Video Article

Chemical Gardens as Flow-through Reactors Simulating Natural Hydrothermal Systems

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Abstract

Here we report experimental simulations of hydrothermal chimney growth using injection chemical garden methods. The versatility of this type of experiment allows for testing of various proposed ocean / hydrothermal fluid chemistries that could have driven reactions toward the origin of life in environments on the early Earth, early Mars, or even other worlds such as the icy moons of the outer planets. We show experiments that include growth of chemical garden structures under anoxic conditions simulating the early Earth, inclusion of trace components of phosphates / organics in the injection solution to incorporate them into the structure, a switch of the injection solution to introduce a secondary precipitating anion, and the measurement of membrane potentials generated by chemical gardens. Using this method, self-assembling chemical garden structures were formed that mimic the natural chimneys precipitated at submarine hydrothermal springs, and these precipitates can be used successfully as flow-through reactors by feeding through multiple successive “hydrothermal” injections.

Video Link

The video component of this article can be found at http://www.jove.com/video/53015/

Introduction

“Chemical gardens” are self-assembling inorganic precipitates developed where two fluids of contrasting chemistries interact[1,2]. These self-assembling inorganic structures have been the subject of scientific interest for over a century partly due to their biomimetic appearance, and many experimental and theoretical studies have been pursued to understand the various complex aspects and possible functions of chemical garden systems[3]. Natural examples of chemical gardens include mineral “chimney” precipitates that grow around hydrothermal springs and seeps, and it has been argued that these could provide plausible environments for life to emerge[4]. To grow a chemical garden simulating a natural hydrothermal vent chimney, a reservoir solution should represent a simulated ocean composition and an injection solution should represent the hydrothermal fluid that feeds into the ocean. The versatility of this type of experiment to different reaction systems allows for simulation of almost any proposed ocean / hydrothermal fluid chemistry, including environments on the early Earth or on other worlds. On the early Earth, the oceans would have been anoxic, acidic (pH 5-6), and would have contained dissolved atmospheric CO_2 and Fe^{2+}, as well as Fe^{III}, Ni^{2+}, Mn^{2+}, NO^{3-}, and NO^{2-}. Chemical reactions between this seawater and the ultramafic ocean crust would have produced an alkaline hydrothermal fluid containing hydrogen and methane, and in some cases sulfide (HS^-)[4-8]. The chimneys formed in early Earth alkaline vent environments could thus have contained ferrous/ferric oxyhydroxides and iron/nickel sulfides, and it has been proposed that these minerals might have served particular catalytic and proto-enzymatic functions toward harnessing geochemical redox / pH gradients to drive the emergence of metabolism[5]. Likewise, on other worlds such as that may host (or may have hosted) water/rock interfaces — such as early Mars, Jupiter’s moon Europa, or Saturn’s moon Enceladus — it is possible that water/rock chemistry could generate alkaline vent environments capable of driving prebiotic chemistry or even providing habitable niches for extant life[5,9-11].

The classic chemical garden experiment involves a seed crystal of a metal salt, *e.g.* ferrous chloride tetrahydrate $FeCl_2 \cdot 4H_2O$, submerged in a solution containing reactive anions, *e.g.* sodium silicate or “water glass”. The metal salt dissolves, creating an acidic solution containing Fe^{2+} that interfaces with the more alkaline solution (containing silicate anions and OH^-) and an inorganic membrane precipitate is formed. The membrane swells under osmotic pressure, bursts, then re-precipitates at the new fluid interface. This process repeats until the crystals are dissolved, resulting in a vertically oriented, self-organized precipitate structure with complex morphology at both macro and micro scales. This precipitation process results in the continued separation of chemically contrasting solutions across the inorganic chemical garden membrane, and the difference of charged species across the membrane yields a membrane potential[12-14]. Chemical garden structures are complex, exhibiting compositional gradients from interior to exterior[13,15-19], and the walls of the structure maintain separation between contrasting solutions for long periods while remaining somewhat permeable to ions. In addition to being an ideal experiment for educational purposes (as they are simple to make for classroom demonstrations, and can educate students about chemical reactions and self-organization), chemical gardens have scientific

significance as representations of self-assembly in dynamic, far-from-equilibrium systems, involving methods that can lead to the production of interesting and useful materials[20,21].

Chemical gardens in the laboratory can also be grown via injection methods, in which the solution containing one precipitating ion is slowly injected into the second solution containing the co-precipitating ion (or ions). This results in the formation of chemical garden structures similar to those of crystal growth experiments, except that the properties of the system and the precipitate can be better controlled. The injection method has several significant advantages. It allows one to form a chemical garden using any combination of precipitating or incorporated species; *i.e.*, multiple precipitating ions can be incorporated into one solution, and/or other non-precipitating components can be included in either solution to adsorb / react with the precipitate. The membrane potential generated in a chemical garden system can be measured in an injection experiment if an electrode is incorporated into the interior of the structure, thus enabling electrochemical study of the system. Injection experiments offer the ability to feed the injection solution into the interior of the chemical garden for controlled time frames by varying the injection rate or total injected volume; it is therefore possible to feed through different solutions sequentially and use the precipitated structure as a trap or reactor. Combined, these techniques allow for laboratory simulations of the complex processes that could have occurred in a natural chemical garden system at a submarine hydrothermal vent, including a chimney formed from many simultaneous precipitation reactions between ocean and vent fluid (*e.g.,* producing metal sulfides, hydroxides, and/or carbonates and silicates)[5,22]. These techniques can also be applied to any chemical garden reaction system to allow for formation of new types of materials, *e.g.,* layered tubes or tubes with adsorbed reactive species[20,23].

We detail here an example experiment that includes the simultaneous growth of two chemical gardens, Fe^{2+}-containing structures in an anoxic environment. In this experiment we incorporated trace amounts of polyphosphates and/or amino acids into the initial injection solution to observe their effect on the structure. After initial formation of the chemical garden we then switched the injection solution to introduce sulfide as a secondary precipitating anion. Measurements of membrane potentials were made automatically throughout the experiment. This protocol describes how to run two experiments at once using a dual syringe pump; the data shown required multiple runs of this procedure. The relatively high flow rates, low pH of the reservoir and reactant concentrations employed in our experiments are designed to form large chimney precipitates on time scales suitable for one-day laboratory experiments. However, fluid flow rates at natural hydrothermal springs can be much more diffuse and the concentrations of precipitating reactants (*e.g.,* Fe and S in an early Earth system) could be an order of magnitude lower[4]; thus, structured precipitates would form over longer timescales and the vent could be active for tens of thousands of years[24,25].

Protocol

1. Safety Considerations

1. Use personal protective equipment (lab coat, goggles, nitrile gloves, proper shoes) to prevent against chemical spills or injury. Use syringes and needles, and take care to not puncture gloves. Take care during experiment setup to check the apparatus for leaks by performing the injection first with double distilled H_2O (ddH_2O), and to check the stability of the reaction vials on the stand, before adding chemicals.
2. Undertake this experiment with any chemical garden recipe, but one of the reactants we use to simulate deep-sea vents is a hazardous chemical, sodium sulfide; therefore do the entire experiment inside a fume hood to prevent exposure.
    1. Only open the bottle of sodium sulfide in the fume hood and place a balance inside the fume hood for weighing sulfide. Always keep sulfide-containing solutions inside the fume hood as they release toxic H_2S gas, and also keep sulfide liquid, sharps, and solid waste containers in the fume hood. Another reactant of interest is $Fe(II)Cl_2 \cdot 4H_2O$, which oxidizes upon exposure to air, so take care to keep solutions anoxic and to grow chemical gardens under an anoxic headspace (*e.g.,* N_2 or Ar), always within a fume hood or glove box.

