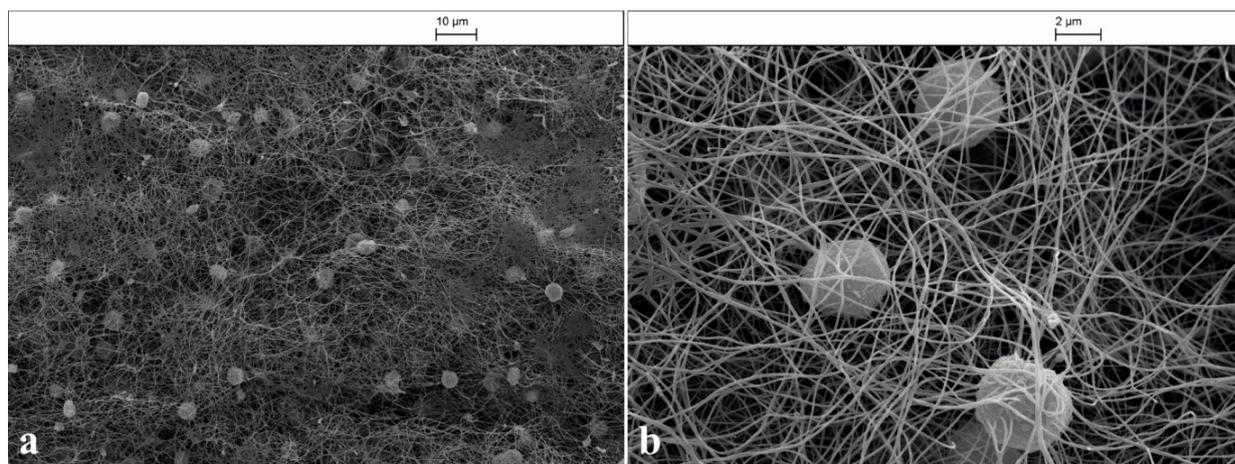


Figure S8 Scanning electron micrograph of the (a) living biomaterial made of cellulose produced from *A. aceti* and immobilised microalgae *C. reinhardtii* cultured in a Petri dish and (b) higher magnification image of (a) showing specific areas with the entrapped microalgae in the cellulose gel.

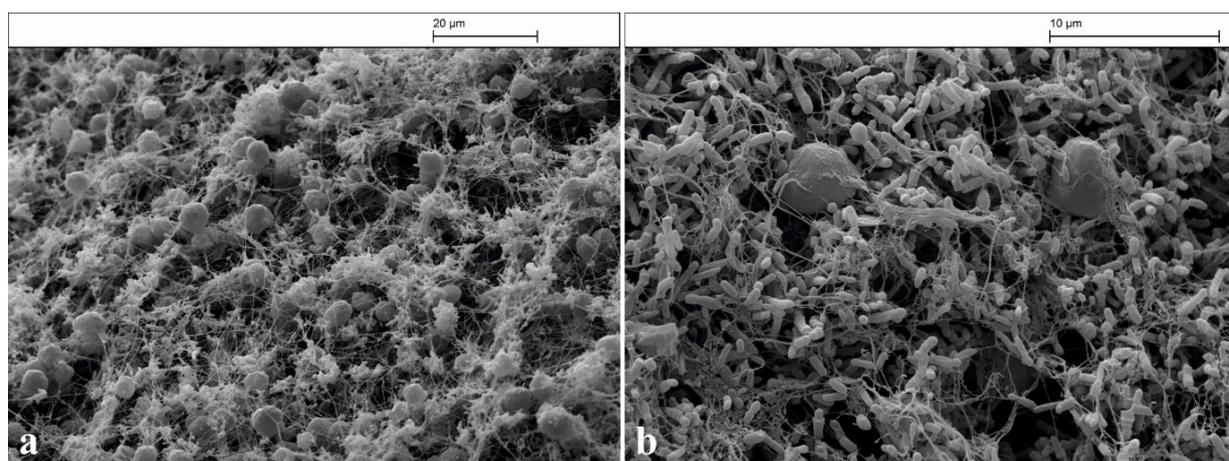


Figure S9 Scanning electron micrograph of the (a) living biomaterial made of cellulose produced from *A. aceti* and immobilised microalgae *C. reinhardtii* cultured in a glass beaker and (b) higher magnification image of (a) showing specific areas with the entrapped microalgae in the cellulose gel.

Mechanical property testing

The mechanical tensile stress properties of the produced cellulose and biomaterial were measured using a tensile machine.

