# Nanocellulose-based mechanically stable immobilization matrix for enhanced ethylene production: A framework for photosynthetic solid-state cell factories

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## Electronic supplementary information (ESI)

## Experimental section

### $CO_2$ measurements by gas chromatography

All samples were prepared as described in Experimental Design #3 in the Methods section, with the exception of preparing samples from both 2% and 1% alginate in addition to 1% TCNF. The amount of $CO_2$ in the vials was monitored similarly as described in the ethylene production assay in the methods section, with the following exceptions: $CO_2$ was measured from both liquid and headspace of the vials every 24 hours. 2N HCL was added in the medium to monitor the $CO_2$ content in the liquid space. For the quantification of $CO_2$, the calibration curve was obtained using different volumes of a commercial gas standard (1% v/v $CO_2$ in $N_2$, AGA, Finland).

## Supplementary figures

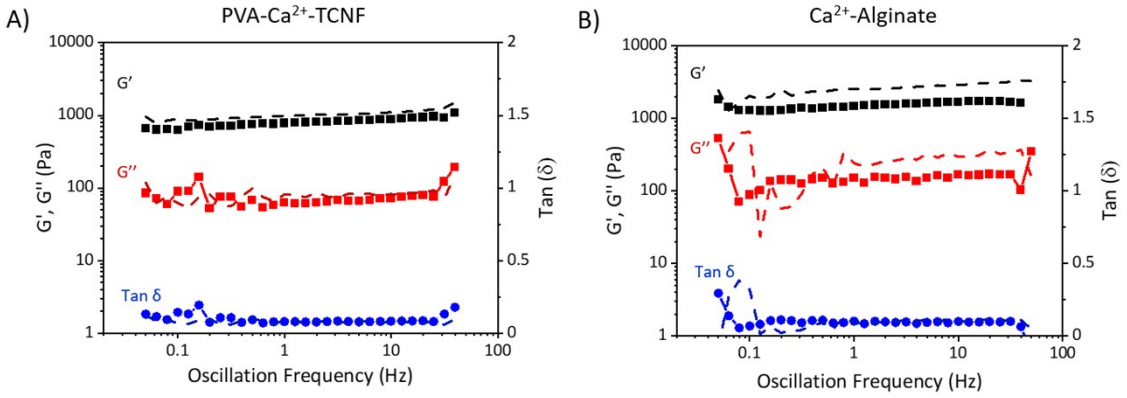


**Fig. S1** Typical frequency sweeps of A) PVA-$Ca^{2+}$-TCNF and B) $Ca^{2+}$-alginate hydrogel films with (solid lines) and without (dotted lines) Synechocystis WT cells, showing elastic moduli (G', black square), viscous moduli (G'', red square) and tan (δ) (blue circle).

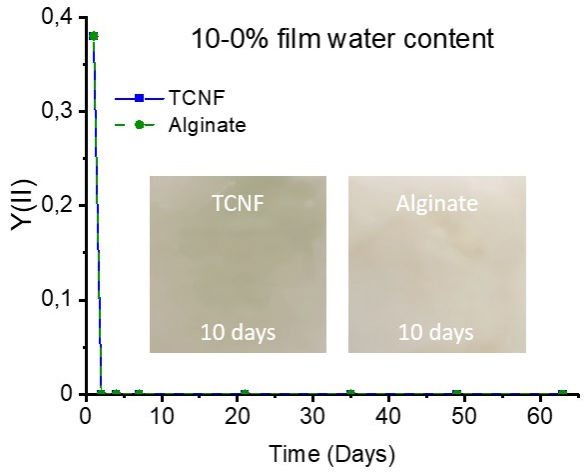


**Fig. S2.** Photosynthetic activity and visual appearance of *Synechocystis* PCC 6803 wild-type cells immobilized within PVA-$Ca^{2+}$-TCNF and $Ca^{2+}$-alginate with 10-0% of original water content.

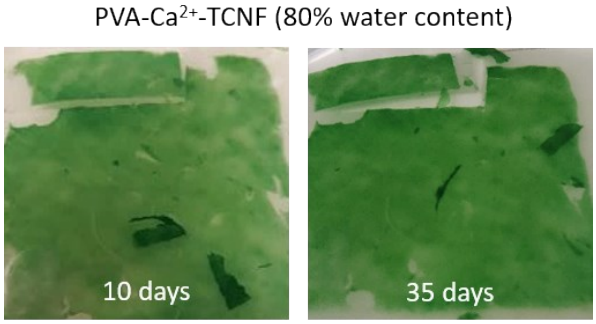


**Fig. S3**. Visual appearance of *Synechocystis* PCC 6803 wild-type cells immobilized within PVA-$Ca^{2+}$-TCNF with 80% of initial water content.