2. Setup for Injection Experiments

1. Create glass “injection vials” by cutting off the bottom 1 cm of a 100 ml clear glass crimp top serum bottle (20 mm crimp seal closure type) with a glass cutter so that, when inverted, the vessel is open to the air. As these are reusable, clean the vials in a 1 M HCl acid bath O/N, and then rinse well with ddH_2O before a new experiment.
2. Prepare the injection vials (**Figure 1**).
    1. Collect a 20 mm septum, 20 mm aluminum crimp seal, and a 0.5-10 µl plastic pipette tip. Using a 16 G syringe needle, carefully puncture a hole through the center of the septum, then remove and discard the needle in the appropriate sharps waste container.
    2. Insert the pipette tip into the needle hole, into the side of the rubber septum that will face inside the crimp top of the vial. Push the pipette tip through the septum so that it pokes out the other side.
    3. Crimp-seal the septum with pipette tip onto the injection vessel to make a watertight seal. When sealed, push the pipette tip further through the septum so that it protrudes outside.
    4. Affix 1/16” inner diameter clear flexible chemical-resistant tubing to the pipette tip (tubing length should reach from the injection vial to the syringe pump); slide it up for a watertight seal.
    Note: This will be the injection tube, fed from the other end by a syringe with 16 G needle.
    5. Check for leaks: Insert a 10 ml syringe filled with ddH_2O with a 16 G needle into the other end of the tubing (smoothly slide the tubing straight onto the needle and be careful not to puncture the wall of the tubing). Slowly inject so that the ddH_2O moves up the tubing and into the bottom of the reaction vessel. Ensure that the syringe/tube, tube/tip, and crimp seals are watertight.
3. Clamp the injection vials on a stand in a fume hood, so that the injection will feed in from the bottom of the vial.
Note: Multiple vials can be set up at once and fed simultaneously by separate syringes.
4. Set up electrodes for measuring membrane potential across the wall of the chemical gardens. Always use the same convention for which lead is “inside” and which is “outside” of the chemical gardens.
    1. Cut lengths of insulated wire (*e.g.,* copper) that reach from inside the reaction vessels to the lead of the multimeter or data logger. Leave a little bit of slack in the wires for positioning.

2. Strip ~3 mm of the wire bare at the ends that will be located inside the reaction vial. At the other ends that will be connected to the multimeter leads, strip ~1 cm of wire.
3. Fix the wires in place to measure membrane potential across the chemical garden. For the wire that will go inside the chemical garden: insert it into the opening of the pipette tip from which fluid will feed into the vessel.
4. Push the wire in lightly to ensure contact with the injection solution, but not so far that it will clog the injection flow. For the outside wire: place it so that it will be in contact with the solution reservoir but not with the chemical garden precipitate.
5. Tape or otherwise secure the wires so that they cannot move inside the injection vial during the experiment (**Figure 2**).
6. Attach the other ends of the wires to the multimeter, and secure the wires so that those ends also do not move throughout the experiment.

5. Set up N_2 gas lines that will each feed into one of the injection vials.
   1. Split the gas feed from a N_2 source into several tubes, so that there is one N_2 feed for each injection vial.
   2. Place each N_2 tube so that it feeds into the headspace of one of the injection vials.

3. Preparation of Solutions for Chemical Garden Growth

1. Prepare the reservoir solution, 100 ml for each experiment. Note: In this example, use 75 mM Fe^{2+} and 25 mM Fe^{3+} as the precipitating cations (**Table 1**).
   1. Create anoxic solutions by first bubbling the ddH_2O with N_2 gas for ~15 min per 100 ml.
   2. Weigh out and add the $FeCl_2 \cdot 4H_2O$ and $FeCl_3 \cdot 6H_2O$, stirring gently to dissolve (not vigorously so as to not introduce oxygen).
   3. After reagents are dissolved, immediately resume light bubbling of the Fe^{2+}/Fe^{3+} solution with N_2 gas while injections are prepared.
2. Choose any two of the primary injection solutions shown in **Table 1**, and prepare 10 ml of each. Fill a 10 ml syringe to the 7 ml mark with each of the solutions (one syringe for each solution). Replace the needle caps and set aside.
3. Prepare 20 ml of the secondary injection solution (sodium sulfide — CAUTION) shown in **Table 1**. Fill two 10 ml syringes to the 7 ml mark with this solution, replace the needle caps and set aside. Always keep sulfide-containing solutions and syringes in the fume hood.
4. Refill the ddH_2O syringes from Step 2.2.5; these will be used to flush the injection tube.

4. Starting the Primary Injection

1. Use desired data logger for membrane potential measurements; measure each experiment's potential on a separate channel, and set the scan rate to give the desired amount of data points (*e.g.*, for a 2-hr injection, recording potential every 30 sec would be sufficient).
2. Secure the primary injection syringes on the programmable syringe pump in the fume hood.
3. Use a waste beaker to catch drips and set the syringe pump to inject at a fast rate until the syringes both begin to drip into the beaker. Then stop the injection (in order to ensure that the two syringes begin injecting at exactly the same level).
4. Re-program the syringe pump to inject at 2 ml per hour (calibrate for the type of syringe being used), but do not hit start.
5. Insert the ddH_2O syringes into the two plastic injection tubes, and inject so that the water fills the clear tubing up to the aperture where it enters the main reservoir. Place the syringes on the stand, above the injection vials.
6. Pour 100 ml of the Fe^{2+}/Fe^{3+} reservoir solution into each vial.
7. Adjust the flow of the N_2 gas lines as desired to keep the experiment anoxic for the duration of the injections.
8. Carefully cover the reservoir vials with an airtight seal (*e.g.*, using Parafilm; not obstructing the view through the glass) and insert an N_2 feed into each vial (**Figure 3**).
9. Bring the ddH_2O syringes (still inserted in the tubing) down next to the primary injection syringes. Carefully slide the plastic injection tubing off the ddH_2O syringe needle, and immediately transfer it directly onto one of the primary injection syringe needles. (Take care to not puncture the wall of the tubing.)
10. Start the injection, and start recording of membrane potential.

5. Starting the secondary injection:

1. Hit stop on the syringe pump after 3 hr (after 6 ml have been injected), once chemical garden structures have formed (**Figure 4**), continually generating a membrane potential (**Figure 5**).
2. Carefully remove the primary injection syringes from the syringe pump (but leave them connected to the tubing so the structures are not disturbed); set them on the stand above the level of the fluid in the vials so that the fluid cannot flow back into the syringe.
3. Secure the secondary injection sulfide syringes to the syringe pump, and repeat Steps 4.3 and 4.4.
4. Remove the secondary syringes one at a time from the syringe pump, and, while holding the syringes above the level of the fluid in the vials, repeat Step 4.9, transferring the tubing from the primary syringes to the secondary syringes (**Figure 6**). Be vigilant that the fluid pressure from the reservoir into the syringe does not cause fluid to flow back into the syringe as this could collapse the chemical garden.
5. When the transfer is complete, carefully secure the secondary syringes to the syringe pump.
6. Re-program the syringe pump to inject at 2 ml per hour, and hit start to continue the injection with the new injection solution.
7. Safely dispose of the primary injection syringes.

6. Ending the Experiment

1. First stop the syringe pump, then stop recording of the membrane potential and save the data.
2. Turn off the N_2 flow and remove the lines and the Parafilm from the injection vessels.
3. If desired, sample the reservoir solution or precipitate for further analysis. To carefully remove the reservoir solution and not disturb the precipitate, use a 25 ml pipette to carefully pipette off the reservoir solution in several aliquots, and discard the solution in a waste beaker.

