Electronic Supplementary Material (ESI) for Environmental Science: Water Research & Technology.

Supporting Information

Achieving high-rate hydrogen recovery from wastewater using customizable alginate polymer gel matrices encapsulating biomass

Kuang Zhu, William A. Arnold, Jonathan Sakkos, Craig Davis, and Paige J. Novak

Materials and methods

Additional methodological details follow.

Figure S1 shows a schematic of the set-up used to make the alginate polymer beads. The mixture of biomass and Na-alginate solution was extruded drop-by-drop by a peristaltic pump through an 18-gauge blunt needle into 0.4 M $CaCl_2$, $SrCl_2$, or $BaCl_2$ solution. After formation of the beads the coating was added if desired as described in the manuscript.

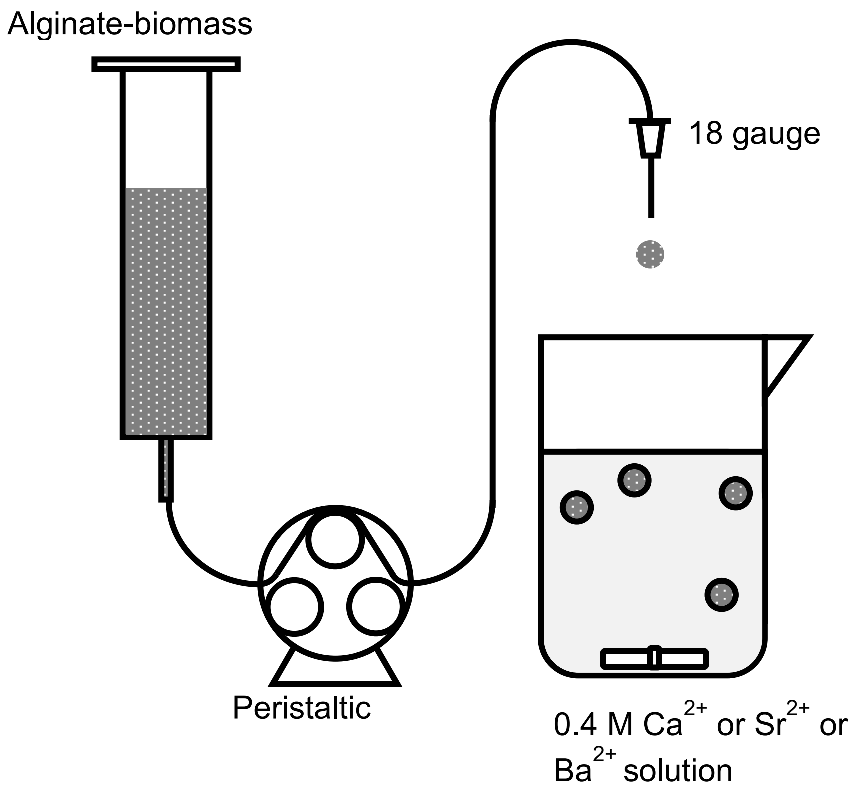

Figure S1. A schematic showing the production of Ca-alginate, Sr-alginate, or Ba-alginate beads.

Figure S2 shows the size of Ca-alginate, Sr-alginate, and Ba-alginate beads. The average beads made from 2% alginate solution cross-linked in 0.4 M divalent metal solution with an 18-gauge needle are 2.5 mm in diameter. No apparent difference in size was observed among the beads cross-linked by different metal ions.

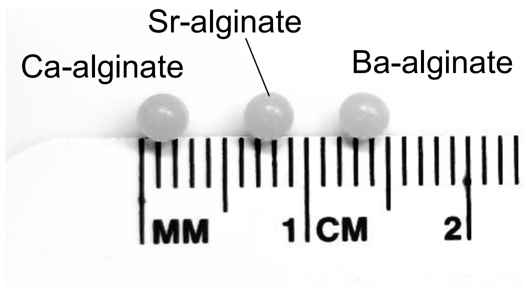

Figure S2. The size of Ca-alginate, Sr-alginate, and Ba-alginate beads.

