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PRESENTS:



THE FULL-GAS MICROBOX





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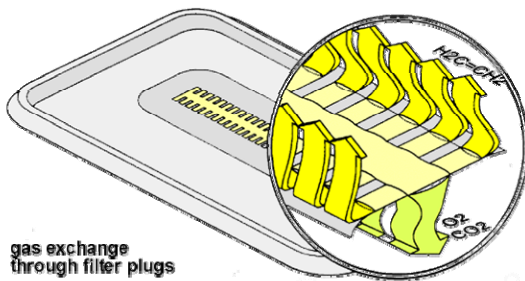
# the FULL-GAS Microbox



a new generation of tissue  
culture vessels with a  
revolutionary breathing system



your guarantee  
for carefree micropropagation!

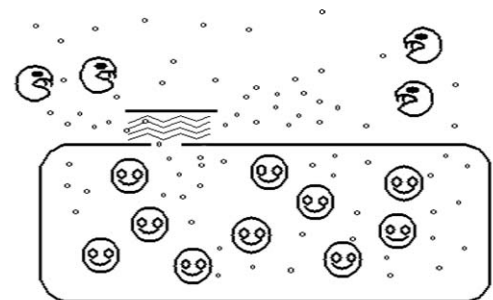


## Description:

- **The Microbox** is a clear polypropylene box, equipped with a hermetically closing polypropylene cover.
- **The cover** is made of crystal-clear plastic, and provided with a filter battery.
- **Each filter battery** consists of a double row of filter wicks, i.e. micro-channels filled with hydrophobic filtering material.

## Its advantages:

- **Adjustable gas exchange:** this occurs by means of depth filtration through the numerous filter wicks. The length of these filter wicks can be adapted to the needs of the plant species being grown, thus avoiding vitrification.
- **No danger of infection:** the wicks are made of resilient filter material, which forms a perfect barrier against pests and contamination. The Microbox makes aseptic growing spaces unnecessary.



- **Transparency:** the transparency of the plastic film, in combination with the narrow filter strips, enables optimal visual quality control.
- **Temperature resistance:** both container and filter retain their properties between -5°C and +135°C (23°F and 275°F), and can be autoclaved several times.
- **Recyclable:** 100%, vessel and filter are both made of polypropylene.

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## Gas exchange through depth filters – determination of $K_v$ value

The principle of our filters is depth filtration, in contrast with membranes, that are surface filters. We only speak of pore size with respect to surface filters.

- with surface filtration, the pore size is of importance because the micro-organisms migrate directly through the membrane;
- with depth filtration, the micro-organisms are retained by the tangled fibres in the same way as cotton plugs do.

Depth filters also limit dehydration as to the length of each of the filter plugs.

The gas exchange capacity of our filters depends on a number of factors that are linked to the filter:

- the nature of the filter material
- the length and width of the filter plugs

But the real gas exchange throughout the filters also depends on external conditions such as:

- quantity and type of medium
- incubation temperature
- ventilation in the rooms
- amount and size of plants per container
- temperature fluctuation due to lighting, etc.

Measurements in this case are difficult to express and Professor Joris Hoozee of the Ghent Engineering College (KAHO) has been working on a standardisation method. This resulted in a calculation system for the Microsacs, which is based on the  $K_v$  value.

### Volumetric gas exchange coefficient

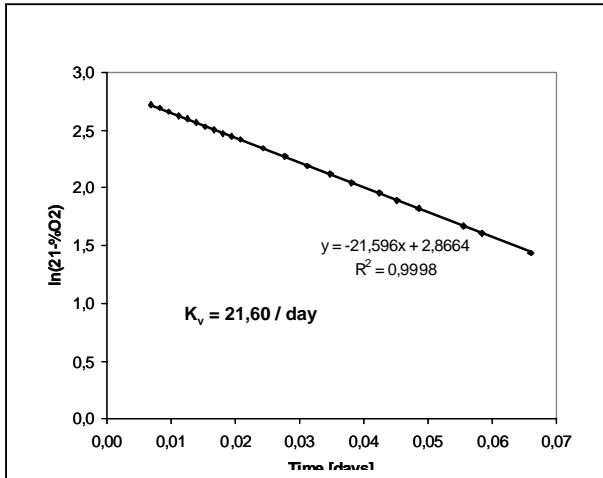
Gas exchange through filters used in Microboxes for the production of mycelium is governed by the law of Fick: the concentration of a gas in the void volume (air space above the plants) inside a box is at all times given by following formula:

