Evaluation of the Microbox : a novel "breathing" tissue culture vessel

Credits: Joris Hoozee, Kaho St.-Lieven, Lab. voor Biotechnologie, Gebr. Desmetstraat 1 B,-9000 Gent, Belgium

Introduction

Current problems in micropropagation are:

- **in the case of hermetically closed containers**: problems caused by poor gas exchange. CO_2, O_2 and especially ethylene-concentrations can be far from optimal. Hyperhydricity is also a result of poor gas exchange.
- **in the case of loose covers**: secondary infections, transported by air or by mites and trips.

Materials and methods

Containers: all incubations were performed in standard polypropylene boxes, 130 mm long,100 mm wide and 70 mm high. They were provided with different types of closures.

- *Type 1*: loose cover without filter, which is snapped on, but not hermetically closed
- *Type 2*: hermetically closed plain cover
- *Type 3*: Microbox-cover, which is equipped with a series of depth filters (fig 2), and which is hermetically closed (fig 1).

Fig 1: Microbox, provided with hermetically closed cover and breathing strip

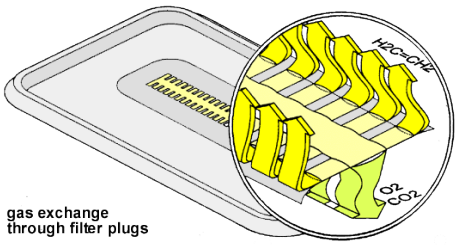
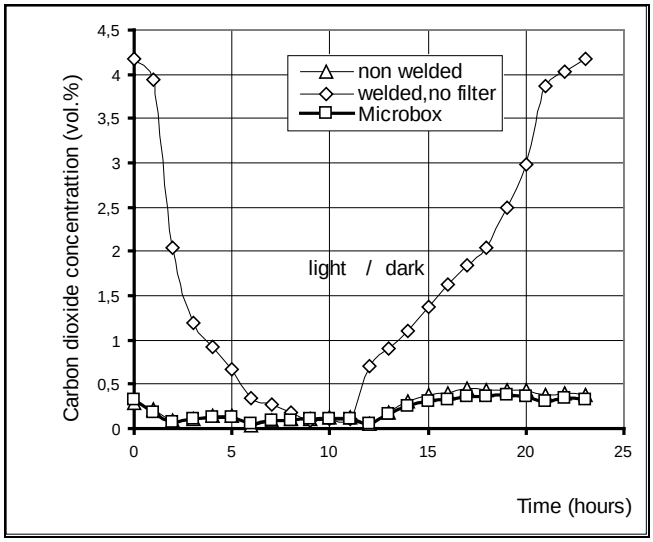


Fig 2: detail of filters

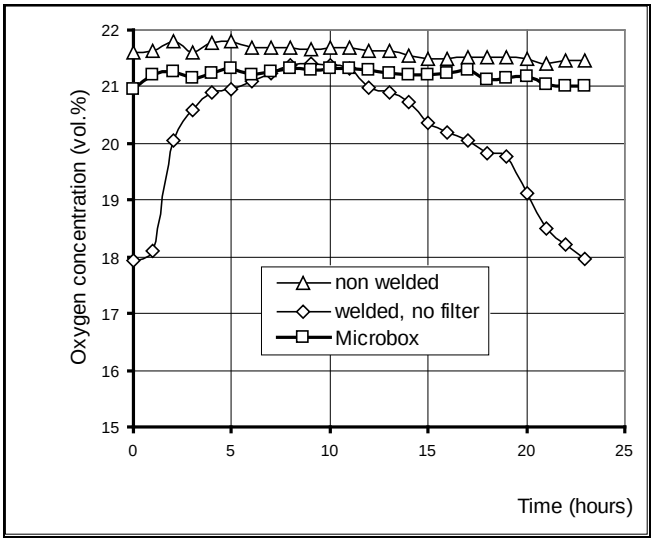
- **Investigated species**: *Anthurium andreanum* cultivar Mylene, which is known to be sensitive for ethylene gas. We prepared shoot cultures with heavy cutting up, in order to produce as much ethylene as possible.
- **Medium**: we used a modified medium according to Murashige and Skoog (1962), solidified with agar.
- **Environmental conditions**:
  - ***Incubation temperature***: 25°C
  - ***Light intensity***: approx.60 µmol.m^{-2}.s^{-1}. We applied a day and night rhythm of 12 h dark and 12 h light. TL- lamps Philips, colour 84, adequate ventilation prevented the CO_2 concentration to rise above 0.2%
  - ***Contamination pressure***: in order to challenge the cultures, the boxes were placed in an environment, which was heavily contaminated with mites and spores of *Aspergillus*.
- **Analitical methods:**
  - ***CO_2 concentration*** was measured with an infra red gas sensor (Engicom Systems)
  - ***O_2 concentration*** was determined with a paramagnetic sensor (Engicom Systems)
  - ***Ethylene*** was detected by means of gas chromatography according to Smalle and Vanderstraeten (1997), using a Delsi Di200 gas chromatograph, equipped with a Porapack type S column, detection with FID, injector temperature 127°C, column temperature 70°C, detector temperature 170°C.

