

NOVEL METHODS FOR BIOFABRICATION AND STIMULATION OF ENGINEERED LIVING MATERIALS

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Abstract

Engineered living materials (ELMs) that contain photosynthetic microorganisms are promising low-energy platforms for biological carbon capture. Here, we report the fabrication and 33-day evaluation of fibrous alginate hydrogels embedding *Chlorella vulgaris*. Each construct contained 0.5 g dry algal biomass in an approximately 40 g hydrated material matrix and provided an effective surface area of about 0.5 m². Under sealed semi-passive chamber conditions, the materials repeatedly depleted injected CO₂ during light periods and maintained visible photosynthetic activity for more than 30 days. The integrated chamber-equivalent CO₂ removal was 89 000 ppm, corresponding to 160.1 g CO₂ under the experimental conversion used, and destructive sampling indicated a final biomass gain of 0.07–0.10 g, or 14–20% relative to the initial dry

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biomass. The results support the use of fibrous alginate architectures for long-lived algal ELMs and motivate future femtosecond-laser experiments aimed at optically modulating carbonic anhydrase-mediated CO₂ hydration and fixation.

Key words: engineered living materials, *Chlorella vulgaris*, alginate hydrogel, biological carbon capture, carbonic anhydrase, femtosecond laser

Introduction. Engineered living materials combine living cells with non-living matrices so that growth, metabolism and material structure jointly generate function [1–4]. For carbon-management applications, photosynthetic ELMs are attractive because microalgae and cyanobacteria can convert CO₂ into biomass and extracellular products using light rather than high-grade thermal or electrical input. Recent work on photosynthetic hydrogels has shown that the geometry of the matrix, optical distribution and cell organization strongly affect growth and gas exchange in three-dimensional materials [5,6], while living photosynthetic composites have also been proposed as dual biological and mineral carbon sinks [7]. Microalgae are established candidates for CO₂ bio-mitigation, with prior studies describing their capacity for carbon uptake, biomass formation and integration into biotechnology platforms [8–10]. Their performance is governed not only by photosynthetic carbon fixation but also by CO₂-concentrating mechanisms, in which carbonic anhydrases catalyse the reversible hydration of CO₂ and support inorganic-carbon supply to the photosynthetic apparatus [11–13]. A second design axis is the immobilization matrix. Alginate hydrogels are widely used for cell immobilization because they gel under mild conditions, maintain high water content and permit molecular diffusion [14–17]. Immobilized microalgae have been used for wastewater treatment and nutrient removal, including *Chlorella vulgaris* in alginate-based systems [18,19], but long-term material vitality, gas-transfer architecture and dynamic stimulation remain open engineering problems. Ultrafast optical methods provide one possible route for future stimulation: femtosecond-laser photoporation has already been demonstrated in live microalgae at single-cell resolution [20], suggesting that controlled optical interaction with algal cells is experimentally feasible. In this short report we describe a fibrous alginate ELM containing *C. vulgaris*, quantify its 33-day chamber CO₂ removal profile, and outline a laser-stimulation configuration intended for future tests of carbonic-anhydrase-linked enhancement.

Materials and methods. *Chlorella vulgaris* biomass was incorporated into a multilayered alginate-based fibrous hydrogel. Each construct contained 0.5 g dry biomass within an approximately 40 g hydrated material matrix and exposed an estimated functional surface area of about 0.5 m². The samples were incubated horizontally in sealed environmental chambers without forced airflow. Chamber conditions were maintained at 27–29 °C and 85–99% relative humidity under natural light-dark cycles of approximately 16:8 to 15:9 h. CO₂ concentration was recorded with a Vernier® non-dispersive infrared sensor. The chambers were pe-

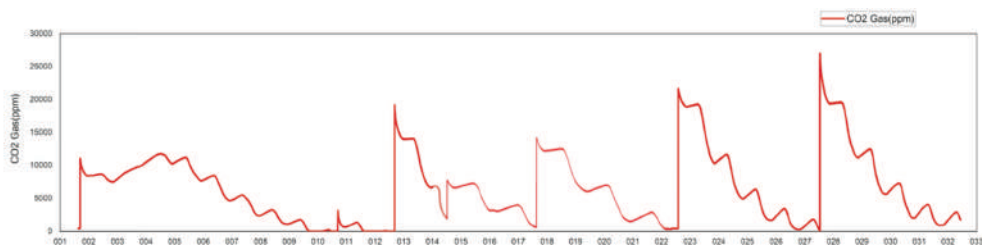


Fig. 1. Long-term CO₂ dynamics in engineered algal living materials. The red trace shows chamber CO₂ concentration over 33 days with periodic CO₂ injections (10 000–25 000 ppm). Diurnal declines during light periods repeatedly approached near-zero values

riodically re-saturated by injecting CO₂ to 10 000–25 000 ppm, which enabled repeated uptake cycles over 33 days. After the monitoring period, destructive sampling and complete dehydration were used to determine the increase in dry biomass. The future optical-stimulation setup consists of a femtosecond-laser source, a step-variable neutral-density filter, steering mirrors, a 70 cm focal-length lens, a motorized X-Y sample stage, and a movable power meter for beam monitoring.