4. Unclamp the injection vessels one at a time and pour the solution into a waste transfer beaker in the fume hood. Use ddH_2O to rinse out pieces of precipitate.
5. Remove the syringes from the syringe pump, and extract them from the tubing, letting extra injection fluid run off into the waste transfer beaker. Empty the syringes into the waste beaker, and dispose of the syringes in a sulfide sharps container kept in the fume hood.
6. Remove the tubing from the experiment vial and dispose of it in a solid waste bag. Uncrimp the seal and dispose of the septum, seal, and pipette tip.
7. Rinse out the glass experiment vial and soak it in a 1 M HCl acid bath O/N. (CAUTION — glassware that has been in contact with sodium sulfide will release toxic H_2S gas when placed in acid. Keep acid baths inside the fume hood.)

Representative Results

Once the injection solution started to feed into the reservoir solution, a chemical garden precipitate began to form at the fluid interface and this structure continued to grow over the course of the injection (**Figures 4-7**). In the experiments reported here, the first injection was sodium hydroxide (which can be modified to include L-alanine and/or pyrophosphate), and the reservoir solution was a 1:3 mixture of Fe^{3+}/Fe^{2+}, yielding a mixed-redox-state iron oxyhydroxide precipitate. The chemical gardens typically exhibited a dual colored morphology — some pieces of the precipitate were dark green (probably indicating a mixed oxyhydroxide) and other pieces were orange (probably indicating mainly a Fe^{3+}-oxyhydroxide/oxide). The iron oxyhydroxide chemical gardens were fairly robust structures and were often able to remain upright when the reservoir solution was removed from the vessel after injection (**Figure 8**). In precipitates containing only Fe-oxyhydroxide, the chemical gardens typically formed several branches; however when the hydrophobic amino acid alanine was included in the injection solution, the chemical gardens tended to form fewer branches or even a single column of precipitate. This inhibition of bursting and branching presumably indicates that the addition of alanine produces a more durable chemical garden wall[26]. Under environmental scanning electron microscopy (ESEM), the precipitates formed in the presence of alanine appeared more rounded and amorphous, whereas pure Fe-oxyhydroxide precipitates (as well as those containing pyrophosphate) appeared more crystalline (**Figure 9**). When pyrophosphate was included in the injection solution, a branched Fe-oxyhydroxide chemical garden formed, and additional green cloudy precipitate (likely iron pyrophosphate) formed and extended from the edges of the structure (**Figure 10**). This green plume precipitate was not part of the chemical garden, and when the reservoir solution was removed, the plumes collapsed and did not aggregate well to the main structure.

The membrane potential in chemical garden experiments was generated as soon as the chemical garden became visible (there was a lag time, as the injection solution traveled through the tubing). In experiments where the injection solution was NaOH, NaOH with alanine, or NaOH with pyrophosphate, the potential tended to peak immediately around 0.45 to 0.55 V and then decreased for about an hour before stabilizing around 0.1 to 0.2 V for the rest of the primary injection. (In experiments where the primary injection was NaOH + pyrophosphate + alanine, the voltage did not peak at the higher value of ~0.45 to 0.55; instead, it remained around ~0.2 for the entire primary injection.) There were differences in membrane potential in repeats of the same experiment (**Figure 11**), but the patterns observed were more or less consistent over four repeats of each injection chemistry.

When the primary syringes were switched to the secondary syringes containing sodium sulfide, the chemical garden continued to grow, except that visible new growths were now black iron sulfide. Rather than contributing to the existing walls, the black sulfide portions of the chemical garden appeared to branch off and grow separately. As soon as the sulfide injection solution reached the chemical garden, the membrane potential immediately jumped to ~0.9 V. The value of the potential reached during the secondary injection was the same for all experiments, regardless of the primary injection solution (**Figure 10**). This is because the potential in chemical garden experiments is mostly due to the chemistry between the two interfacing solutions, and since our secondary injection solutions were all 50 mM $Na_2S•9H_2O$ and the reservoir solution did not change, the voltages generated were similar.

We typically conducted four chemical garden experiments at once, using four reservoir bottles that were fed by four separate syringes and all driven at the same rate by the syringe pump. Using the same chemistry in all four duplicates, we often observed great variations in chemical garden structure (overall size, number of branches) as well as variations in membrane potential within a range of 0.1 – 0.2 V. This lack of reproducibility is to be expected in far-from-equilibrium experiments when so much depends on the intricacies of the initial conditions. It is likely that the random formation of structure in chemical gardens sometimes leads to precipitate membranes with varying permeability to ions; in some cases, the injection and reservoir solutions are probably better separated and thus the membrane potential is able to be maintained for a longer period.

We allowed the injections to continue at 2-3 ml/hr, and the experiment was terminated after 2 hr for the primary injection and an additional 3 hr for the secondary injection. During this time, the membrane potential remained at its characteristic value for whichever injection solution was being used. If injections were stopped, then the potential slowly decayed back to zero as the interior and exterior solutions equilibrated. (In iron-hydroxide systems, if the injection was stopped, the chimneys would sometimes dissolve over several days, due to the very low pH (~2) of the reservoir solution.)

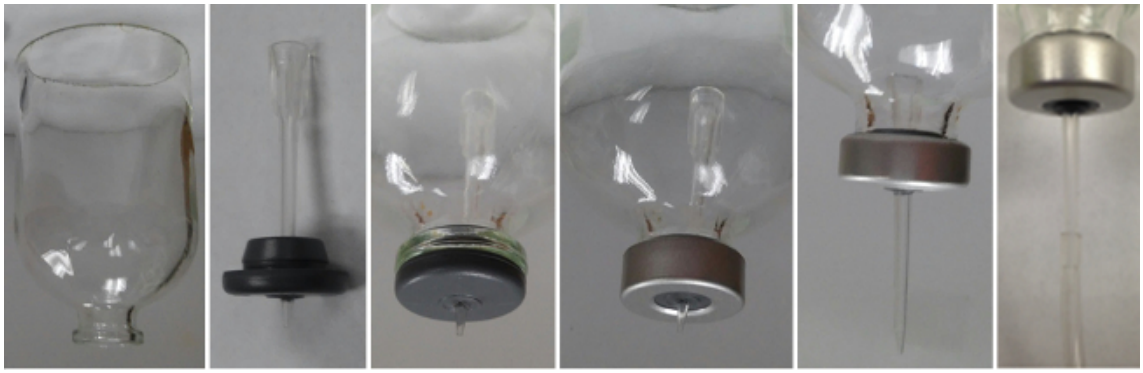

Figure 1. Preparing the reaction vessels. Reaction vessels for injection chemical garden experiments were made by cutting off the bottom of a 100 ml serum bottle, inserting a pipette tip through a septum which was then crimp sealed to the bottle, and attaching a tube through which to feed the injection solution. Please click here to view a larger version of this figure.

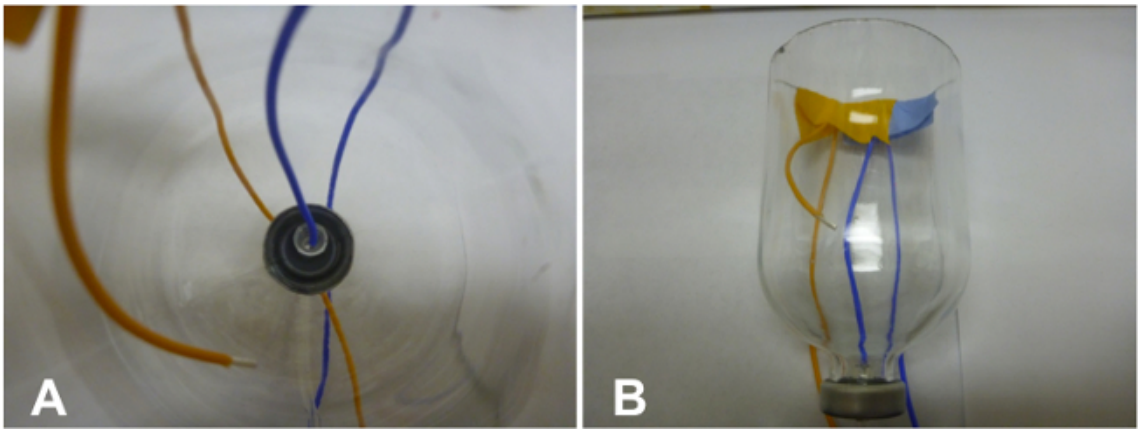

Figure 2. Placement of the wires in the chemical garden reaction vessel. (**A**) View from above, showing the placement of the "inner" electrode into the injection aperture. This wire was enveloped by the chemical garden when it began to grow. The "outer" electrode had to remain farther from the injection point so it was not touched by the growing chemical garden. (**B**) Secure the wires with tape so that they do not move throughout the experiment. Please click here to view a larger version of this figure.