Experimental

This preliminary experiment was carried out in order to evaluate the Microbox as an in vitro culture container. Graphs 1 and 2 represent the carbon dioxide and oxygen levels in the head space during a day and night cycle.

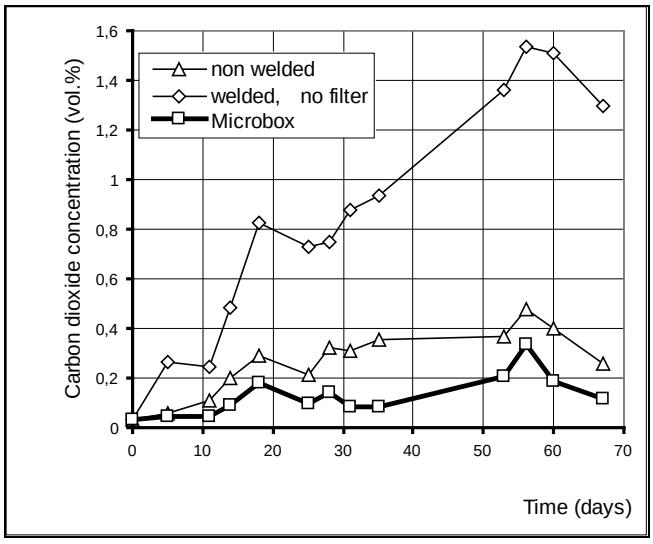


Graph 1: carbon dioxide- concentration in the head space of the vessel, caused by night and day rhythm

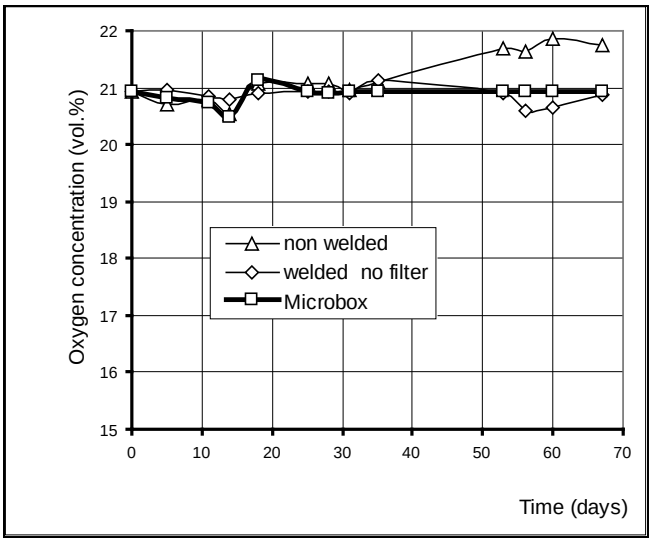


Graph 2: oxygen- concentration in the head space of the vessel, caused by night and day rhythm.

