

Notes & Tips

## Producing and dispensing small quantities of $^{15}\text{N}_2$ gas at atmospheric pressure

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Heavy nitrogen ( $^{15}\text{N}_2$ )<sup>1</sup> is often used as a label for nitrogen fixation experiments in which it is desirable to monitor directly the rate of nitrogen fixation and the destination of the resulting metabolites in an organism or ecosystem [1–4]. Heavy nitrogen can be purchased in a pressurized cylinder or produced in the laboratory with specialized equipment, but both methods of obtaining the gas are expensive. These methods also introduce a complication because a typical nitrogen fixation experiment requires samples of gas at atmospheric pressure. The pressure must be reduced with a pressure regulator in the case of gas in a pressurized cylinder, and traditional laboratory methods produce heavy nitrogen at pressures well below atmospheric that are not generally suitable for direct use in nitrogen fixation experiments [1]. This is because there is no easy way in which to compress the gas to atmospheric pressure for subsequent injection into a sample bottle. This report describes a simple alternative technique that produces small quantities (limited by the volume of available plastic syringes) of heavy nitrogen at atmospheric pressure with readily available analytical chemicals, laboratory supplies, and equipment. The technique is based on that of Wood and Kennedy [4] but does not require their special apparatus.

The technique produces heavy nitrogen through the reaction of  $^{15}\text{N}$ -labeled ammonium sulfate [ $(^{15}\text{NH}_4)_2\text{SO}_4$ ] with lithium hypobromite (LiOBr), and the gas subsequently is sparged first with acidified potassium permanganate ( $\text{KMnO}_4$ ) and then with acidified water to remove contaminating oxides that otherwise would be taken up by

the organisms and thus produce errors in nitrogen fixation experiments [1]. The reagents and product gas are contained and manipulated in large disposable syringes connected by tubing (Fig. 1).

The preparation of solutions necessary for producing  $^{15}\text{N}_2$  from  $(^{15}\text{NH}_4)_2\text{SO}_4$ , and for removing impurities, was described previously by Bergersen [1], who used sodium hypobromite (NaOBr) as oxidizing agent to produce gas at low pressure. In the current procedure, we instead use lithium hypobromite and produce the gas at atmospheric pressure, but otherwise the chemistry is identical.

The procedure to be followed begins here. The following four solutions are prepared at room temperature, unless otherwise specified, with nonionized water: (A) 2.0 M  $(^{15}\text{NH}_4)_2\text{SO}_4$  (98 at%, Cambridge Isotope Labs, Andover, MA, USA), (B) 4.5 M LiOBr, (C) 20 mM  $\text{KMnO}_4$  acidified with 1% (v/v) concentrated HCl, and (D) water acidified with 1% (v/v) concentrated HCl. Disposable plastic syringes with connecting tubing are prepared as follows (Fig. 1). To each of five 60-ml syringes, a polypropylene luer female barb connector is assembled to accommodate 0.0625-inch i.d. tubing (Upchurch Scientific, Oak Harbor, WA, USA) and 5 cm of Tygon transmission tubing (0.0625-inch i.d., Saint-Gobain Performance Plastics, Akron, OH, USA). During the procedure, pairs of the five syringe assemblies are connected together at different times using a polypropylene barbed union as shown in Fig. 1. The amount of gas that can be generated per batch using this method is limited only by the volume of the syringes, and larger syringes can be adapted to the method if they are available.

The two initial reagents A and B are placed separately in two syringes, eliminating all air. The reagents are mixed by connecting the two syringes together with tubing and injecting one into the other. The resulting gas is transferred

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<sup>1</sup> Abbreviations used:  $^{15}\text{N}_2$ , heavy nitrogen;  $(^{15}\text{NH}_4)_2\text{SO}_4$ ,  $^{15}\text{N}$ -labeled ammonium sulfate; LiOBr, lithium hypobromite;  $\text{KMnO}_4$ , potassium permanganate; NaOBr, sodium hypobromite.

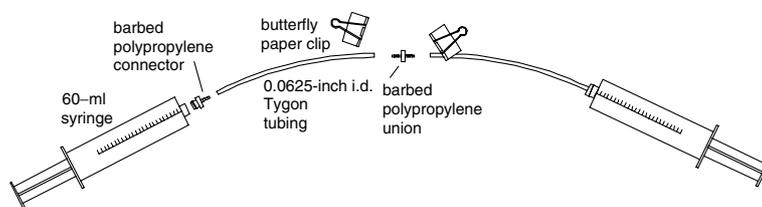


Fig. 1. Apparatus for manipulating reagent solutions and gas produced at approximately atmospheric pressure, consisting of two syringe assemblies connected by a union. A total of five assemblies are required at different times during the procedure.

through connecting tubing to a third syringe, in which it is sparged with solution C, and finally to a fourth syringe, in which it is sparged with solution D. A fifth syringe is used as the storage receptacle for the finished product. Hereafter, these syringes are designated as syringes 1–5.

