

Research Paper

An Inexpensive Apparatus for Growing Photosynthetic Microorganisms in Exotic Atmospheres

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ABSTRACT

Given the need for a light source, cyanobacteria and other photosynthetic microorganisms can be difficult and expensive to grow in large quantities. Lighted growth chambers and incubators typically cost 50–100% more than standard microbiological incubators. Self-shading of cells in liquid cultures prevents the growth of dense suspensions. Growing liquid cultures on a shaker table or lighted shaker incubator achieves greater cell densities, but adds considerably to the cost. For experiments in which gases other than air are required, the cost for conventional incubators increases even more. We describe an apparatus for growing photosynthetic organisms in exotic atmospheres that can be built relatively inexpensively (approximately \$100 U.S.) using parts available from typical hardware or department stores (*e.g.*, Wal-mart or K-mart). The apparatus uses microfiltered air (or other gases) to aerate, agitate, and mix liquid cultures, thus achieving very high cell densities ($A_{750} > 3$). Because gases are delivered to individual culture tubes, a variety of gas mixes can be used without the need for enclosed chambers. The apparatus works with liquid cultures of unicellular and filamentous species, and also works with agar slants. **Key Words:** Cyanobacteria—Algae—Artificial atmospheres—Photosynthesis—Equipment. *Astrobiology* 5, 75–82.

INTRODUCTION

ACCORDING TO NASA Astrobiology Roadmap (Des Marais *et al.*, 2003), one of the primary goals of astrobiological research is to understand the nature and evolution of life on the early Earth. A key difference between present-day Earth and Earth of 3 billion years ago is the composition of the atmosphere. Early Earth probably had a mildly reducing atmosphere primarily consisting of N_2 and CO_2 , possibly with

significant amounts of CH_4 (Kasting, 1993, 1995, 2004; Rye *et al.*, 1995; Hessler *et al.*, 2004; Ohmoto *et al.*, 2004). Re-creating these conditions in the lab can be difficult and costly. However, in our work with cyanobacteria (Martin *et al.*, 1997; Thomas *et al.*, 1998a, 1999, 2001, 2005), we have developed a relatively inexpensive apparatus for growing cyanobacteria and other photosynthetic microorganisms in artificial atmospheres. The basic version of this apparatus can be built for less than \$100 (U.S.).

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In our apparatus, cyanobacteria are grown in liquid culture through which atmospheric gases are continuously bubbled. Waste gases, such as O_2 , are blown out of the tubes by the incoming gases, thus preventing changes in the atmospheric composition due to cellular metabolism. For example, the buildup of O_2 in sealed containers quickly inhibits the growth of a *sodB*⁻ strain of *Synechococcus*. We have shown that we can obtain faster growth and higher yields with this apparatus than with screw-cap slant cultures (for additional information on this mutant, see Samson *et al.*, 1994; Herbert *et al.*, 1992, 1995; Thomas *et al.*, 1998a, 1999). For routine culture growth, the cultures can be bubbled with air. Experimental atmospheres can be mixed on site using calibrated flow meters, or gases can be custom-bottled by commercial suppliers.

Our apparatus is an improvement on the original aquarium-based design once used in David Fork's lab at the Carnegie Foundation in Stanford, CA. We have made several modifications, the most important of which is a new design for air delivery in the culture tubes. The original design used rubber stoppers to close the culture tubes. Air was delivered through a long "airstem" and bubbled through the culture. The air was then exhausted from the culture tube via a bent glass tube (Fig. 1). Theoretically, as long as positive pressure was maintained at the airstem, contaminants were kept out. However, many users of this design (Laudenbach *et al.*, 1990; Herbert *et al.*, 1992, 1995; Samson *et al.*, 1994; Thomas *et al.*, 1998a) found that contaminants could be introduced via the stopper itself. Also, repeated autoclaving caused deterioration of the rubber stoppers.

To help prevent contamination, we redesigned the airstem on the culture tubes. The rubber stopper was replaced with a standard polypropylene slip closure. The airstem tube, also made of polypropylene, passes through the center of the closure and is welded to it. Exhaust air exits the culture tube via the closure—no exhaust tube is needed. Since the closure fits over the culture tube instead of inside it, the chance of contamination via the closure is greatly reduced. Also, because the closure and airstem are both made of polypropylene, they withstand repeated sterilization without deterioration.