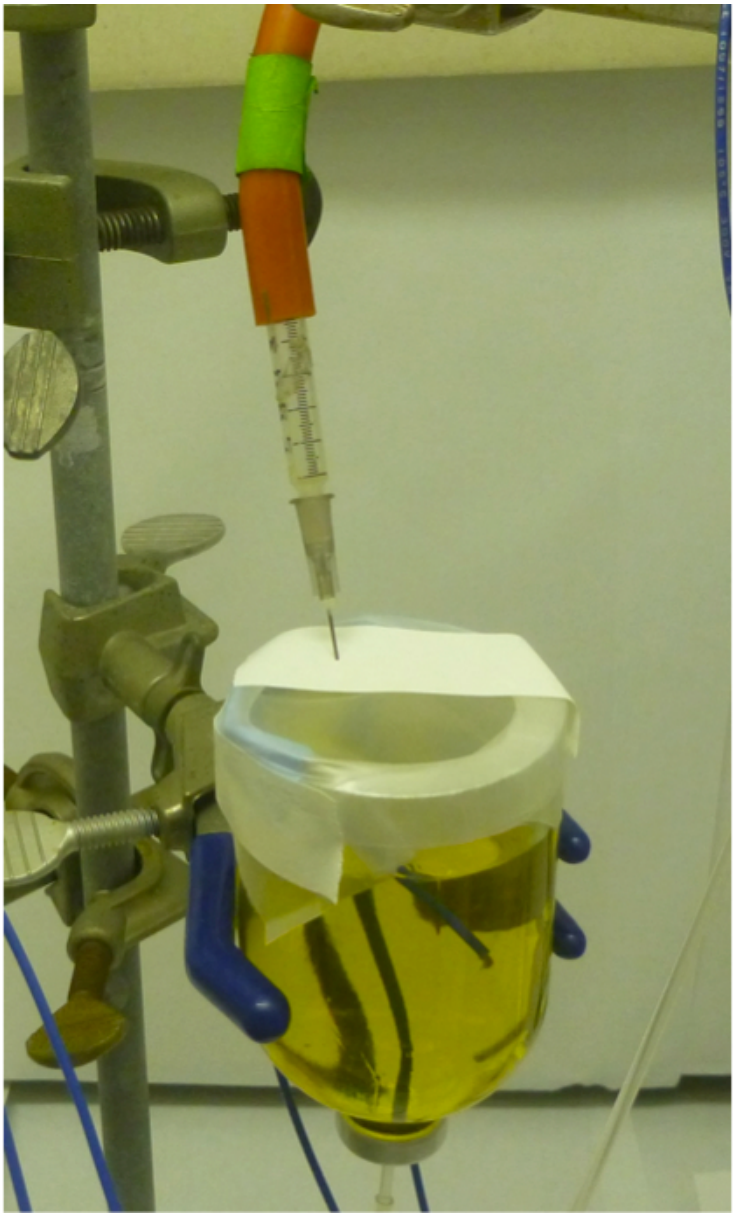

Figure 3. Creating the N_2 headspace. After the reservoir solution was added, an airtight seal was formed over the top of the vessel with Parafilm (covering the electrodes as well), and then a light N_2 feed was inserted to maintain anoxic conditions throughout the chemical garden growth. Please click here to view a larger version of this figure.

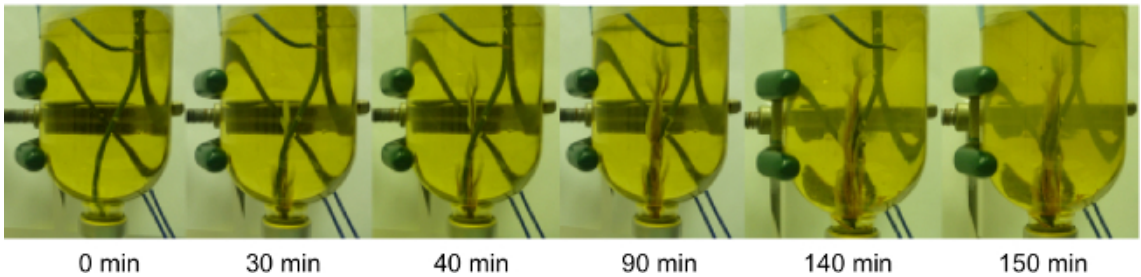

Figure 4. Time-lapse growth of a chemical garden. This experiment contained 75 mM $FeCl_2$•$4H_2O$ and 25 mM $FeCl_3$•$6H_2O$ in the reservoir solution. The first injection was 0.1 M NaOH + 10 mM $K_2P_4O_7$, and after 180 min the injection was switched to 50 mM Na_2S•$9H_2O$. Please click here to view a larger version of this figure.

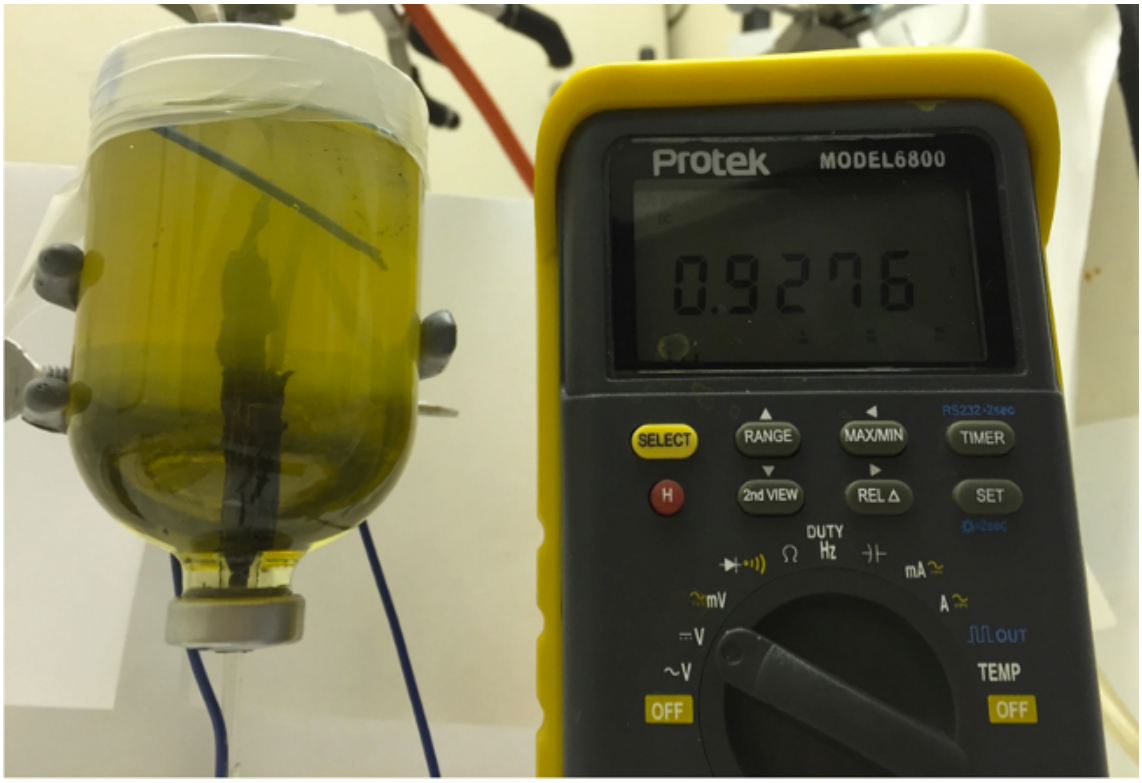

Figure 5. Membrane potentials. Membrane potential was generated as a chemical garden grew around the interior electrode. After the primary injection of hydroxide that first formed the precipitate structure, the syringe was switched with a syringe of sodium sulfide solution. In this experiment the reservoir solution was 75 mM $FeCl_2$•$4H_2O$ + 25 mM $FeCl_3$•$6H_2O$, the primary injection was 0.1 M NaOH, and the secondary injection was 50 mM Na_2S•$9H_2O$. Please click here to view a larger version of this figure.

