

A Medium-Scale Fermentor for Mass Culture of Rhizobia

Inoculants for legumes are produced by blending a broth culture of the root nodule bacteria rhizobia with a suitable carrier material such as peat. A major consideration in inoculant production is the mass culture of Rhizobia which is an important factor determining the scale of production. Mass culture requires suitable fermentors. These must be simple enough to allow for easy sterilization, access for inoculation and sampling, aeration and cleaning operations. A low-cost stainless steel fermentor for mass culture of rhizobia for medium-scale commercial inoculant production is described here.

Description.

The complete assembly of the fermentor is shown in Figure 2. The main body of the fermentor is a 141 liter (37.2 gallon) stainless steel pressure vessel modified to NifTAL's specifications. The height:diameter ratio is 2.25:1. It is domed at the top and bottom and held upright by a welded-on stainless steel skirt. The top dome has an oval opening in its center with snap-type closure which uses a special O-ring seal. The following accessories are also located on the top dome: steam pressure gauge (0-30 psi); pressure relief valve (15 psi, factory adjusted); aeration system with in-line filters for the intake and exit of sterile air; inlet and outlet ports for water passage through the built-in stainless steel cooling coil. The inoculation port, thermometer, and the sampling port are positioned in a vertical plane on the wall of the vessel. The fermentor is situated on a sturdy steel support which also houses a 98,000 BTU 4-ring gas burner.

Operation.

The NifTAL fermentor serves initially as an autoclave to sterilize the growth medium and later for cell multiplication upon inoculation. The fermentor has a maximum working capacity of 100 liters. A standard yeast extract-mannitol medium is used for the growth of rhizobia. The boiling point of the growth medium is reached after one hour with the gas burner turned to maximum heat. An additional 45 minutes are required to reach 15 psi at 121 degrees C. This pressure is maintained with low heat by controlling the burner. The medium is sterilized for 45 minutes. While the growth medium sterilization is in progress, the cotton packed pre-sterilized air inlet filter is attached. Following sterilization of the medium, steam released through the air outlet tube will sterilize the glasswool packed outlet filter. Regular tap water run through the cooling coil will drop the temperature to 30 degrees C within one hour.

One liter of starter culture is aseptically introduced through the inoculation port. Aeration is initiated by gradually letting in compressed air (5 psi) through the inlet filter. This sterile air provides aeration as well as agitation for the growth of the rhizobia. Rhizobial growth can be monitored by aseptically removing samples via the sampling port.

Production Potential.

Usually 1 liter of broth culture from the fermentor is blended with 1.5 kg of finely ground peat to give 2.5 kg of peat inoculant. Therefore, 100 liters of culture can be used to prepare 250 kg of inoculant. Commercial inoculation rates for large seeded legumes, e.g., soybeans, are approximately 0.3 kg inoculant per 65 kg seed which provides 2.5×10^5 rhizobia per seed. Using these figures as guidelines, 250 kg of inoculant will for inoculate 54,166 kg of soybean seed (good for planting 833 ha with soybean).



Figure 1. Inoculating the Fermentor

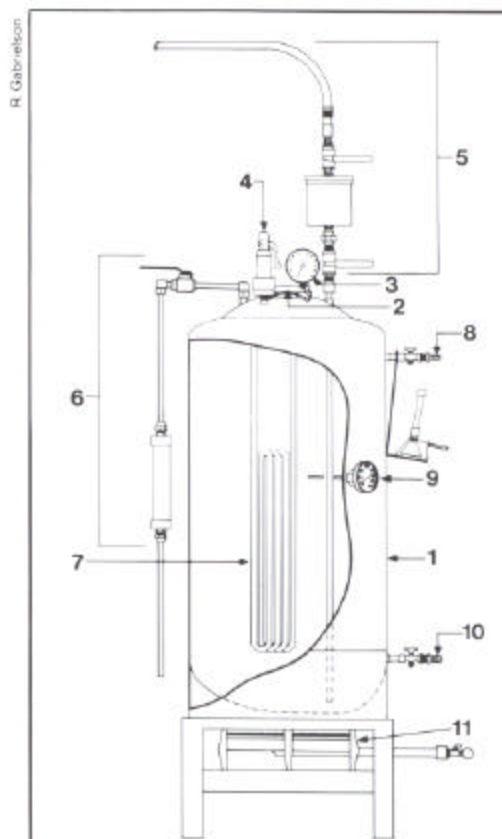


Figure 2. Fermentor Parts

Fermentor vessel (1); snap top closure (2); steam pressure gauge (3); pressure relief valve (4); air inlet assembly (5); air outlet assembly (6); cooling coil (7); inoculation port (8); thermometer (9); sampling port (10); and gas burner (11).

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