$$\frac{dC}{dt} = -k'C$$

$(C_{atm} - C_{measured}) \cdot K_v$  being the volumetric gas exchange coefficient (units:  $\text{time}^{-1}$  or  $\text{h}^{-1}$  or  $\text{days}^{-1}$ )

- $C_{atm}$  being the concentration in the surrounding atmosphere
- $C_{measured}$  being the concentration measured inside the substrate (void space).

After rearranging and integration we find:  $\ln = -K_v \cdot t$ . This equation represents a straight line with a slope =  $-K_v$  or **the number of gas replacements (GR) per time unit**. This is the most valuable method to evaluate filter performance.



The figure shows an example of such a determination.

As a conclusion one can state that there are no significant differences between long and short filters, for the yellow ones as well as for the white ones. There is a difference between the white and the yellow filters, which has always been the purpose.

The table below gives a comparison of Kv value:

- of different filter types
- in round and oval Microboxes

Kv value for:	round Microboxes	oval Microboxes
White filter (Large)	9,87 GR / day	7,44 GR / day
Yellow filter (XL)	13,09 GR / day	9,84 GR / day
Red filter (XXL)	15,58 GR / day	10,83 GR / day
Green filter (XXL+)	81,35 GR / day	62,87 GR / day



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## GUIDELINES CONCERNING THE USE OF THE MICROBOX

1. To sterilise in the autoclave ("to autoclave")

**Preferable procedure: to autoclave containers and medium separately.**

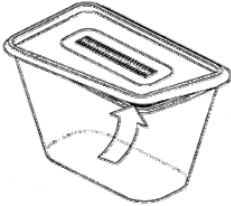
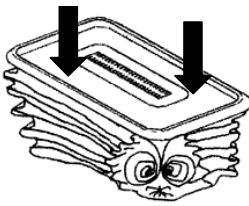
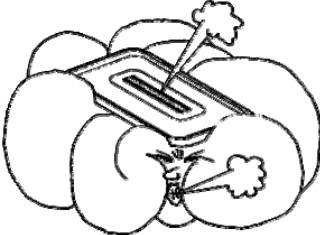
This way the PP containers perfectly keep their shape and they can be used an infinite number of times over and over again:

- pack stapled containers and lids separately in an autoclavable wrapping
- fill containers under LAF with warm sterilised medium
- click to close lid brims all around
- keep containers with medium in a well ventilated room

**Alternative procedure: to autoclave containers with medium.**

The following procedures avoid that the containers are deformed or burst while autoclaving:

- fill containers with medium
- place the lids on the containers and press gently along 3/4 of the surface leaving one border of the lid open
- after sterilisation, slowly reduce the pressure in the autoclave back to atmospheric pressure

<b>Corner of lid left open: No deformation</b>	<b>Hermetically closed lid: containers garble with pressure changes</b>	
		
gas has free passage	quick pressure increase	quick pressure reduction

- immediately after emptying the autoclave, click to close the lids all around
- keep containers with medium in a well ventilated room

2. Gas exchange

**This is done by means of diffusion throughout a series of depth filters.**

Their function is optimal in the following conditions:

- keep the filter zones free of labels or any other object
- keep the filters well dry:
  - before re-using the lids, clean them by means of a moist cloth (do not soak)
  - leave a border of the lid open during sterilisation (see above)
  - dry filters wet by accident in circulating air
- choose the suitable filter type (L, XL, XXL, XXL+) and adjust the ventilation ratio in order to obtain an ideal gas exchange in combination with a minimal dose of deshydration



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## FREQUENTLY ASKED QUESTIONS

### ***Can the Microbox be re-used?***

The vessels made of polypropylene are autoclavable at 121°C, and can be re-used 7 to 10 times. In order to prolong the life of the containers and the covers, the best is to sterilise them separately, and fill them with sterilised medium afterwards. Just before autoclaving, make sure to leave one corner of the lid open. This is to avoid the formation of a vacuum in the container deforming the plastic.