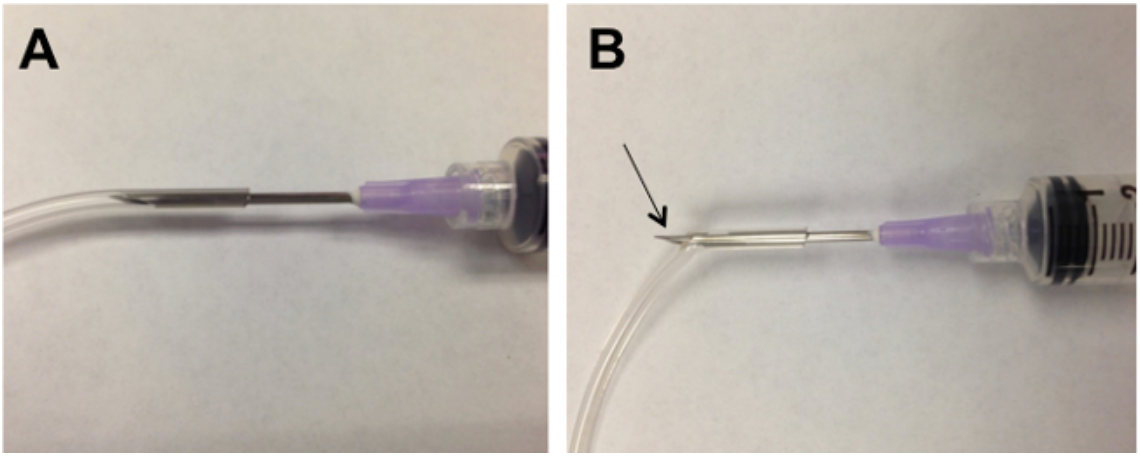

Figure 6. Syringe. (**A**) Correct insertion of the syringe needle into the flexible plastic tubing. Care must be taken not to puncture the tubing — example of incorrect insertion is shown in (**B**). Please click here to view a larger version of this figure.

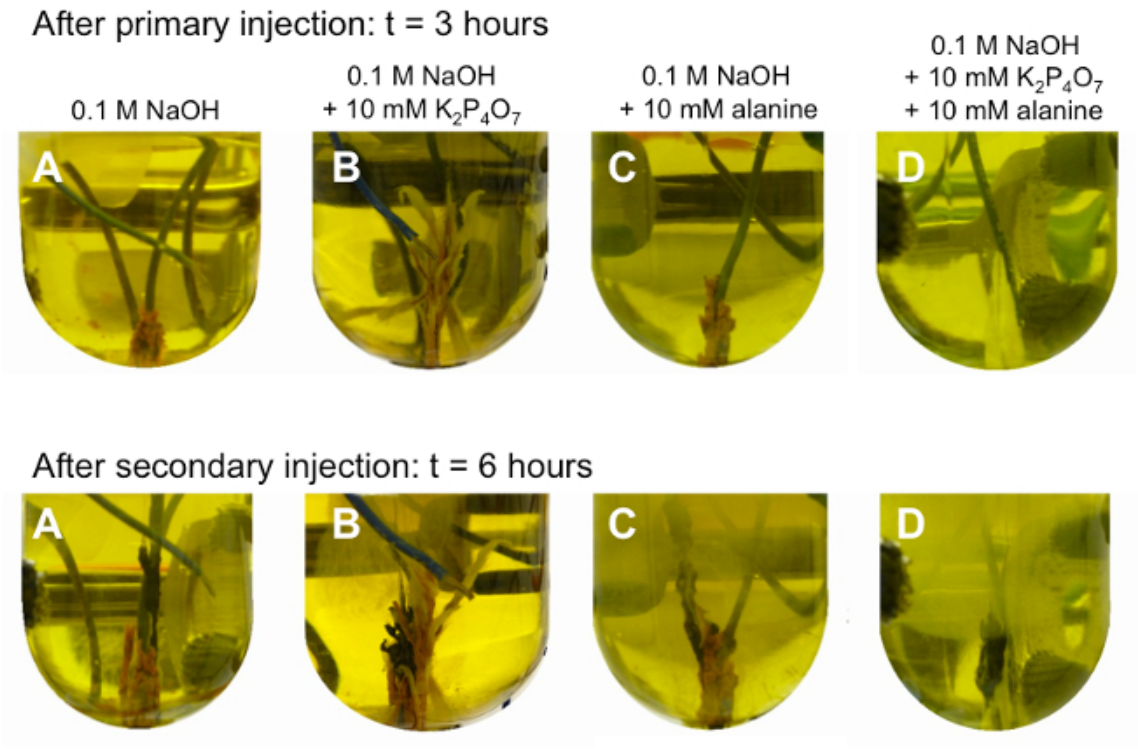

Figure 7. Chemical gardens after primary and secondary injection. Chemical gardens grown in reservoir solutions of 75 mM $FeCl_2$•$4H_2O$ + 25 mM $FeCl_3$•$6H_2O$, shown after the primary injection of 0.1 M NaOH (plus the additives of alanine and/or $K_2P_4O_7$ listed in **Table 1**) and after the secondary injection of 50 mM Na_2S•$9H_2O$. Please click here to view a larger version of this figure.