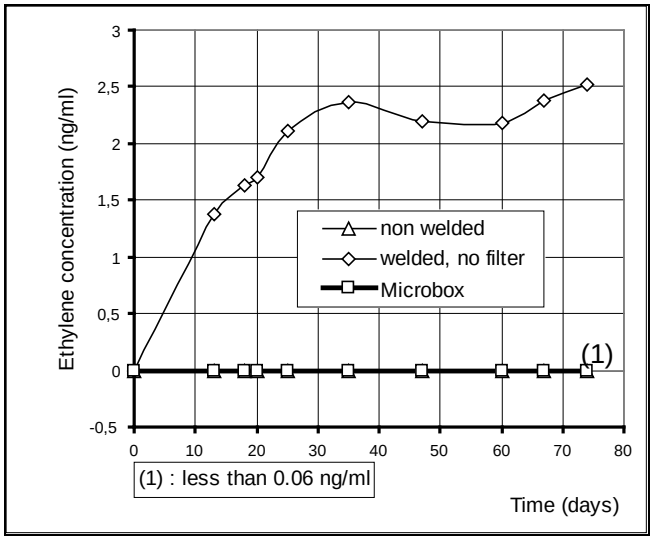
Graphs 3, 4 and 5 show the concentration of resp. carbon dioxide, oxygen and ethylene gas in the head space of the different containers in a long term time series experiment. All measurements were taken at the beginning of the light phase.



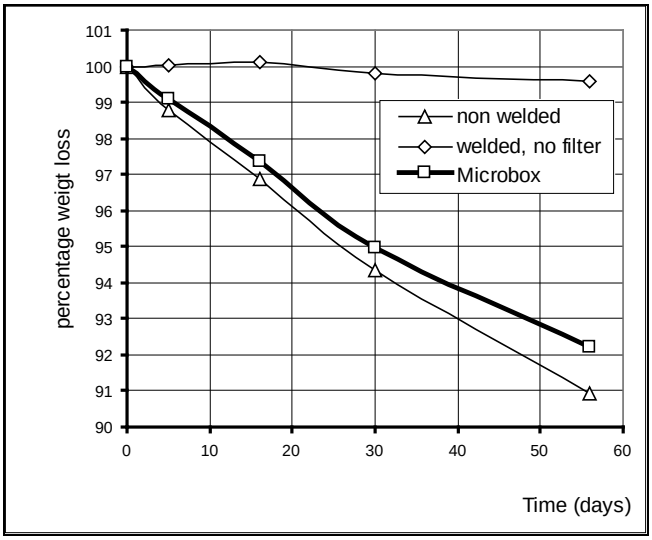
Graph 3 : carbon dioxide concentration in the head space of the vessel during long term experiment



Graph 4 : oxygen concentration in the head space of the vessel during long term experiment



Graph 5: ethylene- concentration in the head space of the vessel



Graph 6 : weight loss due to evaporation, relative humidity in the incubator was between 40 and 50 %

Observations:

- As could be expected, the ***carbon dioxide concentration*** decreased as soon as the light was switched on, and increased in the dark phase, indicating that besides dark metabolism there was also some photosynthetic activity in this culture. The carbon dioxide never rose above 0.6 % in all vented containers. A slight rise in carbon dioxide is considered beneficial.
- The ***oxygen concentration*** was in all containers almost at the same level, allowing dark reactions.
- The ***ethylene concentration*** was in all vented systems lower than the detection limit of 0.0625 ng/ml of gas phase, which is too low to have a visible effect on the plantlets. In our comparative series with completely closed containers ethylene concentrations as high as 1.5 ng/ml were measured. On photographs 1 to 4 the effects of high ethylene concentrations are clearly visible, such as long internodia, pale green leaves, adventitious air roots and shoot formation in the medium.
- The Microbox showed an even **better gas exchange** than the non hermetical cover.

Conclusions

- **Gas exchange capacity**: with respect to gas exchange the Microbox was found to be at least as good, if not better than the classically used containers with non welded cover.
- **Barrier against pests and diseases:** a very important advantage of the Microbox is the perfect protection against micro-organisms, mites and trips.

Microbox at the end of the test period

Photograph of a detail of the filter system of a Microbox