Another possibility is to fill the vessel with medium and close it, leaving one corner open while autoclaving. Immediately after autoclaving, the lids should be closed completely! This way there is no risk for deformation of the plastic or contamination of the medium.

### ***Does the medium in the Microbox dry out with plants that stay in the boxes for a long time?***

The principle of the filters is depth filtration, in contrast with membranes, that are surface filters. Depth filters limit dehydration as to the length of each of the filter plugs (see folder).

### ***Do the plantlets grown in the Microbox have problems with weaning?***

No: thanks to an adequate gas exchange, the plantlets are already autotrophe during their stay in the vessels, so that weaning is no problem.

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## ABOUT THE FILTERS

Please note the **subdivisions of our filters** in the following colours:

L (large) will be *WHITE*

XL (extra large) will be *YELLOW*

XXL (extra extra large) will be *RED*

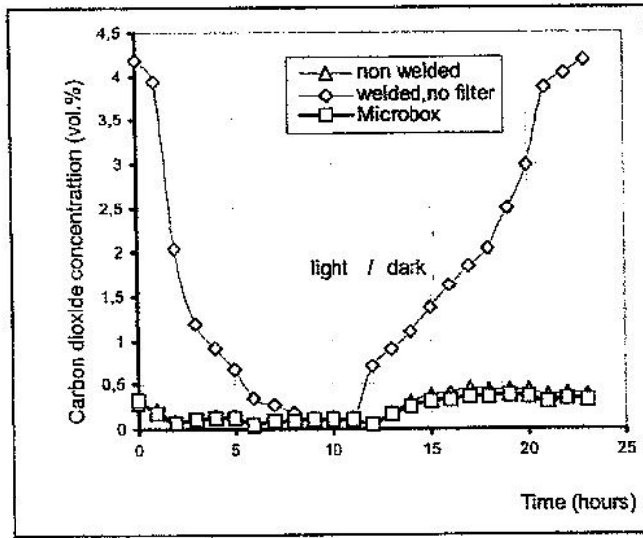
XXL+ (with extra gas exchange) will be *GREEN*

P.S.: XXL+ leaves very much air through the filter, which could possibly cause dehydration.

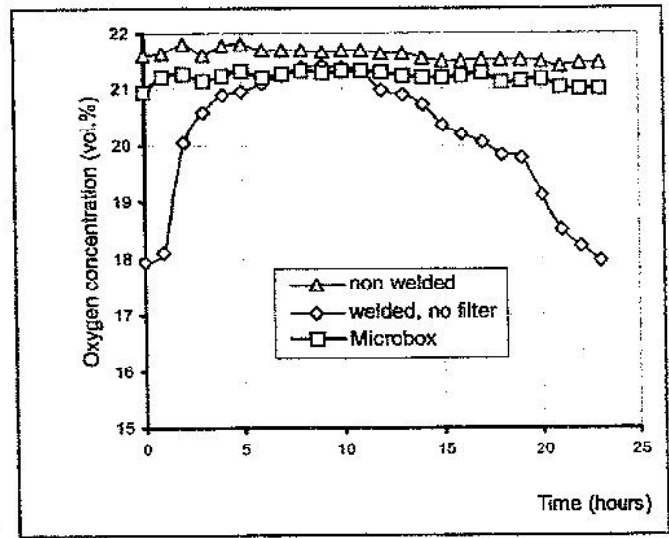
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## Experimental

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.