Figure S3 shows the diffusion cell apparatus used in experiments to measure the diffusivity of hydrogen and dissolved organic carbon through the coated and uncoated alginate polymer sheets cross-linked by Ca^{2+} and Sr^{2+}. The design of the experimental apparatus was based on the methods described by Shimotori and coworkers.[1] The polymer sheets were placed between the two compartments. The large compartment was 600 mL and the small compartment was 60 mL. Both compartments were continuously stirred. Samples were taken from ports at the top of each compartment.

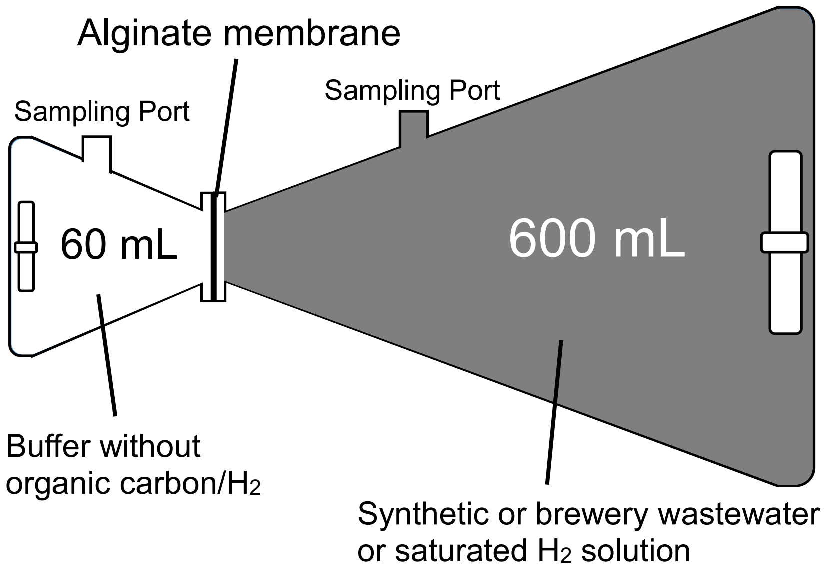

Figure S3. The diffusion cell apparatus used in experiments.

Figure S4 shows the experimental set-up used in the flow-through hydrogen production experiments. Sterilized brewery wastewater was fed continuously to the bottom of all reactors by a peristaltic pump, pushing the mixture of effluent and the gas produced by biomass out from the top of the reactor. Effluent was acidified to stop all biological reaction, the gas and liquid were separated, and the gas volume produced was then measured via volume displacement.

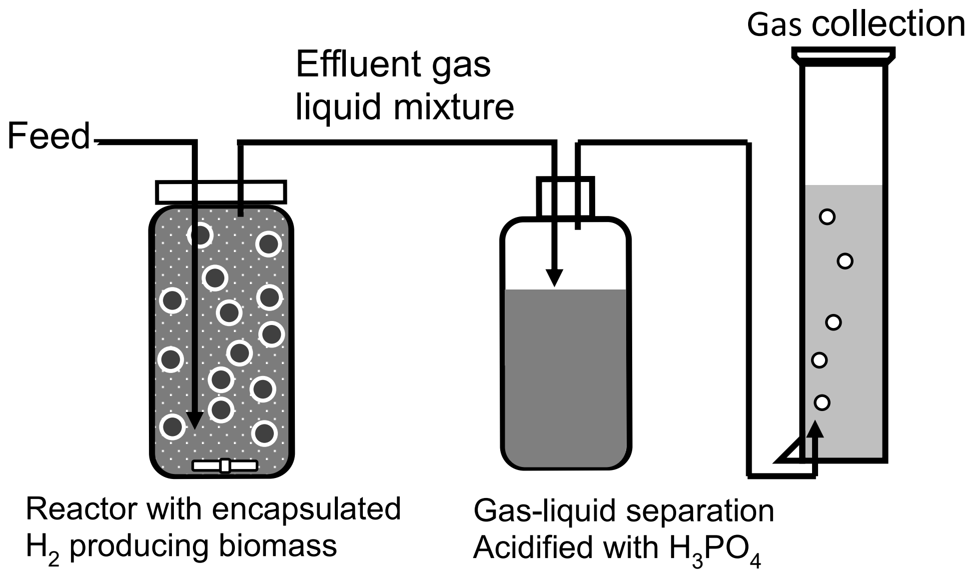

Figure S4. Diagram of the flow-through reactor set-up used in the hydrogen production experiments.