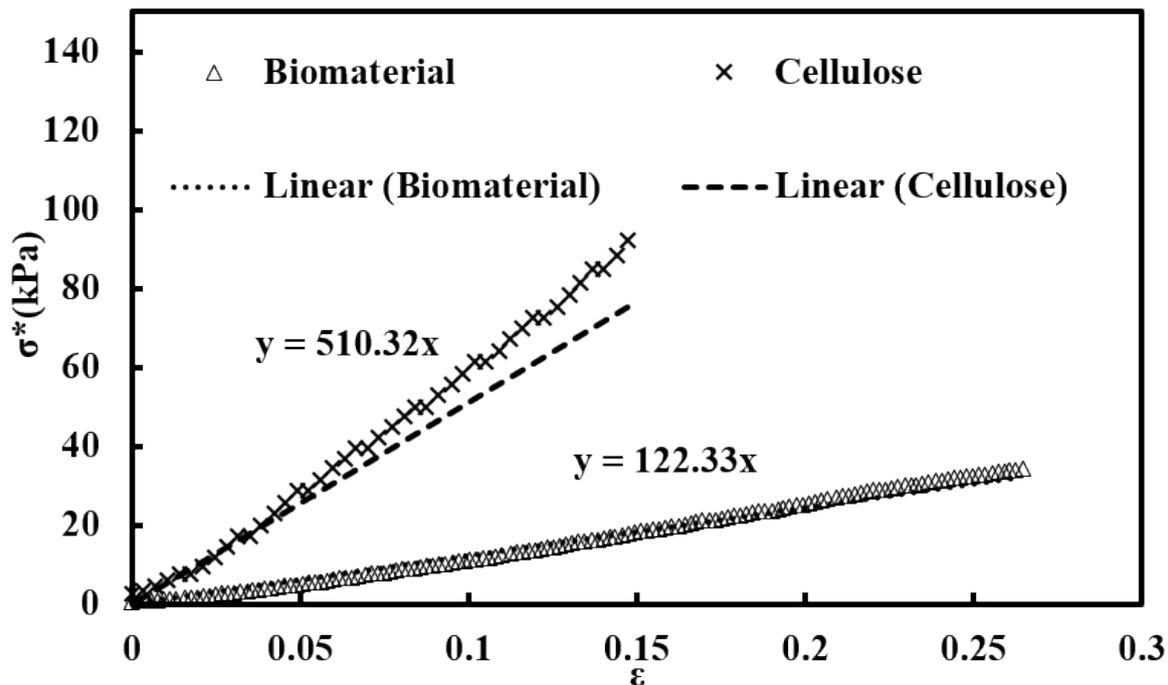


Figure S10 Typical stress-strain graph of the produced cellulose and the living biomaterial at room temperature in which σ^* is the tensile stress and ϵ is the tensile strain. The materials were pulled to rupture in both test.