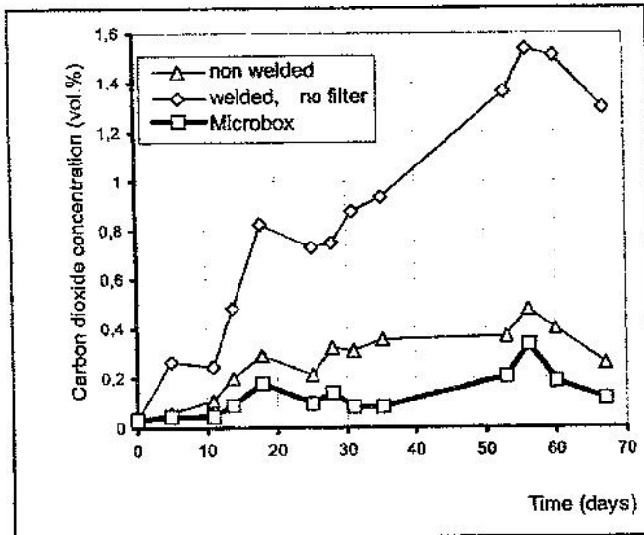


Graph 1: carbon dioxide- concentration in the head space of the vessel, caused by night and day rhythm

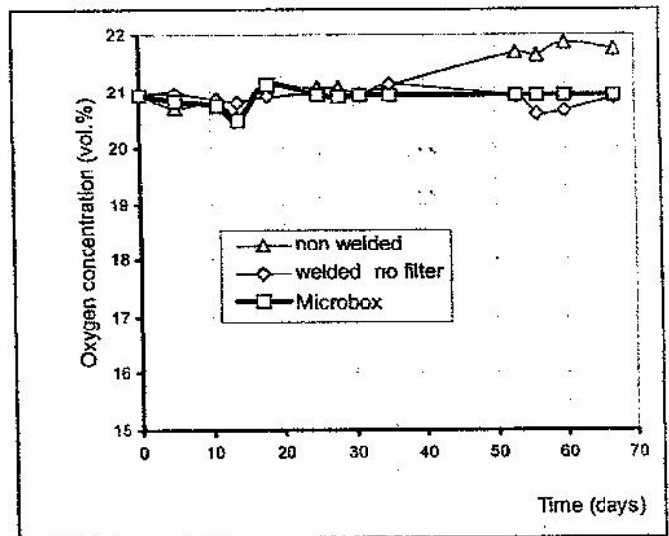


Graph 2: oxygen- concentration in the head space of the vessel, caused by night and day rhythm.

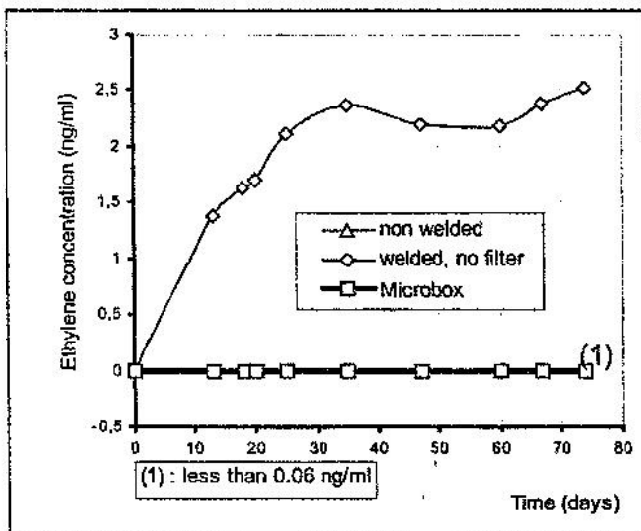
Graphs 3, 4 and 5 show the concentration of resp. carbon dioxide, oxygen and ethylene gas in the head space of the different containers in a long term time series experiment. All measurements were taken at the beginning of the light phase.



