ILLUSTRATED CONCEPTS IN AGRICULTURAL BIOTECHNOLOGY

A series from the NifTAL Project*-MIRCEN, Department of Agronomy and Soil Science
College of Tropical Agriculture and Human Resources
University of Hawaii

A SMALL GLASS FERMENTER FOR THE PRODUCTION OF RHIZOBIUM INOCULUM

Legumes can obtain a substantial amount of nitrogen for their growth and nutrition in symbiosis with effective N-fixing rhizobia in their root nodules. Rhizobial inoculants for seed or soil inoculation may be prepared (Figure 1) by blending fermenter cultured rhizobia with suitable carriers such as finely ground peat. A glass fermenter has been devised which may be used for the production of sufficient volumes of broth cultures to meet the requirements of large field experiments on *Rhizobium* inoculation. In addition, this glass fermenter is also suitable for the production of starter cultures for medium sized commercial fermenters.

Materials

All materials required are readily available. The items needed are: One 4 liter Erlenmeyer flask to which a 35 mm long glass tube (7 mm OD) has been fitted near its base and parallel to the bottom; glass tubing, 800 mm x 7 mm OD (cut into 6 pieces: 1 @ 400 mm, 5 @ 80 mm); two pieces of larger diameter glass tubing, 120 mm x 30 mm OD (the barrels of two disposable 50 ml plastic polypropylene syringes may be used instead); a piece of solid glass rod, 3 cm x 8 mm OD; surgical rubber tubing, 800 mm x 12 mm OD (6 mm ID); four #6.5 rubber stoppers; one #10 rubber stopper; small roll aluminum foil; small roll of non-absorbent cotton wool; tubing clamp; 300 mm flexible steel wire; autoclavable masking tape; small air pump such as an aquarium pump; YMB broth (mannitol - 10 g; yeast extract - 0.5 g; $MgSO_4 \cdot 7H_2O$ - 0.2 g; K_2HPO_4 - 0.5 g; NaCL -0.1 g. Dissolve in 1 liter of distilled H_2O, adjust pH to 6.8 and autoclave.)

Assembly

The assembly is shown in Figure 2 (diagram) and Figure 3 (photograph). It is important to pack the cotton in the filter barrels uniformly but loosely enough to facilitate air passage. All glass tubes and stoppers have to be tightly fitted to avoid leaks. Support for the filters is achieved by a wire ring surrounding the lower neck of the vessel. Two small pieces of wire are bent into little hooks which are taped to the ends of the filters. The filters may then be hooked into the wire ring.

Operation

A maximum of three liters of yeast mannitol broth may be filled into the vessel. The mouth of the flask is then tightly stoppered and secured with a wrapper of cotton wool followed by aluminum foil. The air inflow is closed with a clamp near that point where the inflow filter connects to the 400 mm inlet tubing. The unit is then autoclaved at 15 lbs. and 121° C for 1 hour. Inoculation is done on a clean bench after the broth has cooled to room temperature. Twenty (20) ml of inoculant broth from a shaker culture is aseptically injected through the latex inlet tubing with a sterilized syringe fitted with an 18 g needle. An aquarium pump is then attached to the inflow tubing, the clamp removed, and the airflow activated. A fully grown culture of 1×10^9 cells/ml can be expected after 4 days for fast growing rhizobia and 6 days for most slow growers.

Production Potential

For irradiated peat, approximately 40 ml (a trial injection of broth to peat ratio must be done to insure correct moisture content) of fermenter culture is aseptically injected into 50 g peat to give 90 g of inoculant. This results in 75 bags or 6.75 kg of moist inoculant from 3 liters of broth culture. Commercial inoculation rates for large seeded legumes, e.g., soybean, is around 0.3 kg inoculant per 65 kg seed (good for planting 1 hectare) which provides approximately 2.5×10^5 rhizobia per seed. Using these figures as guidelines, 6.75 kg of inoculant prepared from 3 liter of fermenter culture can be used to inoculate 1527.5 kg soybean seeds for planting 23.5 hectares.

For inoculating small-seeded species, e.g., alfalfa, the commercial rate is 70 g per 16 kg seed (good for planting 1 hectare) to provide approximately 5×10^3 rhizobia per seed. In this case, the 6.75 kg of alfalfa inoculant from 3 liters of culture will be sufficient to inoculate 1542.4 kg of alfalfa seed for planting 96.4 hectares.

Comparative Economic Value

The net economic gain per inoculated crop was estimated by using U.S. commercial production yields and a cost of US$.20 per Kg of Urea fertilizer. Thus, for an equivalent benefit from nitrogen, the per hectare cost of urea fertilizer would be US$435 for alfalfa (2176 Kg per hectare) and US$87 for soybean (435 Kg per hectare).

By Heinz J. Hoben and Padma Somasegaran

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H. Hoben

Figure 1. Inoculant preparation with broth culture from fermenter.

R. Gabrielson

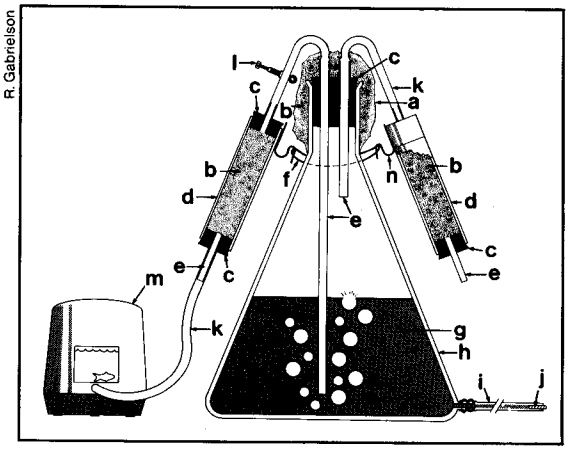

Figure 2. Scheme of simple fermenter unit. a. aluminum foil outer wrap; b. nonabsorbent cotton wool; c. autoclavable stoppers; d. glass or plastic syringe filter housing; e. glass tubing; f. wire ring; g. growth medium; h. Erlenmeyer flask; i. sampling tubing; j. glass plug; k. latex tubing; l. hose clamp; m. aquarium pump; n. wire hook.

R. Gabrielson

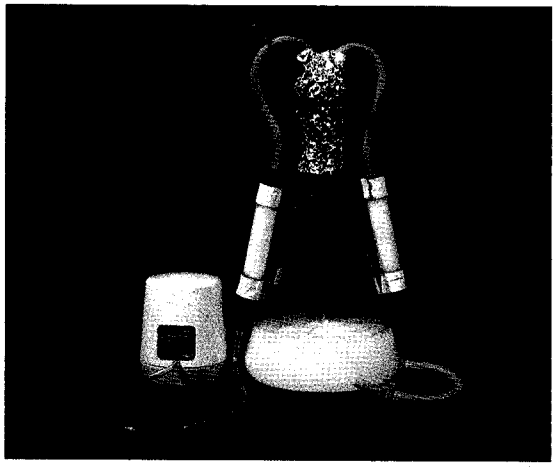

Figure 3. Assembled fermenter containing inoculum.