

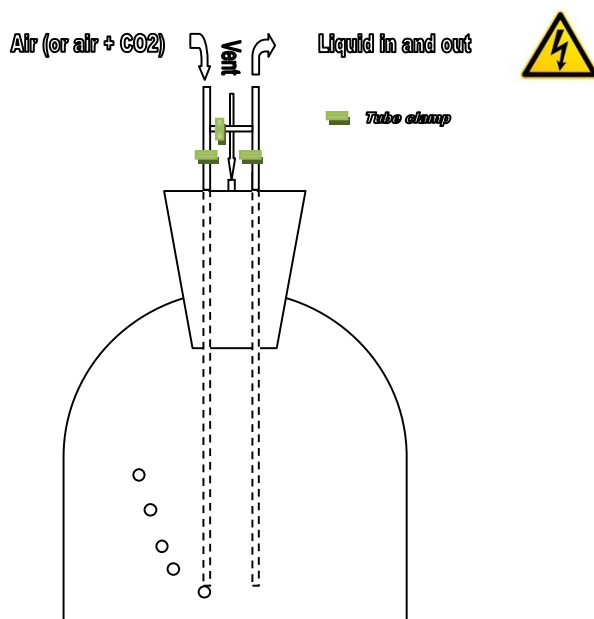
# Medium scale culture of micro-algae in polycarbonate carboys

PREAMBLE: 10 litre polycarbonate carboys are a useful vessel for growing medium volume cultures aseptically in a closed system. Also required are silicon tubing, tube closures, clamps and air filters.

1. 10 litres filtered good quality seawater for marine organisms, or 10 litres pure water for non-marine, are added to the vessel and the lid with 3 ports is fitted. The ports are:
  - Air supply. One tube is connected to an air pump via a 0.22 micron filter. Inside the vessel a long tube bubbles the air out at the base of the carboy.
  - Liquid in and out. Samples can be extracted and more medium added.
  - Vent. This allows pressure to be maintained and should be covered by a sterile hydrophobic air filter attached via a short tube.

All open ends should be covered with aluminium foil.

2. The whole vessel including tubes should be sterilised by autoclaving at 121 °C for 15 minutes. Be careful to loosen the lid (to avoid explosion) and tighten all clamps (to prevent liquid siphoning out into the autoclave). On removal from the autoclave, do not tighten the lid until the contents have fully cooled or the bottle will implode.



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*Apparatus: 10 litre clear polycarbonate carboy (Nalgene) lid with 3 ports, silicon tubing, clamps and sterile filter.*

*Illuminated constant temperature room at 18-25 °C ; a class II biological safety cabinet.*

*Culture media: 10 l filtered seawater or deionised water. Concentrated nutrients such as Walne's, f/2 or 3NBBM+V (all at [www.ccap.ac.uk/media/pdfrecipes](http://www.ccap.ac.uk/media/pdfrecipes)).*

*Algae: Require 500ml of a healthy micro-algal culture to inoculate carboy.*

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3. Nutrients should be sterilised separately and can be added to the basic medium by unscrewing the lid in a laminar flow or class II cabinet and pouring in using aseptic technique. Walne's medium is convenient for production of dense cultures of marine algae to be used in aquaculture, but any suitable growth medium can be used. 3NBBM+V is recommended for freshwater cultures.
4. Leave the vessel for at least 24 hours before inoculating with culture to allow cooling and equilibration of gases.