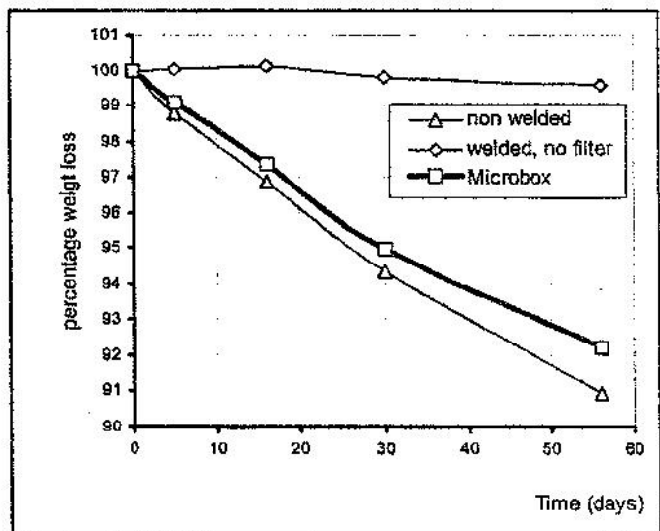
Graph 3 : carbon dioxide concentration in the head space of the vessel during long term experiment



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 welded 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.

## 1. CLEAR POLYPROPYLENE CONTAINERS WITH COVER

Hermetic cover and filter:  
*gamma irradiated*

Code	Dimensions	Packaging
 <b>OV80+OVD80</b>	Lid: 150 x 90 mm Base: 125 x 65 mm Height: 80 mm	<b>1 case (59x40x45cm; 13kg) = 350 covers + vessels:</b> 14 bags with 25 covers 14 bags with 25 vessels
 <b>OS140+ODS140</b>	Lid: 110 mm diam Base: 100 mm diam Height: 140 mm	<b>1 case (59x40x45cm; 8.5kg) = 180 covers + vessels:</b> 12 bags with 15 covers 12 bags with 15 vessels
 <b>O118+OD118</b>	Lid: 110 mm diam Base: 100 mm diam Height: 80 mm	<b>1 case (59x40x45cm; 10.6kg) = 240 covers + vessels:</b> 12 bags with 20 covers 12 bags with 20 vessels
 <b>OS60+ODS60</b>	Lid: 90mm diam Base: 80mm diam Height: 60mm	<b>1 case (59x40x45cm; 10kg) = 500 covers + vessels:</b> 10 bags with 50 covers 10 bags with 50 vessels
 <b>OS40+ODS40</b>	Lid: 90mm diam Base: 80mm diam Height: 40mm	<b>1 case (59x40x45cm; kg) = 500 covers + vessels:</b> 10 bags with 50 covers 10 bags with 50 vessels

Code filters			
L	Large (white)	XXL	Extra Extra Large (red)
XL	Extra Large (yellow)	XXL+	Extra gas exchange (green)



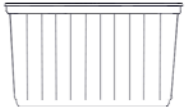





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## 2. POLYSTYRENE CONTAINERS AND COVERS

One way, *gamma irradiated*

<b>RA 40</b>	<b>Dimensions</b>	<b>Packaging</b>
	L: 145 mm B: 100 mm H:40 mm	<b>1 case</b> (59x40x45cm; 6.8kg) = <b>600</b> pcs: 12 bags of 50 containers
<b>RA 60</b>	<b>Dimensions</b>	<b>Packaging</b>
	L: 145 mm B: 100 mm H: 60 mm	<b>1 case</b> (59x40x45cm; 8.8kg) = <b>600</b> pcs: 12 bags of 50 containers
<b>RA 85</b>	<b>Dimensions</b>	<b>Packaging</b>
	L: 145 mm B: 100 mm H: 85 mm	<b>1 case</b> (59x40x45cm; 10.9kg) = <b>600</b> pcs: 12 bags of 50 containers
<b>RDA 145</b>	<b>Dimensions</b>	<b>Packaging</b>
	L: 145 mm B: 100 mm H: Plain	<b>1 case</b> (60x40x36cm; 3.5kg) = <b>600</b> pcs: 12 bags of 50 covers
<b>RDA 28</b>	<b>Dimensions</b>	<b>Packaging</b>
	L: 145 mm B: 100 mm H: 28 mm	<b>1 case</b> (60x40x36cm; 7kg) = <b>600</b> pcs 12 bags of 50 covers
<b>RDA 60</b>	<b>Dimensions</b>	<b>Packaging</b>
	L: 145 mm B: 100 mm H: 60 mm	<b>1 case</b> (59x40x45cm; 8kg) = <b>600</b> pcs: 12 bags of 50 covers