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# Anoxic Biomass Carbon Storage: Fundamental feasibility and environmental impact

Morgan Reed Raven*, Aaron M. Martinez, Natalya Evans
Marine Science Institute, University of California Santa Barbara
* correspondence to: raven@ucsb.edu

Results presented below are in provisional and in prep for publication. Long-term breakdown experiments for which initial timepoints are reported are ongoing through January 2025.

## Key Questions:

1. **What happens to Carboniferous' bagasse briquettes when they are soaked in Orca brine?**
2. **How are bagasse briquettes broken down by microorganisms in anoxic seawater vs. brine?**

## Question 1. What happens to bagasse briquettes when they soak in brine?

The primary result of soaking bagasse briquettes in both seawater and brine is that a small fraction of the biomass dissolves, producing Dissolved Organic Carbon (DOC).

We quantified the rate of carbon released and the chemical form of the leached DOC using both laboratory experiments and field incubations. The process of DOC leaching is abiotic and reproducible, which means that we can mathematically model DOC release over time (Figure 1). Leachable components of the bagasse fall into two categories. Highly soluble materials, representing 0.43% of total C, leach with a half-life of ~11 hrs; somewhat soluble materials, representing an additional 0.83% of C, leach with a half-life of ~38 days. We validated the rates from our lab experiments by comparing the predicted accumulation of DOC field incubation bottles (0.76% of initial C) with observations from the laboratory experiments in Figure 1 (0.77% of initial C). Using this approach, we can conclude that:

- Expected losses of DOC to the oxic water column during biomass placement are less than 0.08% of initial C (allowing a conservative 3 hrs of transit).
- Approximately 1.3% of the initial C in bagasse is soluble over timescales of months, even in the absence of microbial activity. This represents the proportion of initial biomass that would be expected to dissolve into the brine on timescales of years.