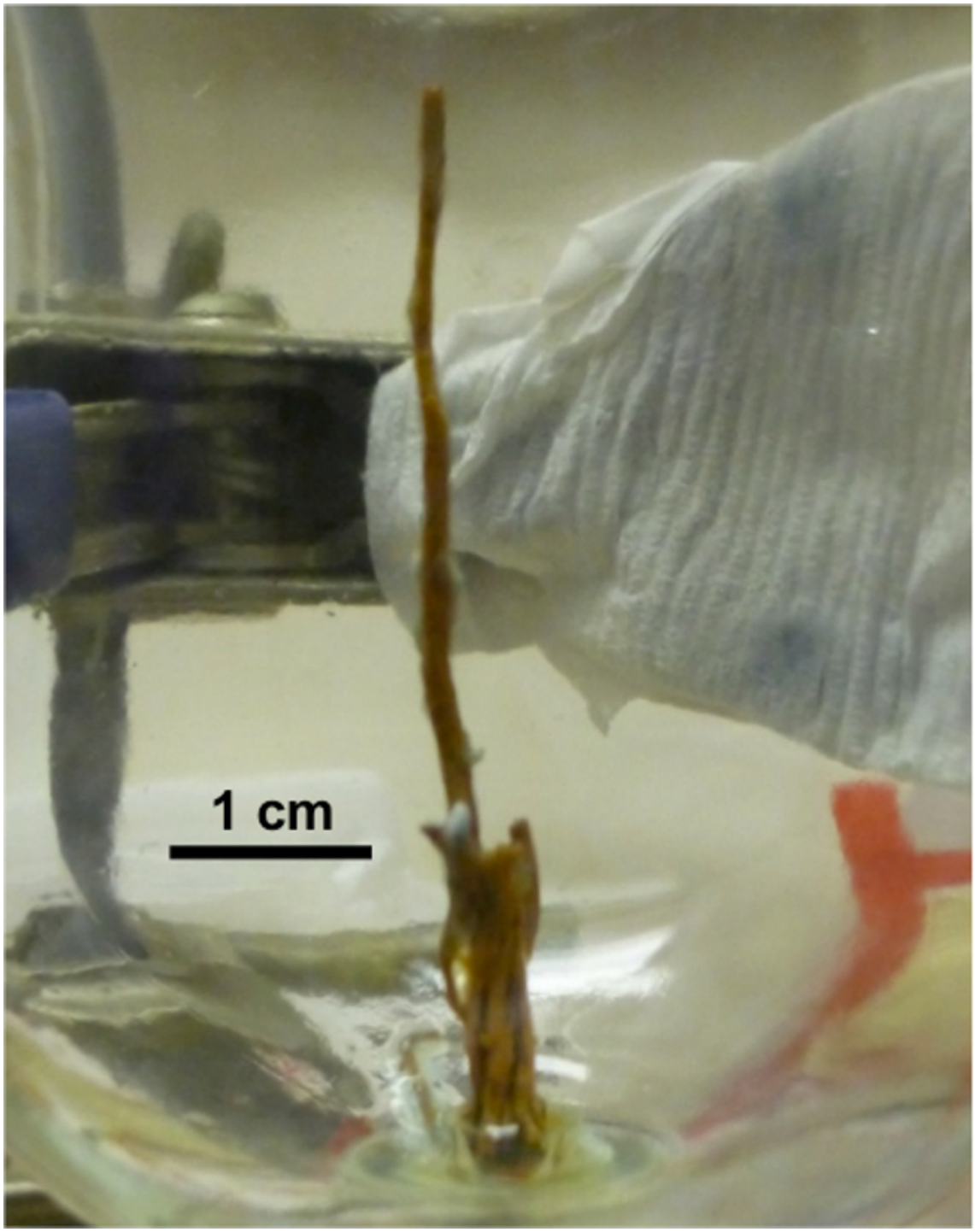

Figure 8. Precipitate stability. Fe(II/III)-hydroxide chemical gardens sometimes can maintain structural stability after the reservoir solution is carefully removed. The precipitate can then be sampled for further analysis if desired. Please click here to view a larger version of this figure.

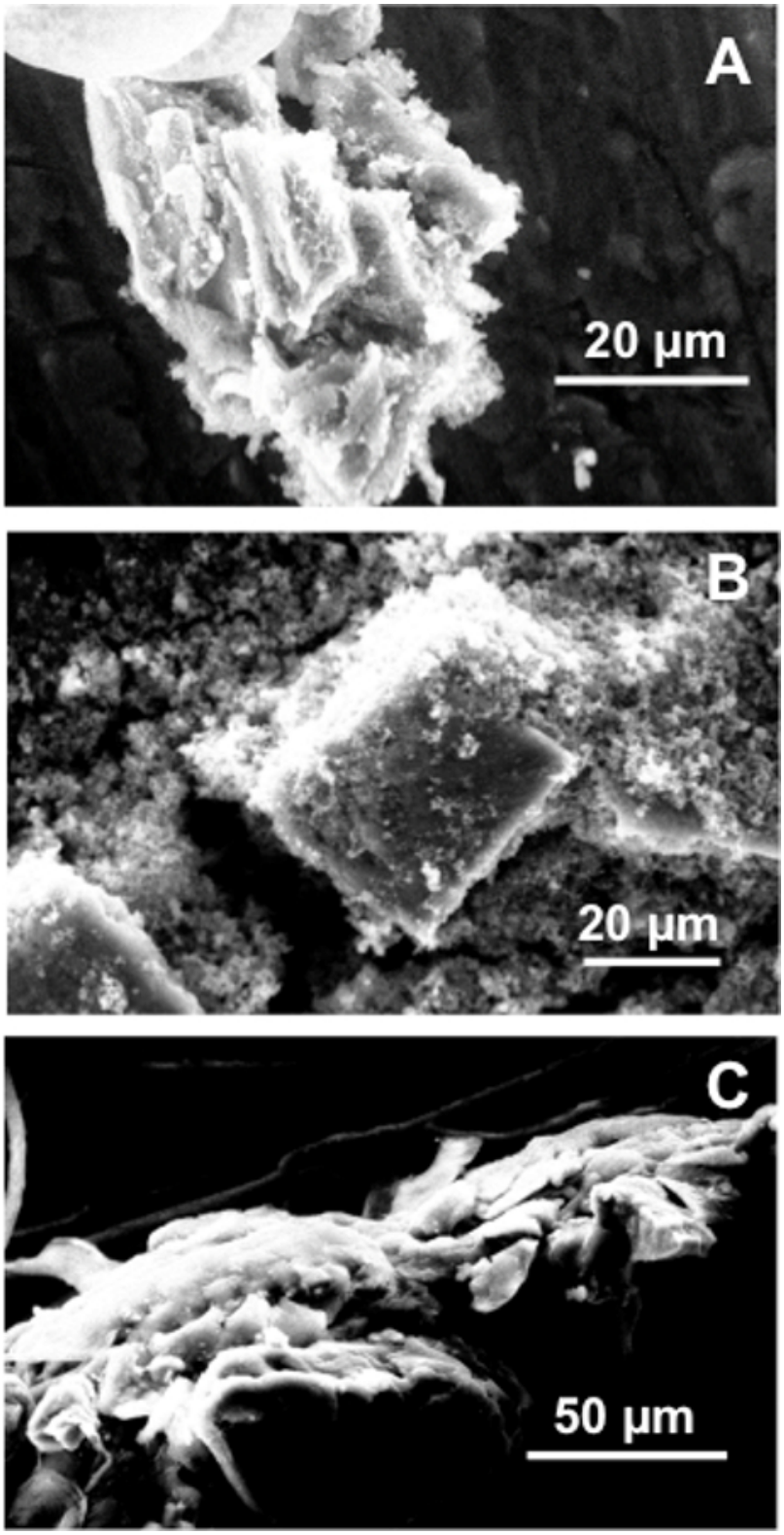

Figure 9. Environmental Scanning Electron Microscopy imaging. (**A**) Fe(II/III)-hydroxide chemical gardens, (**B**) Fe(II/III)-hydroxide chemical gardens containing $K_2P_4O_7$, and (**C**) Fe(II/III)-hydroxide chemical gardens containing alanine. All images are of chemical gardens after the primary injection only. The precipitates that incorporated alanine appeared rounded and less crystalline than the precipitates of only Fe(II/III)-hydroxide and Fe(II/III)-hydroxide containing $K_2P_4O_7$. Please click here to view a larger version of this figure.