Results

The escape of biomass from the metal-alginate encapsulation matrices over time is shown in Figure S5. Most of the escape occurred in the first 10 – 20 days of the experiment. There was little escape observed during the rest of the experiment. It is thought that the biomass encapsulated in the outer layer of the beads migrated out in the beginning of the experiment and reached equilibrium after 10 – 20 days.

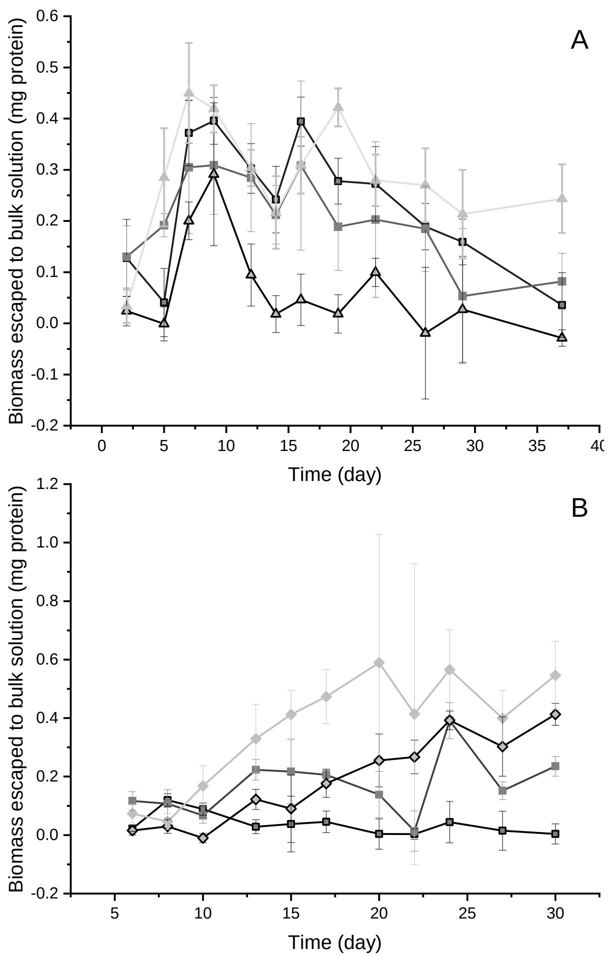

Figure S5. The escape of biomass-protein to the bulk solution with time. The squares represent Ca-alginate, the triangles represent Sr-alginate, and the diamonds represent Ba-alginate. The symbols with a black border represent coated beads and the symbols without a dark border represent uncoated beads. Panels A and B represent 2 different sets of experiments performed under the same condition.

Figure S6 shows the average cumulative leaked biomass from each treatment after 30 days (light grey bars) and the encapsulated biomass that remained within the beads after 30 days (dark grey bars). Each treatment was set-up in triplicate and the standard deviation of the average is shown as the error bar. Results from the abiotic beads containing no biomass are also shown (denoted with the symbol "-" on the x-axis).