The heart of the incubator, or "culture tank," is a common 10-gallon aquarium (Fig. 2). The aquarium is topped with a high density polyethylene cover in which holes have been bored for

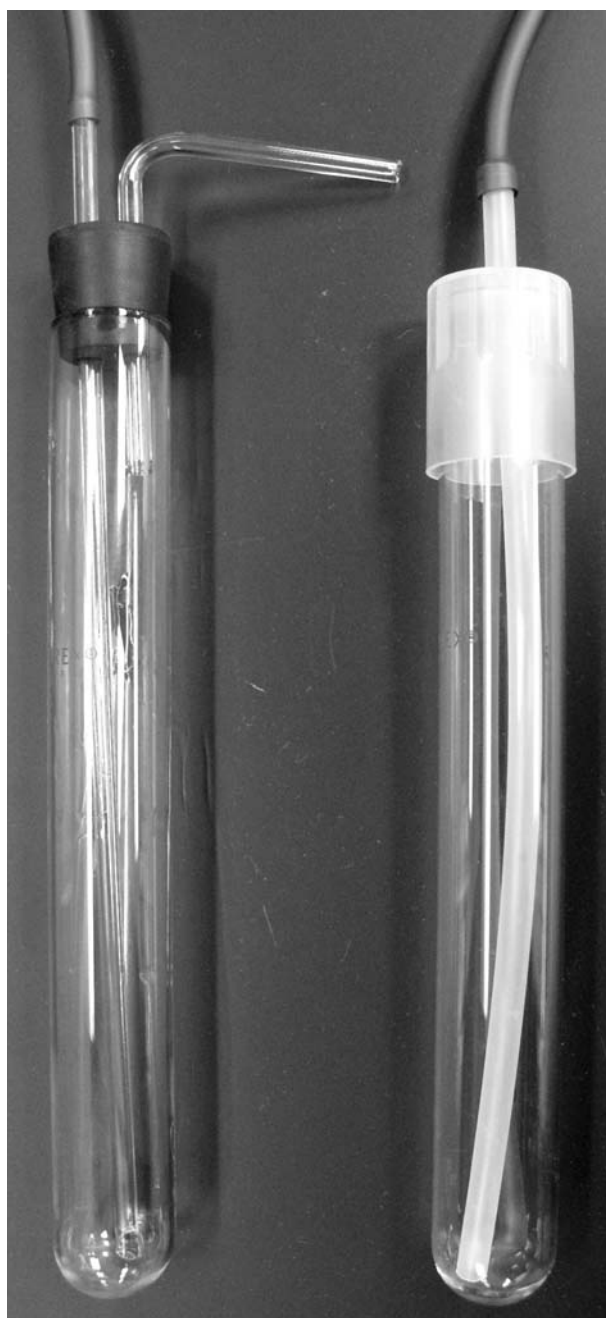


FIG. 1. Comparison of old and new airstems in culture tubes. The old style culture tube (left) delivered gas through a long glass tube, and then vented through a short, bent tube. The rubber stopper had a tendency to introduce contaminants as it was inserted, and also deteriorated after repeated autoclaving. The new design (right) is entirely made of polypropylene, and withstands repeated autoclaving. The gas vents through the rim of the closure, which helps prevent contamination.

placement of the culture tubes. In the original design, a Plexiglas® cover (available at local glass shops) was used. Over time though, the Plexiglas absorbed water on the interior side, causing the



FIG. 2. Fully assembled culture tanks made from 10-gallon aquaria. Each tank supports 16 culture tubes. Lighting is provided by standard 24" cool-white fluorescent tubes. The black objects on the fronts are digital thermometers that record high and low temperatures and produce an audible alarm when the temperature exceeds a set range. As shown, each tank cost \$100–\$150 (U.S.) to construct.

cover to warp and crack. Polyethylene does not suffer from this problem. A 100-W heater and a submersible pump maintain constant temperature throughout the tank. Fluorescent tubes provide illumination from the sides of the tank, and gases are distributed to the individual culture tubes via standard aquarium gang valves.

CONSTRUCTION

Our apparatus consists of three functional parts: individual culture tubes with airstems, a growth chamber based upon a 10-gallon aquarium, and a gas delivery manifold. Most of the parts can be purchased at a hardware store or discount store. Here, we describe the construction of a culture tank for use with 25- × 200-mm culture tubes. Other sizes of culture tubes may be used with minor modifications to the design.

Airstems

Drill holes in polypropylene tube closures that are the same diameter as the outside diameter of

the tubing to be used. Polypropylene tubing with an outer diameter of 1/4" is typically used by D.J.T. for the airstems. Place the drilled closure on a culture tube and then insert the polypropylene tubing through the closure. Leave an approximately 5-mm gap between the end of the tubing and the bottom of the culture tube. Leave approximately 7 cm of tubing on the outside of the closure. Weld the tubing to the closure.