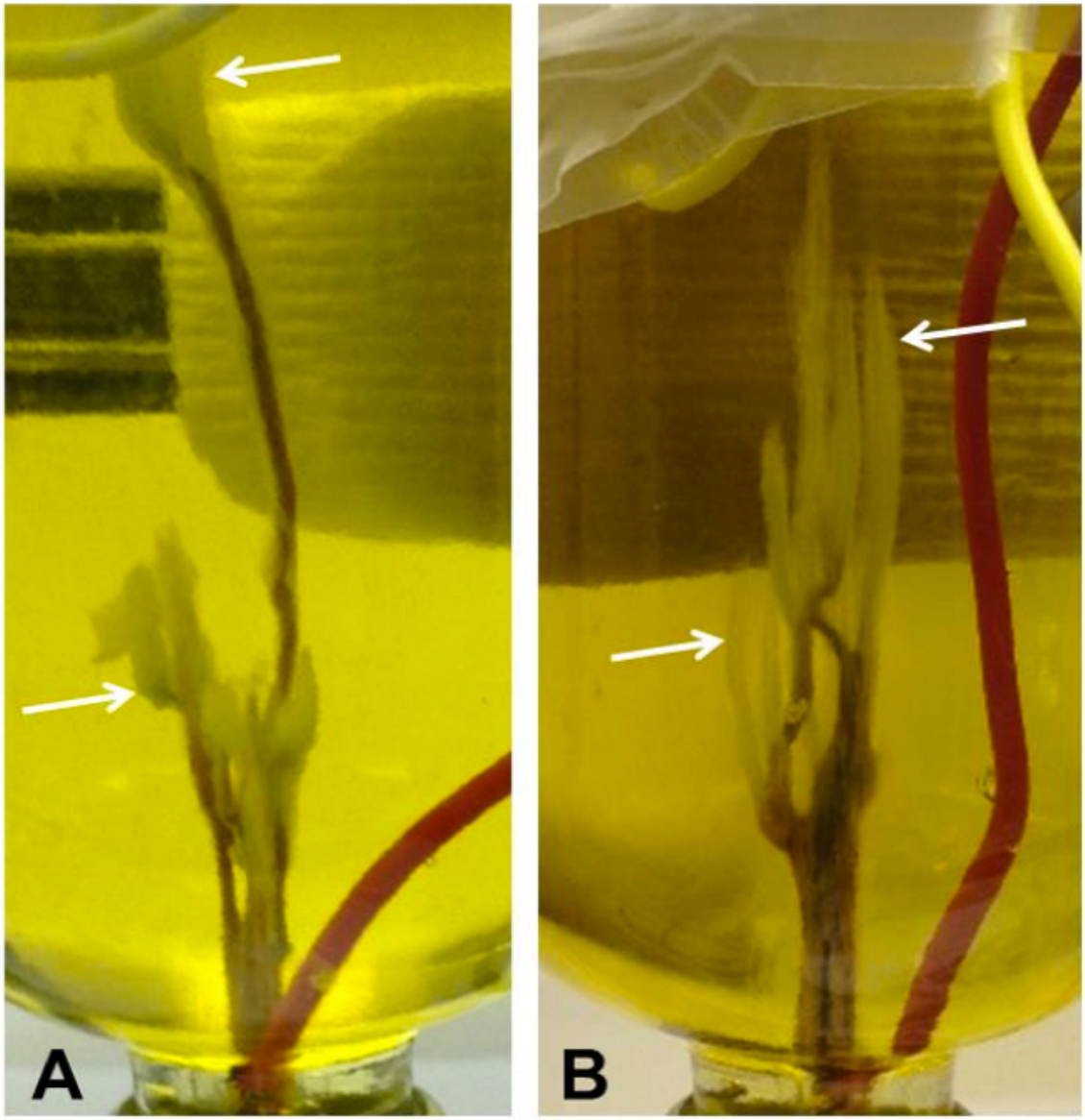

Figure 10. **Chemical gardens grown in a reservoir solution of 75 mM $FeCl_2$•$4H_2O$ + 25 mM $FeCl_3$•$6H_2O$.** (**A**) Injection solution contained 0.1 M NaOH + 10 mM $K_2P_4O_7$. (**B**) Injection solution contained 0.1 M NaOH + 10 mM $K_2P_4O_7$ + 10 mM alanine. In chemical gardens where the injection solution contained $K_2P_4O_7$, green precipitate plumes (arrows) formed near the solid precipitate branches, but these plumes were not fully aggregated to the main structure and collapsed when the reservoir solution was removed. Please click here to view a larger version of this figure.

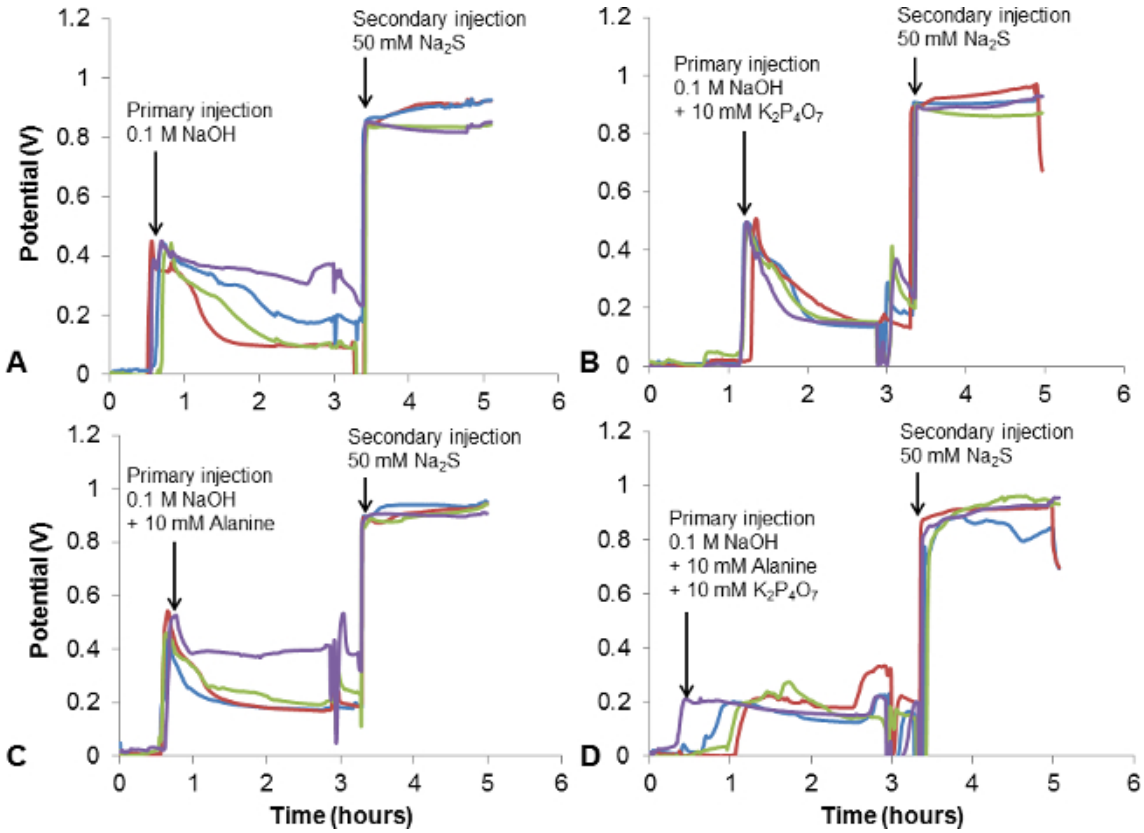

Figure 11. **Membrane potential generated by chemical gardens grown in reservoir solutions of 75 mM $FeCl_2$•$4H_2O$ + 25 mM $FeCl_3$•$6H_2O$.** Four repeats of each experiment are shown. The potential was generated as soon as the injection solution traveled up the tube and contacted the reservoir solution to produce a precipitate structure enveloping the inner electrode. The structure continued to grow as the primary injection proceeded. When the syringe was swapped to sodium sulfide solution and the secondary injection began (arrows), the potential increased to 0.9 - 1.0 V. Please click here to view a larger version of this figure.

Reservoir Solution (100 ml)	Primary Injection (6 ml)	Primary Injection Rate	V_{1max} (avg)	Secondary Injection (6 ml)	Secondary Injection Rate	V_{2max} (avg)
75 mM $FeCl_2$•$4H_2O$ + 25 mM $FeCl_3$•$6H_2O$	0.1 M NaOH	3 ml/hr	0.431 V, σ=0.002	50 mM Na_2S•$9H_2O$	2 ml/hr	0.881 V, σ=0.047
	0.1 M NaOH + 10 mM $K_4P_2O_7$	3 ml/hr	0.473 V, σ=0.016		2 ml/hr	0.914 V, σ=0.040
	0.1 M NaOH + 10 mM alanine	3 ml/hr	0.485 V, σ=0.044		2 ml/hr	0.929 V, σ=0.015
	0.1 M NaOH + 10 mM $K_4P_2O_7$ + 10 mM alanine	3 ml/hr	0.239 V, σ=0.061		2 ml/hr	0.923 V, σ=0.033

Table 1. Voltages generated by chemical gardens generated by slowly injecting first a primary, then a secondary, solution into a reservoir. V_{1max} (avg) and V_{2max} (avg) are the averages of the highest voltages produced during the primary and secondary injections, respectively; σ is the standard deviation.

