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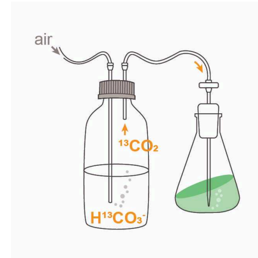
Isotopically labelled inorganic carbon delivered to algal cultures via bubbler bottle



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We use this protocol and it's working

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Abstract

This protocol describes a method for delivering labelled inorganic carbon as $^{13}CO_2$ to algal cultures by bubbling air through a solution of $H^{13}CO_3^-$, and then into the culture. We developed this method to deliver label to cultures grown under continuous bubbling with air, without the use of $^{13}CO_2$ labelled gas and the necessary equipment to mix labelled gas with air at near-atmospheric levels. Bubbling precludes the more common approach of adding $H^{13}CO_3^-$ label directly to the media, because dissolved HCO_3^- is in equilibrium with atmospheric CO_2. Thus, excess HCO_3^- added to a solution will leave the solution as CO_2 gas as it equilibrates. Bubbling rapidly accelerates this equilibration which is typically diffusion limited. The method described here takes advantage of this aspect of carbonate chemistry, and uses a solution of $H^{13}CO_3^-$—which is less expensive and more convenient than $^{13}CO_2$ gas—to generate a flux of $^{13}CO_2$ that can be bubbled into a culture.

Materials

Aquarium pump
Bottle with vent and fill port assembly lid
Tubing and luer locks
Bubbling flask assembly (Erlenmeyer flask, foam plug, serological pipet, syringe filter)
Culture medium & inoculum
Na13CO3
H2KPO4
HK2PO4

Troubleshooting

The purpose and problem of bubbling cultures

1 Bubbling air into algal cultures stimulates photosynthetic growth by ameliorating diffusion limitation for CO_2 (fig. 1A-B). Stable isotope probing (SIP) experiments examining carbon fixation often involve ^{13}C label introduced directly into the culture medium as a $H^{13}CO_3^-$ salt. Unfortunately, because dissolved inorganic carbon (DIC) is in equilibrium with atmospheric CO_2 (eq. 1), this labelling method does not work for cultures that are bubbled in an open system. Bubbling accelerates equilibration, causing excess HCO_3^- to rapidly exit the solution so that the label is lost before it can be fixed into biomass (fig. 1C). This presents some inconvenience, as $H^{13}CO_3^-$ salts are cheaper and easier to work with than is $^{13}CO_2$ gas.

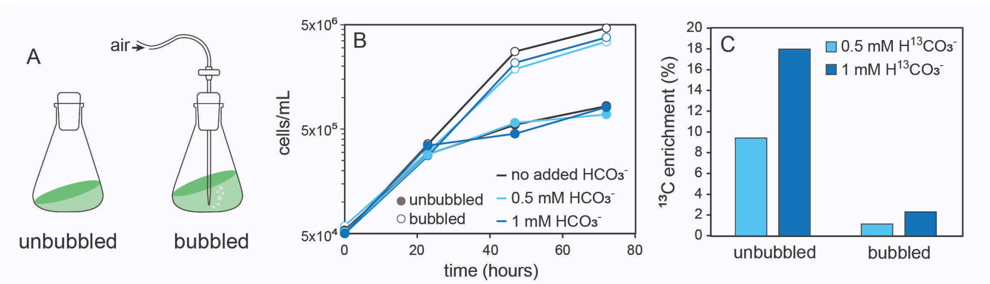

Figure 1. Bubbling enhances photosynthetic growth but precludes stable isotope probing with labelled bicarbonate. **A.** Diagrams of unbubbled vs. bubbled culture formats. Culture data presented in this protocol are of the unicellular green alga *Chlamydomonas reinhardtii*, grown in minimal medium under continuous light at 28°C and shaken at 125 RPM. **B.** Growth curves of *C. reinhardtii* in unbubbled vs. bubbled culture formats, with and without added bicarbonate. Bubbling greatly enhances autotrophic growth. At the concentrations shown, bicarbonate addition does not impact growth. **C.** ^{13}C enrichment of *C. reinhardtii* biomass grown with ^{13}C labelled bicarbonate added directly to the culture medium for 24 hours, measured by IRMS on lyophilized cell pellets. Unbubbled cultures exhibit substantial ^{13}C enrichment; ~90% of that signal is lost when bubbled.

Equation 1. $CO_{2(g)} + H2O \leftrightharpoons H_2CO_3 \leftrightharpoons HCO_3^- + H^+ \leftrightharpoons CO_3^{2-} + 2H^+$

Label delivery via bubbler bottle

2 We developed a SIP method that takes advantage of this liability of bubbling and carbonate chemistry (figure 2). Using an aquarium pump, we bubble air into a solution of $H^{13}CO_3^-$. The DIC in the solution exchanges with the air, releasing label in the form of $^{13}CO_2$ gas. That air is then bubbled into algal cultures. Using this method, we achieved substantial biomass ^{13}C enrichment in bubbling *C. reinhardtii* cultures (fig. 2B).