Results. The algal ELMs remained functionally active throughout the 33-day observation period. After each CO₂ re-saturation event, chamber concentration declined during subsequent light periods, and in several cycles the concentration approached 0–100 ppm (Fig. 1). The integrated chamber-equivalent uptake over the experiment was 89 000 ppm, corresponding to 160.1 g CO₂ according to the experimental conversion applied to the chamber system. The repeated uptake cycles indicate that the fibrous hydrogel matrix preserved photosynthetic function under high humidity, semi-passive and non-forced-airflow conditions. Final destructive sampling showed a net dry-biomass increase of approximately 0.07–0.10 g, equivalent to a 14–20% increase relative to the initial 0.5 g biomass loading. The poster-derived optical scheme for the next experimental stage is shown in Fig. 2.

Discussion. The main result is the preservation of repeatable CO₂ uptake for more than one month in a hydrated, fibrous algal material. This is relevant for ELM development because many photosynthetic materials fail through drying, nutrient limitation, light attenuation or loss of gas-transfer efficiency. The fibrous architecture probably contributed to increasing the exposed area while maintaining water-rich alginate microenvironments around the cells. The observed biomass gain is consistent with sustained net photosynthetic assimilation, although the magnitude of biomass accumulation is modest relative to the cumulative chamber CO₂ decrease. This difference should be interpreted cautiously: ppm depletion in a sealed chamber measures gas-phase removal under a defined volume and re-saturation protocol, whereas final dry biomass measures retained cellular matter after the experiment. Some carbon may remain as dissolved inor-

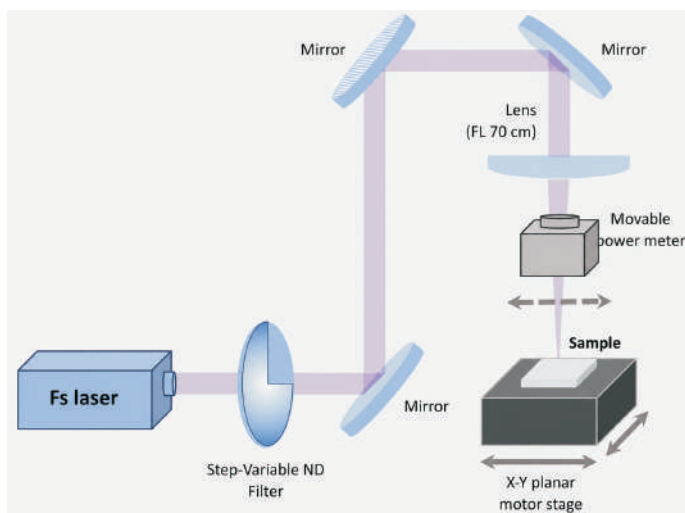


Fig. 2. Femtosecond-laser setup for algae-laden membrane stimulation. The beam is intensity-controlled via a neutral-density filter, redirected by mirrors, focused by a 70 cm lens onto the motorized X-Y sample stage and monitored by a movable power meter

ganic carbon, extracellular products or carbonate species in the hydrated matrix, and part of the signal may depend on chamber volume, leakage control, sensor calibration and temporal integration. Therefore, the present data should be treated as a proof-of-principle demonstration of material longevity and repeated uptake rather than as a final open-air productivity estimate. Future work should add biological and material controls, replicate chambers, continuous light and humidity logging, normalization to photon flux, exposed area and initial biomass, and direct carbon-mass-balance measurements. The proposed femtosecond-laser stage should be tested conservatively, with dark and sham-irradiated controls, thermal monitoring, chlorophyll-fluorescence readouts, cell-viability assays, and direct carbonic anhydrase activity assays. If a non-damaging optical regime increases CO_2 hydration or fixation, it could provide a tunable external stimulus for photosynthetic ELMs; if not, the same setup will still define the safe optical operating window for algal hydrogel materials.

Conclusion. A fibrous alginate hydrogel containing *C. vulgaris* maintained repeated CO_2 uptake and biomass increase during a 33-day semi-passive chamber experiment. The system reached an integrated chamber-equivalent removal of 89 000 ppm CO_2 and preserved material vitality for more than 30 days. These results justify further optimization of fibrous algal ELMs and provide the experimental basis for controlled femtosecond-laser stimulation studies targeting carbonic-anhydrase-linked carbon capture.

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