To produce 40 cc of 98%  $^{15}\text{N}_2$  enriched nitrogen gas, 0.9 ml of solution A, containing 1.8 mmol of  $^{15}\text{N}$ , is loaded into a 1-ml syringe and injected into syringe 1 with the barbed connector momentarily removed to allow access to the tip. The headspace and tubing air is removed, and a tubing clamp is placed at the liquid–air boundary as close to the open end of the tubing as possible, but leaving room for the barbed union. Syringe 2 is loaded with 20 ml of solution B, again eliminating air and clamping. Syringe assemblies 1 and 2 are then connected with the barbed union. Both clamps are removed, and the solution in syringe 2 is pumped into syringe 1, resulting in gas immediately forming in syringe 1 and displacing the plunger. Care is taken not to allow the gas to push the plunger out of syringe 1, so that any excess gas will transfer to syringe 2. The syringes are shaken to ensure complete reaction of the reagents. When the reaction stops, all gas is collected in syringe 1 and all liquid is collected in syringe 2. The tubing of syringe 1 is clamped at the liquid–gas boundary to avoid losing gas or introducing air. The liquid in syringe 2 is jettisoned.

Then 20 ml of solution C is drawn up into syringe 3 and air eliminated. The gas is transferred to syringe 3 from syringe 1 by connecting them with the barbed union, as described above, and removing and reapplying clamps as necessary. Syringe 3 is shaken vigorously to eliminate oxides from the gas. Finally, 20 ml of solution D is loaded into syringe 4, the gas is transferred from syringe 3 to syringe 4, and syringe 4 is shaken vigorously. With care

being taken to eliminate all liquid, the gas is transferred to syringe 5, which is used for storing and dispensing the gas.

The following procedure is used to extract small volumes of gas from the reservoir syringe 5 into a small syringe at exactly atmospheric pressure for injection into sample bottles. To maximize the accuracy of injection volumes, the smallest syringe that will accommodate the desired sample size should be chosen. The tubing of syringe 5 is punctured with the small syringe close to the tubing clamp (Fig. 2). Gas is forced into the small syringe by retracting its plunger while depressing that of syringe 5. The friction between the plunger and barrel of the syringes is used to ensure pressure above atmospheric in the system. Somewhat more than the desired volume of gas is transferred to the small syringe, and then the plunger of syringe 5 is forced back with the small syringe, using the internal pressure of the system to do so, until the small syringe has the desired volume. It is important that the pressure in the system be above atmospheric to prevent contamination of the gas with air when the small syringe is withdrawn. A second tubing clamp is placed on the tubing to isolate the puncture point and thus not lose gas from syringe 5. The small syringe is withdrawn from the tubing, allowing some gas to escape and equalizing the pressure to atmospheric. Then the gas in the small syringe is injected immediately into a sample bottle of the subject experiment or, if desired, the small syringe can be imbedded in a rubber stopper for up to several hours to prevent dilution of the gas with air. Subsequent samples are taken from the same puncture hole.

The technique described here is practical for generating volumes of  $^{15}\text{N}_2$  up to that of the largest available syringe. It has the primary advantage over traditional methods of producing  $^{15}\text{N}$  gas at atmospheric pressure and, thus, is

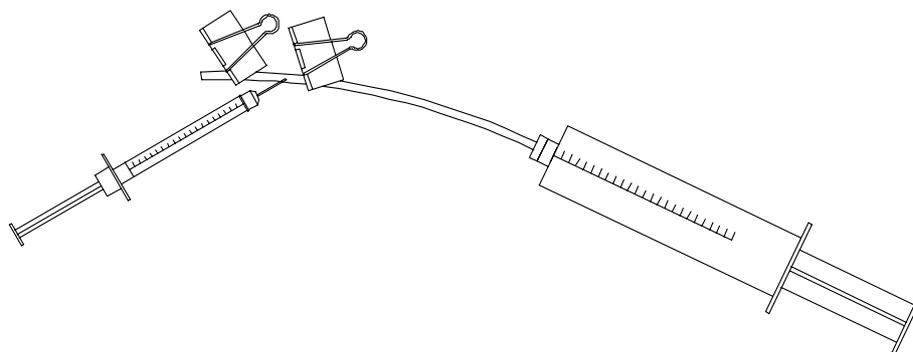


Fig. 2. Isolation of puncture point to prevent gas loss from reservoir syringe.

usable directly for nitrogen fixation experiments. In addition, the technique does not require any special equipment such as pressure regulators, vacuum equipment, or glassware. Finally, these advantages result in a method that is easy and inexpensive.

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