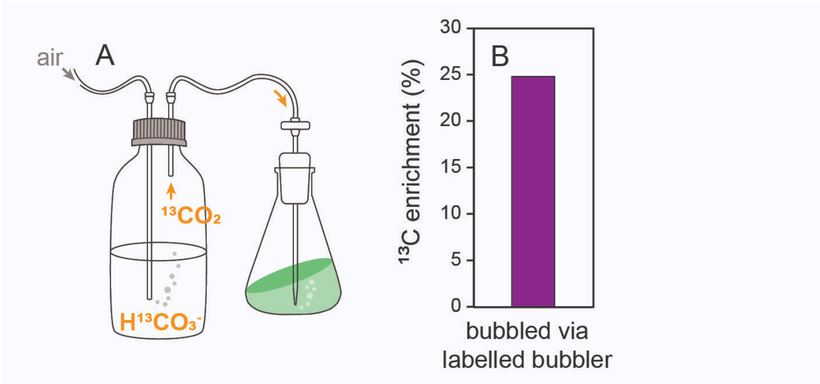

Figure 2. Bubbling air through a solution of $H^{13}CO_3^-$ is an effective strategy to label bubbling cultures. **A.** Diagram of our bubbler label delivery approach. Bubbler bottle contains 500 mL of 1.5 mM $NaH^{13}CO_3$, air flow is ~1L/min. **B.** ^{13}C enrichment of *C. reinhardtii* biomass labelled by this approach after 24 hours.

3 The rate of label release in this method can be tuned by buffering the bubbler solution at different pH values (fig. 4A). In lower pH solutions, carbonate equilibria (eq. 1) shift towards a higher fraction of the DIC pool speciated as dissolved CO_2, which increases the rate at which that DIC exchanges into the bubbled air. We identified solution conditions suitable for experiments requiring rapid incorporation of ^{13}C into algal biomass over short timescales (minutes) and for steady-state release over longer timescales (days). With a bubbler solution buffered with phosphate at pH 7.5, we obtained ^{13}C enrichment detectable in algal biomass by IRMS in <15 minutes (fig. 4B, green). With a bubbler solution devoid of any additional buffer beyond the HCO_3^- itself, we obtained slower but longer-lived steady-state incorporation of the label (fig. 4B, orange).

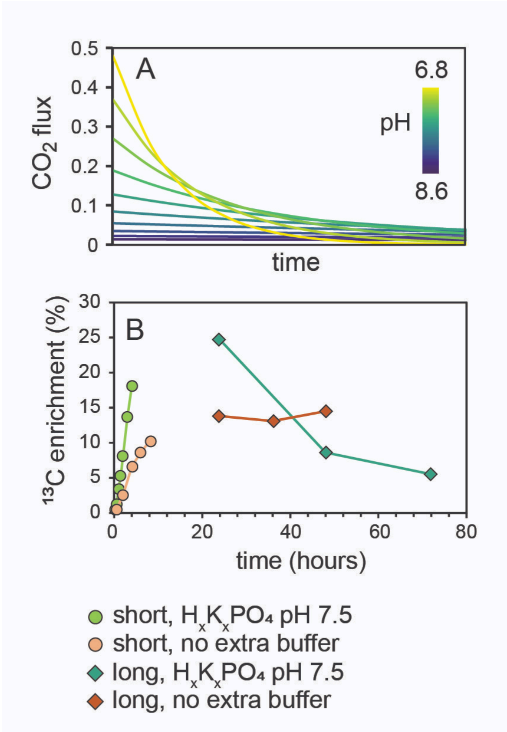

Figure 3. Label release from the bubbler solution can be modulated by pH. **A.** Model describing the flux of CO_2 released from the bubbler solution when buffered at different pH values, presented as the fraction of initial DIC leaving the solution over time. At lower pH values, the DIC leaves the solution more rapidly. **B.** Data from four separate experiments illustrating the biomass ^{13}C enrichment of *C. reinhardtii* cultures over time, with bubblers buffered with 10x excess $H_xK_xPO_4$ at pH 7.5 (green) vs no additional buffer (orange), examined over long (diamonds) or short (circles) timecourses. At pH 7.5, the equilibrium DIC concentration is >10x lower than the DIC added to the bottle as $H^{13}CO_3^-$, so most of the label leaves the solution rapidly. This results in rapid labelling of biomass but also rapid depletion of label from the system. Without a separate buffer, DIC leaving the system drives the pH up to the pH at which the DIC concentration in the solution is at equilibrium with the air, and then exchanges with the air at a steady-state rate. This results in a longer-lived signal of label in biomass.