The welding can be done in two ways. One way is to use a commercial plastic welding kit (*e.g.*, Chicago Electric hot air welder, Harbor Freight Tools, Camarillo, CA). While welding, a steel rod (*e.g.*, small Phillips screwdriver) is inserted into the airstem from the top of the closure to prevent the tube from collapsing. The region around the insertion point is heated until the plastic softens, and then the ends of the airstem are pushed slightly inward (Fig. 3). After the plastic cools, the steel rod can be removed. The other way to weld the airstem is to use high-temperature glue in a hot-melt glue gun. The glue may soften during autoclaving, but as long as the airstems are kept upright, they can be welded without any additional concerns. Alternatively, a piece of poly-

FIG. 3. Airstem weld using a commercial plastic welder. A plastic welder was used to partially melt the polypropylene tube and the top of the closure. While soft, pressure was applied to both ends of the tube in order bond it to the closure. Some distortion of the closure may occur, but this does not interfere with proper function.



propylene rod or tubing can be inserted into the glue gun in place of the glue. The molten polypropylene does not flow as well as the glue does, but with practice one can form a good plastic weld with it (Fig. 4). After the weld is completely cooled, attach a 15-cm piece of flexible, autoclavable tubing (PVC or silicone rubber) to the outside end of the airstem. This will be used to connect the culture tube to the air manifold (Fig. 5).

Culture tank

The actual dimensions of the tank cover will vary somewhat, according to the dimensions of the aquarium. Cut a piece of $\frac{1}{4}$ " or thicker poly-

ethylene or polypropylene slightly smaller than the dimensions of the top of the aquarium so that the cover rests on the inside lip of the aquarium trim. Plastic kitchen cutting boards are an inexpensive source of the polyethylene. If necessary, the cover can be constructed in two halves that rest side-by-side on the aquarium. Bore a row of equidistant holes along the long edges of the cover using a spade bit in a hand-held electric drill (a drill press could be used as well). We used a 1" spade bit to drill the 25-mm-diameter holes for the culture tubes used in our labs (Fig. 6). Hole saws should not be used since they generate too much heat. Leave a space of at least one tube di-



FIG. 4. Airstem weld using hot glue gun. A hot-melt glue gun was used to make this weld. A piece of polypropylene rod was used in place of the normal glue stick. This method is less expensive, and produces a neater weld. However, the weld usually is not as strong as one made with a plastic welder.



FIG. 5. Culture tubes attached to the gas manifold. Air or other gas is delivered via standard PVC aquarium tubing. A T-connector is used to split the delivery line into two sections, which are then joined at the front, forming a loop. Note the “rubber bands” below each closure, which prevent the culture tubes from falling through the cover. These were cut from $\frac{3}{4}$ ” diameter surgical tubing. The black line in the foreground is a thermometer probe cable.



FIG. 6. Drilling a hole in the tank cover. A 1” spade was used to make holes in the polyethylene cover. In this case, the holes are just large enough for 25-mm-diameter culture tubes. Forstner bits or large twist bits would probably work as well, but hole saws generate too much heat, causing the plastic to melt instead of cutting through it.

ameter between adjacent holes and between the holes and the edges of the cover. Cut a notch at one end of the cover to allow passage of the pump and heater cords.

An optional divider can be suspended from the cover to prevent stray light from illuminating the backs of the culture tubes (not needed for routine growth). Using a router, cut a groove along the center of the cover that is slightly wider than the thickness of the divider and half as deep as the thickness of the cover. Attach the divider to the cover with stainless steel wood or sheet metal screws (#6 or smaller).

A submersible heater can be inserted through one of the culture tube holes. Alternatively, a smaller heater can be attached to the end of the aquarium with suction cups. In either case, the heating element and thermostat must be covered by water when the aquarium is filled. The thermostat should be accurate to within $\pm 1^\circ\text{C}$. A submersible circulating pump can be attached to one end of the aquarium with suction cups or simply placed on the bottom of the tank. The circulating pump does not have to be large; an aquarium "powerhead" pump is more than adequate.

After installation of the pump and heater, fill the aquarium with distilled or deionized water. Leave approximately 2 cm at the top for expansion when the culture tubes are added. *Do not turn on the heater until the aquarium is filled.* For a 10-gallon aquarium, 10 ml of either 1 M CuSO_4 or 50% benzalkonium chloride can be added to the water to prevent incidental microbial growth in the tank. Under "normal" usage, water should

be added to the tank as needed to compensate for evaporation. The entire tank should be emptied, cleaned, and refilled every 3 months or so. Keep this in mind when choosing culture tank locations.

Place an 18" or 24" single-tube fluorescent light fixture on each side of the tank to provide illumination. The 18" fixtures are usually much less expensive and are adequate for routine culture growth. However, the 24" fixtures provide more uniform lighting, which is necessary for most experimental applications.