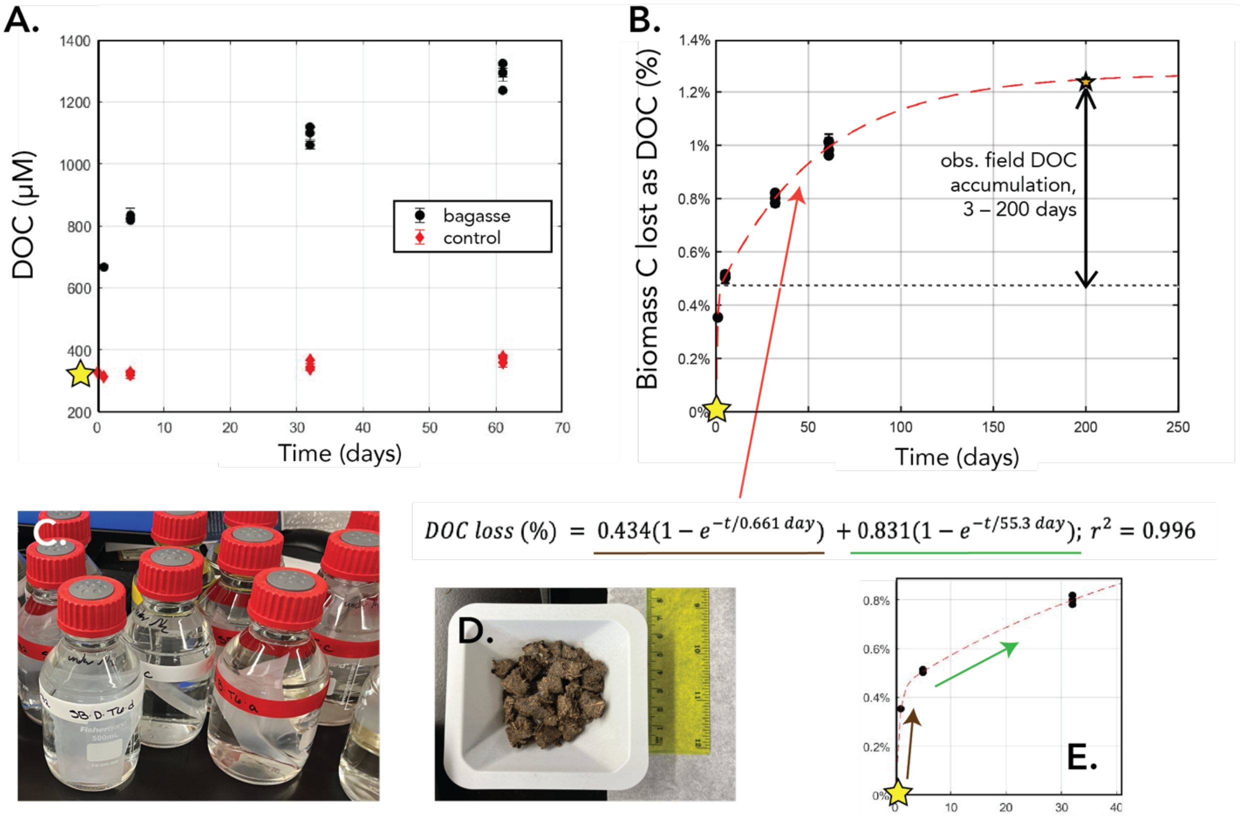


**Figure 1.** Experimental results showing DOC concentrations over 60 days of incubation in anoxic Orca brine (Long-term breakdown experiment "LTB-2", ongoing since June 2024). (A.) Black circles indicate bagasse experiments and red circles show biomass-free brine controls. These data were used to calculate panel (B) in units of the fraction of biomass carbon that has been converted to DOC over time. The dashed best-fit line is defined by the equation shown, see text. (C) Incubations were conducted in 600-mL glass bottles with no headspace, stored at 4°C in the dark. (D) Bagasse pieces prior to experiments. Approximately 2 g were sealed into 35-µM nylon mesh bags, degassed under $N_2$ overnight and then incubated in anoxic Orca brine. Bottles were sacrificed in triplicate at each time point. (E) Data from (B) over the first 40 days. The brown and green arrows illustrate the change caused by dissolution of highly soluble and slowly soluble organic matter, respectively.

The data above tell us about our total carbon budget, but to understand the significance of DOC release for marine ecosystems, we also need to understand the chemical form and reactivity of the molecules within the leached DOC pool.

We are using several analytical tools to characterize DOC chemistry, including fluorescence spectroscopy, to compare the composition of bagasse leachate with that of background DOC in Orca Basin. These data show that:

- DOC in bagasse leachate, as expected, chemically resembles typical terrestrial, plant-derived molecules like humic acids, lignins, and tannins.

- Orca brine naturally accumulates high concentrations of DOC (to ~300 μM). As seen by fDOM spectroscopy, the vast majority (98%) of DOC in Orca Basin brine appears to represent very similar, terrestrial or "humic" materials to those in bagasse leachate.

- An addition of bagasse DOC to Orca brine is relatively unlikely to impact microbial communities because similar molecules are already abundant in the environment. However, because bagasse DOC contains organic acids, their effects on local pH should be included in evaluations of impact.

## Question 2. How do microbes break down bagasse in Orca brine?

Over several months, bagasse briquettes begin to be consumed by microorganisms. In the absence of $O_2$, these microorganisms "breathe" iron and then sulfate as they oxidize organic carbon in the biomass to $CO_2$. During this reaction, iron-reducing microbes also convert iron minerals to dissolved $Fe^{2+}$, and sulfate-reducing microbes convert dissolved sulfate to sulfide.

We quantified the extent of microbial breakdown of the bagasse in long-term breakdown experiments by monitoring changes in the mass of biomass over time and the rates of accumulation of $Fe^{2+}$ and sulfide. Laboratory experiments tested compressed bagasse (with and without γ-irradiation to remove any on-board microbes), processed sargassum, and controls (no biomass) in bottles containing either Orca brine or "normal" anoxic seawater from the deep Santa Barbara Basin (California, U.S.). Breakdown is expected to proceed faster in normal seawater than in brine, so these data provide a conservative maximum for breakdown rates in brine.

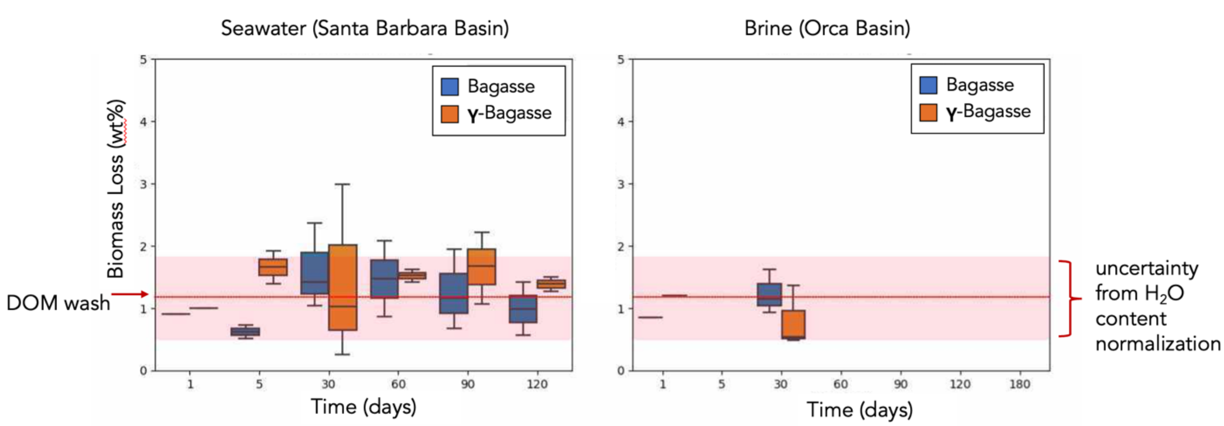


**Figure 2.** Experimental results to date (later Orca timepoints ongoing) showing changes in the mass of bagasse contained within 30-μm nylon mesh bags in incubations (LTB-2) with anoxic seawater (left) and anoxic Orca brine (right). All bags are extensively washed and freeze-dried to remove salt; this also removes the DOC quantified in Figure 1 from all bags, which is shown as the horizontal red line. Bag masses are

compared with controls to account for the water loss from freeze-drying. This water content varies among biomass sub-samples and limits the precision of this method to +/- 0.6 wt% (red shading).