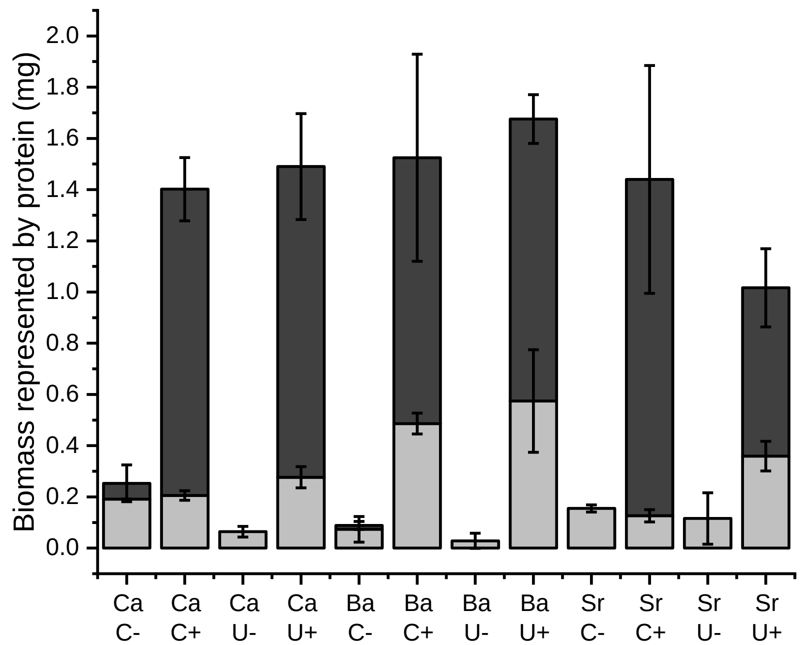

Figure S6. The biomass at Day 30 that was present encapsulated in the alginate beads (dark grey) versus the biomass that had leaked from the system after 30 days (light grey). Ca and Sr denote the divalent cation used cross-link the alginate polymer (Ca^{2+} and Sr^{2+}, respectively). C and U denote the beads that were coated with three layers of the composite coating and beads that were not coated, respectively. The symbol "-" denotes the abiotic beads that contained no encapsulated biomass, while the symbol "+" denotes the beads containing encapsulated hydrogen-producing biomass. The average from triplicate treatments is shown with the error bar denoting the standard deviation.

Figure S7A shows the average daily hydrogen production from synthetic wastewater and Figure S7B shows the average bulk biomass concentration in the batch reactors. Triplicate reactors were set up for each treatment, with the average shown and the error bars showing the standard deviation. Coated and uncoated Ca-alginate beads containing encapsulated biomass and coated and uncoated Ca-alginate beads containing no biomass (abiotic controls) were investigated. The synthetic wastewater was replaced every 24 hours with the exception of the last 48 hours of the experiment, in which the synthetic wastewater was not replaced, allowing for degradation of the dissolved organic carbon in the bulk solution over a longer period of time.

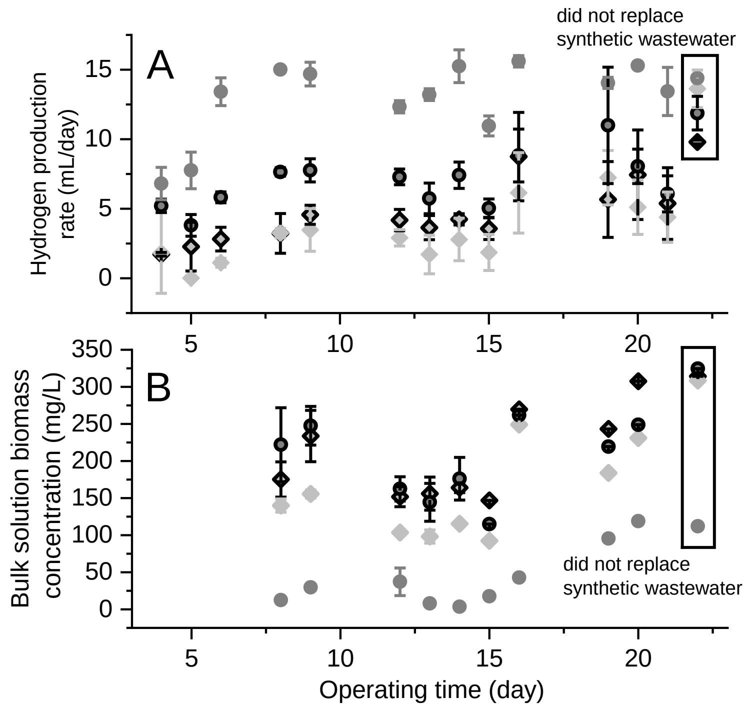

Figure S7. The (A) daily hydrogen production rate from synthetic wastewater, and (B) bulk biomass concentration in the batch reactors. Coated (with border) and uncoated (without border) Ca-alginate beads encapsulating biomass (circles) and Ca-alginate beads containing no biomass (diamonds), again, both coated (with border) and uncoated (without border) are shown. The synthetic wastewater was not replaced for the last 48 hours. All treatments were in triplicate, with the average shown. The error bars represent the standard deviation.

References

1. T. Shimotori, E. L. Cussler, W. A. Arnold and A. M. Asce, High-Density Polyethylene Membrane Containing Fe 0 as a Contaminant Barrier, *Environ. Sci. Technol.*, 2006, **38**, 803-810.