Gas delivery manifold

At minimum, the gas supply consists of an air pump, a microfilter, and manifold valves. For a tank with 16 culture tubes, the air pump should move at least 2 L of air/min. Most small- to medium-size aquarium pumps are adequate. Connect a 0.2–0.45- μm (pore size) inline HEPA filter to the output of the pump. The filtered air is delivered to the culture tubes via a manifold made from inline, four-gang, chrome-plated brass aquarium air valves (Fig. 7). Brass valves are used because they can be autoclaved. Connect tubing together with standard aquarium fittings or specialty hose barb fittings.

Optionally, an activated carbon filter tube and a humidifying flask can be added inline before the HEPA filter. If atmospheres other than ambient air are needed, mixing valves can be used to adjust the atmospheric content. Correlated rotameters [*e.g.*, Cole-Parmer (Vernon Hills, IL) or

FIG. 7. Gas manifold gang valve detail. The gas manifold is made from brass aquarium gang valves. They can be autoclaved and/or disinfected with ethanol or isopropanol.



Omega Engineering (Stamford, CT)] allow precision mixing of a variety of gases, including air, Ar, CO₂, H₂, He, N₂, and O₂. Most manufacturers can provide correlation data for other gases as well.

Additional options

As described, this culture tank works for most general-purpose applications, but it does have limitations. The minimum temperature of the tank is 2–3°C above ambient. If lower temperatures are needed, a heat exchanger made from a coil of stainless steel, or copper tubing can be suspended in the tank and connected to a circulating chiller. Copper tubing is available at most hardware stores and costs less than stainless steel. However, it will discolor and corrode over time. The maximum temperature is limited by the heater and construction materials. The materials described here are usable to approximately 65°C. At higher temperatures, the polyethylene cover and the PVC airlines may soften. Keep in mind that most aquarium heaters are thermostatically limited to a high temperature of around 35°C, and that the heater materials may not be rated for higher temperatures. Higher temperatures may require a more specialized heating system. A heat exchanger—similar to that described for cooling—could provide the means for higher temperatures. One or more electrical power strips with individually switched outlets make control of pumps, lights, and heaters easier.

If the airlines are properly disinfected, and the air or other gases are properly filtered, contamination is rarely a problem. However, for experiments in which sterility is absolutely essential (*e.g.*, molecular genetics), we recommend adding a small, inline 0.2- μ m (pore size) filter to the inlet of each culture tube. Although we usually use the tanks with liquid cultures, agar slant cultures also can be suspended in the tanks for incubation. Although usually done with ambient air, slants can be grown under artificial atmospheres by shortening the airstems. However, the agar slants will desiccate more rapidly under these conditions.

USING THE CULTURE TANK

After the tank has been filled, and the heater has been adjusted to the appropriate temperature, the culture tubes can be inserted. Fill the cul-

ture tubes two-thirds full with liquid medium [*e.g.*, BG-11, Allen's medium, etc. (Culture Collection of Microorganisms from Extreme Environments, 1999; American Type Culture Collection, 2004; Pasteur Culture Collection of Cyanobacteria, 2004; University of Texas Culture Collection of Algae, 2004)], and inoculate them with the appropriate microorganisms. The culture tubes are suspended in the water with small rubber bands made from 3/4" latex surgical tubing. Cut small circles of tubing and slip them over the culture tubes to just below the bottoms of the closures.

The gas manifold with the brass gang valves can be autoclaved prior to use, and/or disinfected with 70% ethanol or isopropanol. Connect the gas delivery tube of each airstem to a valve outlet. After all tubes are connected, turn on the air pump or open the gas valves. Adjust each gang valve to provide equal volumes of gas to each culture tube. If bottled gases are used, we recommend a secondary pressure of approximately 75 kPa (10 psi). If noxious gases are used, the entire apparatus can be placed in a fume hood.

We have used these culture tanks in both research and teaching settings to cultivate a wide variety of algae and cyanobacteria, including *Anabaena*, *Chlamydomonas*, *Chroococciopsis*, *Nostoc*, *Plectonema*, *Spirulina*, *Oscillatoria*, *Synechococcus*, and *Synechocystis* (Laudenbach *et al.*, 1990; Herbert *et al.*, 1992; Martin *et al.*, 1997; Hall *et al.*, 1998; Thomas *et al.*, 1998a,b, 1999, 2001, 2005; Price *et al.*, 2001). In general, unicellular, filamentous, and colonial species can be grown with little difficulty. We have noted that as cultures age and become denser, they often become viscous, and may splatter out from beneath the closures. This can be minimized by decreasing the total volume of the culture medium, and by harvesting the cultures before they become viscous. However, because some cells may still escape, *we do not recommend using these culture tanks for pathogenic microorganisms.*

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