Discussion

The formation of a chemical garden structure via injection method can be accomplished by interfacing any two solutions containing reactive ions that produce a precipitate. There are many possible reaction systems that will produce precipitate structures and finding the right recipe of reactive ions and concentrations to grow a desired structure is a matter of trial and error. The flow rate of the injection solution is controlled by a programmable syringe pump and this can also be varied between experiments to simulate different rates of fluid flow in a natural system. The structure of chemical gardens is dependent on many factors, including composition and flow rate, and it is possible to grow structures in as little as a few hours and over longer periods of days to weeks. One can also add other trace components of interest into the injection or reservoir solution, such as organic molecules or other components thought to be geologically or biologically relevant[27,28]. Depending on the chemistry, these components may be incorporated into the precipitate and/or undergo reactions.

There are various methods that have been utilized in previous work for growing chemical garden precipitates, including direct growth from dissolving crystals or 'pellets'[18,29] and injection experiments like those featured here[30,31]. To design a chemical garden experiment where it

is possible to reliably measure the membrane potential, one must create some way of completely enveloping the "interior" wire within the precipitate membrane throughout the entire experiment. This is difficult (though not impossible[14]) to accomplish in crystal growth experiments. In previous injection experiments[13], we have generally observed that the wire must be placed directly into the injection point, otherwise the chemical garden often "avoids" the wire as it grows, thus leaving both wires in the reservoir solution and no membrane potential can be measured. Chemical gardens grown via injection vary in structural stability depending on the chemical reactant system(s) used — *e.g.*, iron-silicate or iron-hydroxide systems give more robust structures that remain standing when the reservoir fluid is decanted, whereas pure iron-sulfide systems tend to give a much more gelatinous, delicate precipitate that readily collapses if the solution is disturbed. A collapse of the chemical garden or any significant breaking of the membrane will cause immediate effects in the membrane potential, as the unequal distributions of charged species across the membrane bleed out. Thus, in this type of experiment, it is very important that the wires are carefully secured prior to injection so that they will not move as the chemical garden grows, and that the experimental / injection setup is stable and not jostled during growth.

Because following the injection of the fluid flowing into the reservoir is instructive, flexible transparent Tygon tubing is recommended over other possibilities such as stainless steel. The clear tubing allows for observation of precipitate particles forming within the tubing, allows one to dislodge clogs, and permits detection / removal of air bubbles. The downside of this tubing is that it can be easily punctured by the syringe needle (**Figure 6**). We experimented with switching syringes by inserting the second needle directly into the tubing from the side ahead of the first injection, rather than actually moving the tubing from one syringe to another, but this technique was very difficult to accomplish without puncturing. Another benefit of the Tygon tubing is that, in case of accidental puncture while inserting a needle, one can simply cut the punctured part of the tube off and re-insert the needle.

The growth of the membrane is directed by the buoyancy and, to a lesser degree, the pressure of injection. A drastic change in the injection pressure can cause a collapse of the chemical garden, especially in systems that do not produce robust precipitates. When switching syringes, it is important to hold the syringe being removed *at, or just above,* the fluid level to prevent flow back and the likely disaggregation. Such an event could be also be avoided by setting up the experiment such that the syringe pump is at the approximate level of the reservoirs. It makes little difference to the membrane potential data if the experiment "pauses" for a length of time while switching syringes, so long as the chemical garden remains undisturbed. Thus it is recommended to switch syringes carefully, one at a time, and secure the syringe that is holding the internal pressure of the chemical garden so that it cannot "flow back", before moving on to the next. The injection rate should be kept fairly constant between the first and second injections, and in general should not be too fast (minimum experiment time ~ several hours), since excess injection pressure will rupture the membrane.

This experiment is versatile in that it allows for investigation of self-assembling precipitate growth in a variety of reaction systems, including those in which one or more reactants are present in the same solution. The swapping of syringes allows for the possibility of growing a stable chemical garden using one reaction chemistry, then using that structure as a "chemical reactor" for a second component passing through. For example, if one wanted to investigate whether organic molecules can become absorbed and/or react within a hydrothermal chimney composed of iron minerals[26], one could grow a chemical garden of relevant inorganic components and then feed through a second syringe of solution containing, for example, nucleotides, amino acids, peptides, or RNA[28]. This would have the effect of adsorbing and absorbing the organic components into the precipitate rather than them dissipating into the reservoir. In our experiments, we observed that the secondary injection caused iron sulfide chimneys to grow on top of the existing iron hydroxide chimneys, presumably through ruptures in the original membrane due to fluid pressure. Thus, the interiors of the different chimneys could be at least somewhat connected and the sections of different minerals in the membrane might serve different functions in an origin-of-life scenario, for example, metal sulfides oxidizing hydrothermal H_2 / reducing oceanic CO_2[32,33] and iron oxyhydroxides driving phosphate reactions and reducing nitrate to ammonium on site[5,34,35]. Materials science investigations can be conducted using this type of experiment as well; for example, deliberately forming chemical gardens of catalytic components (*e.g.,* aluminosilicates) and then feeding other components (*e.g.,* organic molecules or phosphates) through them to react. One could also explore forming layered materials by alternating syringes to produce different inorganic precipitates (as in Roszol and Steinbock 2011[23]). It is a simple matter to keep the individual reaction vessels under anaerobic conditions or any desired gas headspace during chemical garden formation.

The limitations of this type of experiment are mostly due to the fact that chemical garden structures in systems driven by inflation, buoyancy and convection are very difficult to control. The precipitate structures can be fragile and difficult to remove and analyze after the experiment. Additionally, since the growth of the chemical garden is always unpredictable, in order to ensure measurement of membrane potential, the "outer" wire in the reservoir must be distanced from the injection point, to prevent the chemical garden enveloping both wires. However, taking this precaution means that the wires are usually not ideally close to the membrane. Instead, precise inorganic membrane potential measurements can be achieved by growing the membrane on a parchment paper template between the two solutions[36]. In chemical garden experiments it is not usually possible to sample and/or otherwise measure (*e.g.,* pH) the interior solution; detailed real-time analysis can only be done on the reservoir solution.

Natural vents also would host thermal gradients between the heated hydrothermal fluid (~70-100 °C) and the ocean[4], and so to simulate hydrothermal systems it may be desirable to grow the chemical garden at a higher temperature and pressure[37], which poses challenges with the setup described here. It would be possible to wrap the reservoir bottle in a heating coil in order to regulate the temperature before starting; however, a different type of pump might be necessary in order to similarly heat the injection solution. To simulate a natural system, it might be necessary to include dissolved gases (*e.g.,* CO_2) in either solution; while this might be easier to accomplish within the reservoir (ocean simulant), it would require more careful preparation for the injection (hydrothermal simulant). In deep-sea systems, the high pressure could affect chimney growth and chemistry, and, depending on the experiment, increasing gas pressure in both fluids could have a significant effect (*e.g.*, dissolved CO_2 could result in iron carbonate precipitation in the chemical garden, also dependent on hydrostatic pressure[6]). Incorporating increased temperature and pressure in chemical garden experiments of this type would lead to many interesting possibilities, since temperature and pressure affect the solubility, precipitation, and specific properties of many minerals.

Disclosures

The authors declare that they have no competing financial interests.

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