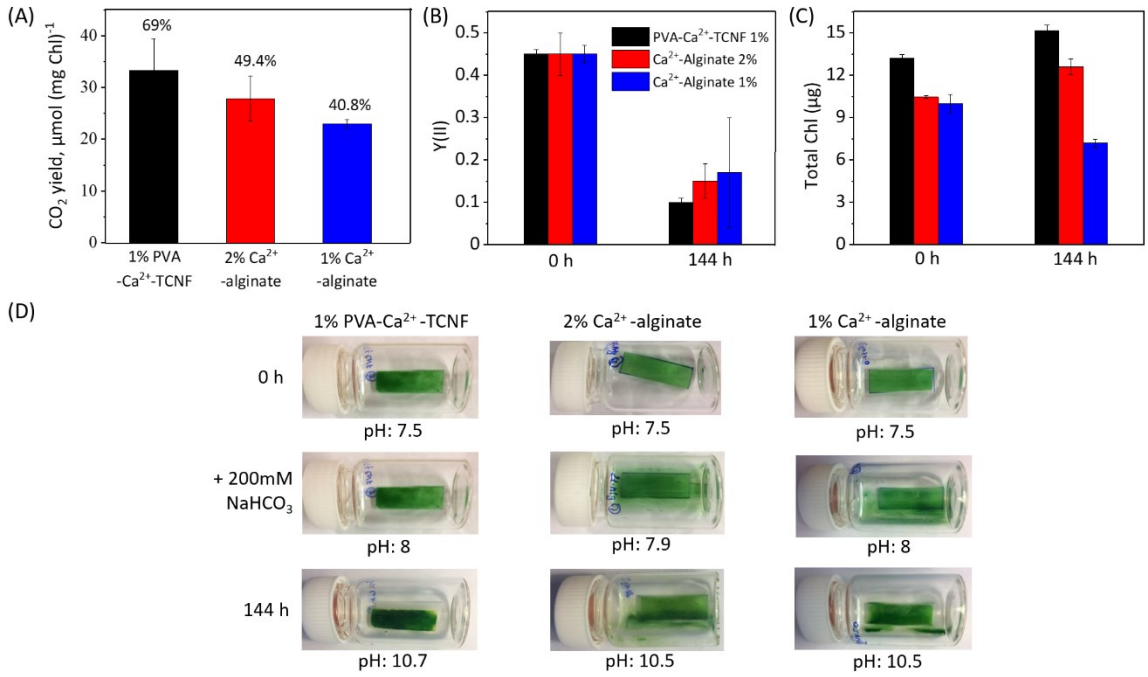


**Fig. S4**. A) Estimation of $CO_2$ uptake (at 144 h) of *Synechocystis sp. PCC 6803* wild-type (WT) cells entrapped within PVA-$Ca^{2+}$-TCNF and $Ca^{2+}$-alginate, calculated based on their initial total Chl content. The presence of $CO_2$ was measured with respect to the total $NaHCO_3$ (200 mM) supplementation in the tightly sealed vials containing 3ml of BG11 medium (pH 7.5). B) Chl, and c) photosynthetic activity Y(II) were determined at 0 and 144 h. d) Visual appearance of the entrapped cells in the different matrices. Each experimental point represents at least 3 independent measurements from 3 vials. The error bar represents the mean of all replicates (±SD).