- There is no statistically significant change in the solid mass of bagasse over 120 days of incubation in anoxic seawater. In other words, the total loss of bagasse biomass was less than 0.6 wt% over four months.

We can achieve higher precision for this estimate by monitoring concentrations of dissolved species.

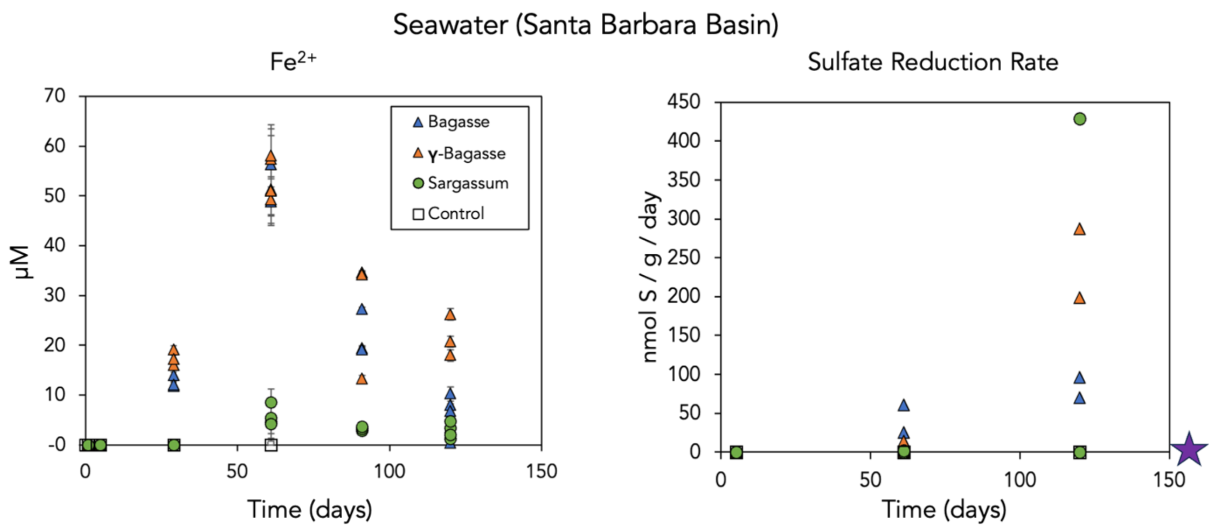


**Figure 3.** Indicators of microbial iron and sulfate reduction in long-term biomass breakdown experiments with anoxic seawater. Iron reduction leads to the accumulation of $Fe^{2+}$ over the first 60 days. At this point, the microbial community switches to sulfate reduction, and the resulting sulfide precipitates out the dissolved $Fe^{2+}$ as solid iron sulfide minerals. Sulfate reduction rates are measured by injecting radioactive sulfate ($^{35}SO_4^{2-}$) into the bottles and measuring the amount of radioactive sulfide produced after 24 hours. The purple star shows the rate of sulfate reduction in brine through the 60-day timepoint (ongoing).

- In anoxic seawater, we observe the expected sequence of microbial metabolisms. Concentrations of $Fe^{2+}$ indicate an approximate breakdown rate of 0.0003% of biomass carbon per day through about 60 days. After this time, microbial sulfate reduction becomes the principal mechanism of breakdown. Maximum observed rates of sulfate reduction (0.002% of biomass C per day at 120 days) are ten times higher than peak iron-reduction rates and are likely to continue to increase.

- In brine, rates of microbial sulfate reduction are below detection limits of ~2 nmol C per day per gram biomass after 60 days of lab incubation. Additionally, no sulfide was detected in the in-situ bagasse incubation after 200 days on the Orca Basin seafloor, indicating that sulfate reduction rates remain insignificant on that timescale.

- Observations indicating no sulfate reduction in Orca brine align with our thermodynamic calculations, which show that typical pathways of sulfate reduction are energetically unfavorable and cannot support microbial growth under brine conditions.

## Summary

Both lab and field results indicate that bagasse briquettes placed in Orca Basin would experience no significant microbial breakdown due to $O_2$, nitrate, iron, or sulfate reduction over the first year of sequestration. Approximately 1.3% of bagasse carbon would dissolve and lead to a slight increase in the concentration of humic species (tannins, lignins) in the brine.

Upcoming results will address potential impacts of bagasse placement on nutrients and methane cycling.

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