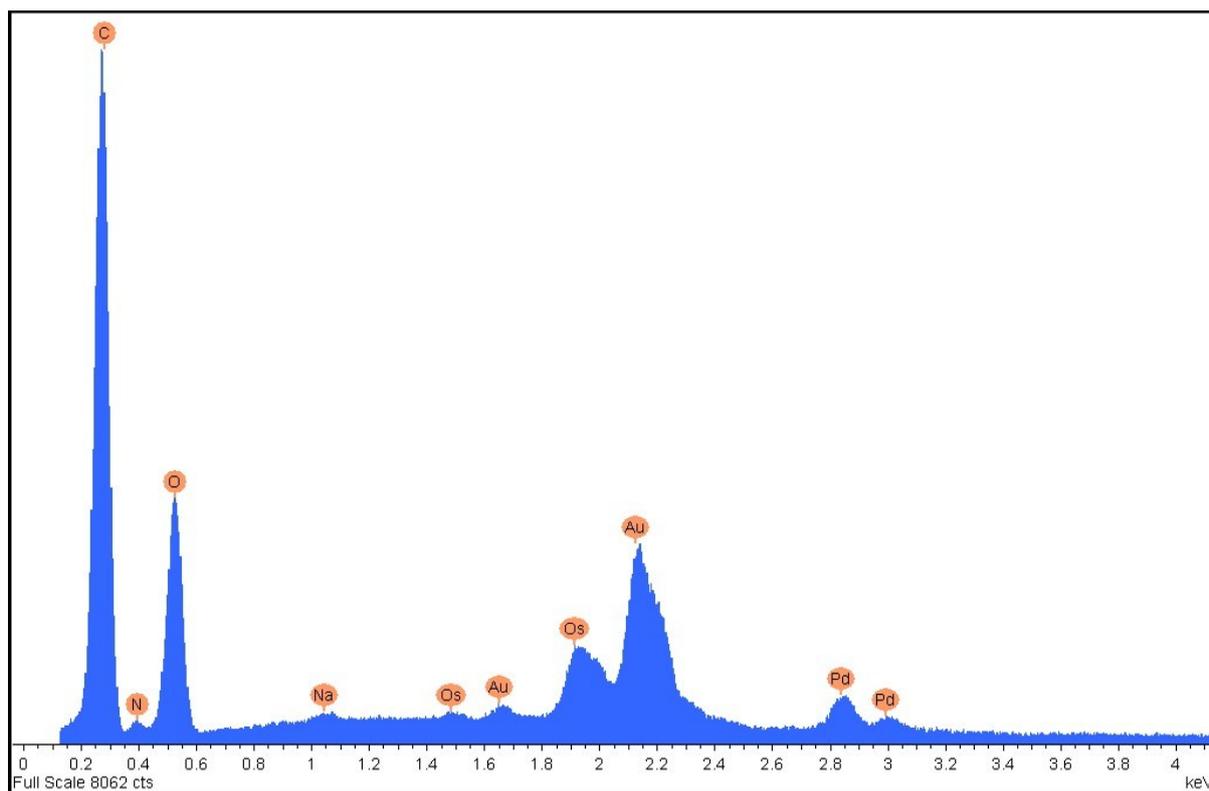


Figure S11 EDX analysis of cellulose fibres produced by *A. acetii*.

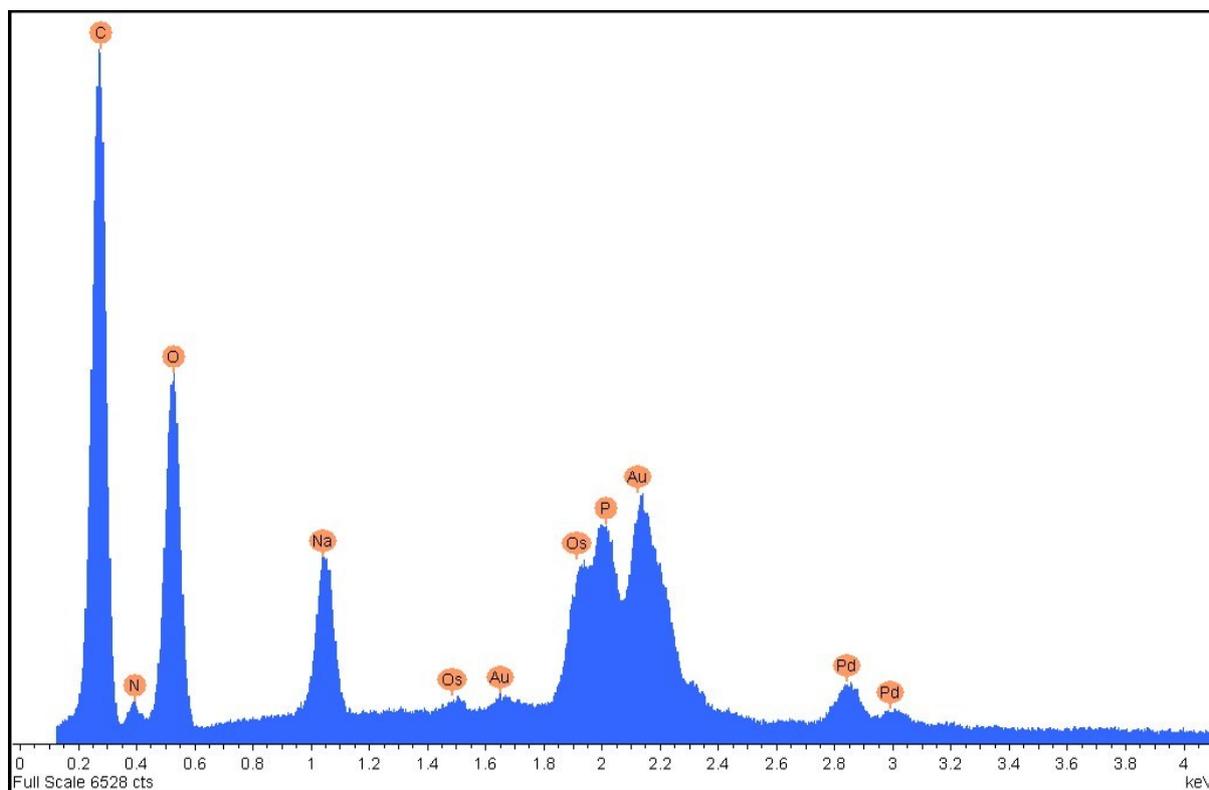


Figure S12 EDX analysis of the living biomaterial produced by the symbiotic growth of *A. acetii* and *C. reinhardtii*.