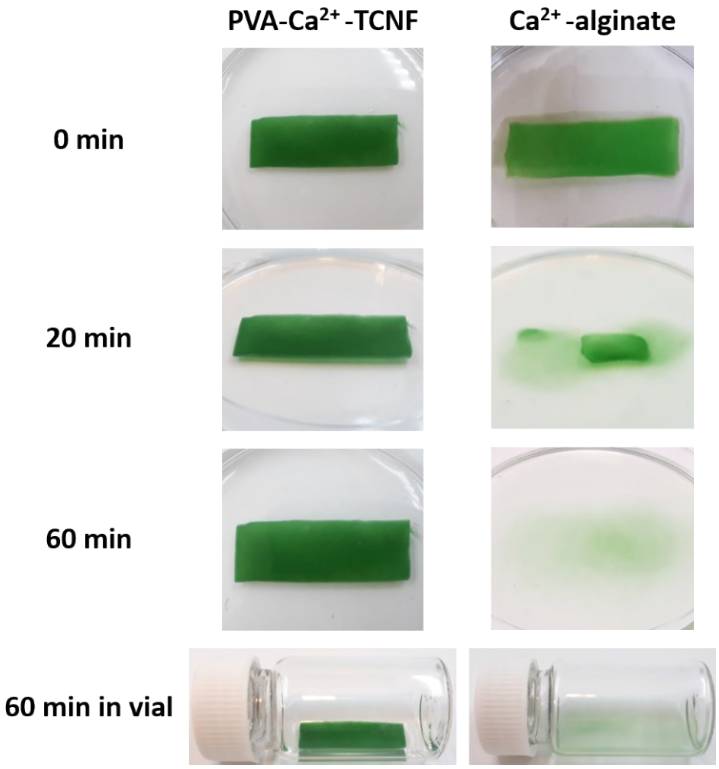


**Fig. S5.** Visual appearance of *Synechocystis sp. PCC 6803* wild-type (WT) cells entrapped within PVA-$Ca^{2+}$-TCNF and $Ca^{2+}$-alginate matrices without external support after immersion in BG-11 medium with supplemented 200 mM $NaHCO_3$ as inorganic Ci source. After 20 minutes, $Ca^{2+}$-alginate begins to disintegrate, leaking cells into the medium, and after 60 minutes, the matrix is completely disintegrated. In contrast, PVA-$Ca^{2+}$-TCNF materials remains stable and intact, demonstrating excellent applicability in submerged production conditions with $NaHCO_3$ supplement.

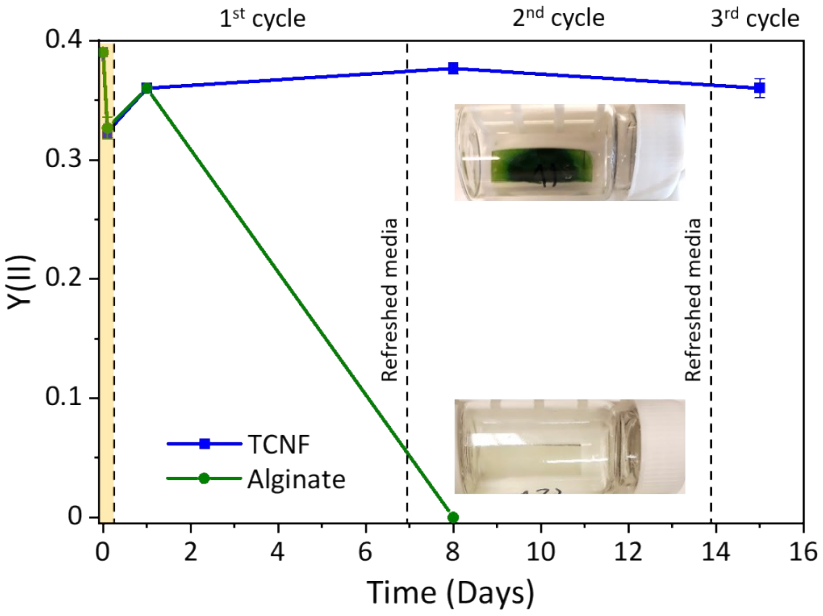


**Fig. S6.** Photosynthetic activity of *Synechocystis* sp. PCC 6803 wild-type (WT) cells entrapped within PVA-$Ca^{2+}$-TCNF and $Ca^{2+}$-alginate matrices in tightly sealed vials, measured 24 hours after the introduction (day 0) or replacement (day 7 and 14) of medium (200 mM $NaHCO_3$ in 3ml of BG11, pH 7.5). The lightly coloured part during the first day denotes the drying and recovery period of the cells during matrix preparation. The photosynthetic activity of cells in $Ca^{2+}$-alginate could not be measured after 7 days, as the matrix had been destroyed and the leaked cells were washed away during the media exchange. The photographs inserted in the graph show the visual appearance of the matrices after 8 days.