INFORS HT

Application note

Analysis of evaporation in Box for Microtitre Plates

University College London, Prof. Gary Lye, 2010, and INFORS HT, CH-Bottmingen, www.infors-ht.com

1. Introduction

The use of microtitre and deep well plates for cell culture on the one hand and for bacteria, yeasts and funghi on the other hand is gaining ever more significance. Advantages are the possibility of running many cultures in parallel, resulting in savings of time and costs in screening programs. This is especially useful e.g. if the target is the identification of the ideal clone.

For all applications it was demonstrated, that employment of incubation shakers will yield superior culture results compared to static incubation. A technical difference is, that incubation shakers always use blowers to gain a high quality of temperature distribution and humidification is only possible up to 85%. These differences may lead to evaporation losses and contaminations in microtitre plates. If these problems will occur or not, strongly depends on the experimental conditions: Deep well plates with up to 2 mL of liquid, especially if covered with gas permeable membranes and incubated over night only, are usually not critical. 96 round well plates with very small amounts of liquid e.g. 150 µL per well and plastic lids can be sensitive to evaporation and contamination. They might require additional protection to yield satisfactory results. Cell culture dishes like 6 well plates are extremely sensitive to both evaporation and contamination. They will not yield satisfactory results if used with plastic lids in incubation shakers without further protection.

2. Experimental conditions

All experiments were performed with a Multitron 2 with Direct Steam Humidification (INFORS HT, CH-Bottmingen). Unless stated otherwise, the experiments were performed at 37°C, 250 rpm, 25 mm shaking diameter, humidity setpoint 85% and with a Teflon filter in the top plate of the box.

To demonstrate the gas transfer capability of the filter in the top lid of the box, CO_2 gas was used. The box for microtitre plates was placed inside the Multitron 2; CO_2 control for the chamber was set to 5% and the box for microtitre plates was gassed with nitrogen to keep CO_2 out. Then, the nitrogen gas supply was stopped and CO_2 concentration build up in the incubation chamber and in the box was measured.

E. coli: BL21 genetically modified for the expression of transaminase. Inoculum was grown using LB medium, with a glycerol stock culture of *E. coli*. TB was used for the microwell culture, because it had previously been shown to yield higher growth and productivity for this cell clone. *E. coli* cell concentration is expressed as wet weight.

The microtitre plates used were: Costar 3799, 96 well cell culture cluster, round bottom with lids, non-pyrogenic polystyrene. 150 µL of water and culture medium respectively were used per well. Gas permeable film: BrandTech Film Seal Gas Permeable PK100, Art. No. 701364.

Unless stated otherwise, all data courtesy of University College London, Prof. Gary Lye, 2010.

Fig. 1: Box for Microtitre Plates

3. Gas transfer capability

The graph demonstrates, that a CO_2 concentration of 4% inside the box is reached in about 10 minutes and full saturation to 5% in about 30 minutes, which indicates an entirely sufficient gas transfer for cell and microbial cultivations. There is no significant difference between filter types or with/without shaking movement.

Removal of the filter from the top plate, however, leads to a much faster gas transfer: 5% CO_2 is reached in about 4 minutes (top plate of the box is in place).

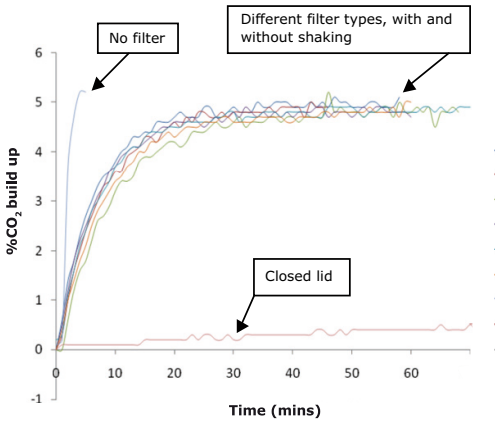

Fig. 2: Plot of CO_2 build up in box against time at varying conditions

- A: no shaking, Teflon filter
- B: no shaking, paper filter
- C: no shaking, cotton filter
- D: 250 rpm, Teflon filter
- E: 250 rpm, paper filter
- F: 250 rpm, cotton filter
- G: no shaking, no filter
- H: no shaking, closed

Humidity build up inside the incubation chamber and inside the box were recorded after closing the incubator door (Fig. 3). The comparison shows, that humidity inside the box follows the humidity inside the chamber with an offset of about 5%. Saturation inside the chamber and inside the box is reached within 15 minutes.

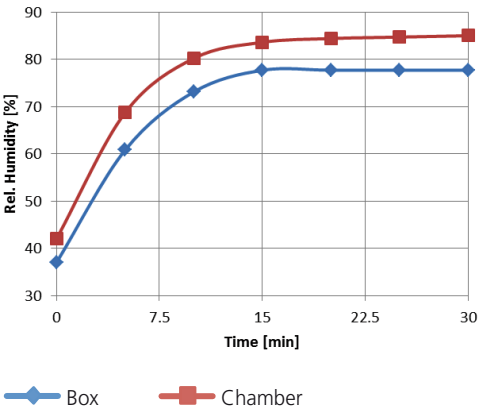

Fig. 3: Increase of humidity in chamber and box (with Teflon filter in lid)

4. Evaporation

a) With active humidification

Analysis of evaporation data showed, that evaporation was minimal with an average of about 0.6% over 24 h (Fig. 4) using active humidification at 85%, thus a negligible value. At a relative humidity of 65%, the evaporation rate rises to 3,6% over 24 h.

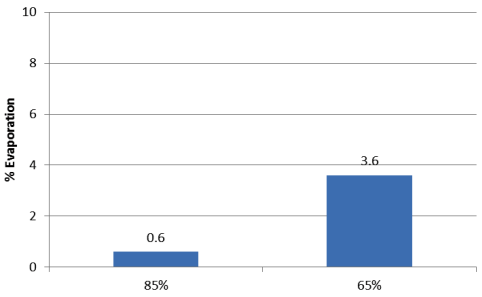

Fig. 4: Average, percentaged evaporation per 24 h in 96 well plates with 150 µL per well at 85% and 65% relative humidity

Use of the gas filter in the top lid is recommended for contamination-sensitive cultures, e.g. cell cultures, and to provide a strictly sterile incubation environment if this is desired. As a standard, a disposable paper filter is delivered. If the box is autoclaved regularly, it is recommended to put a new paper filter in place prior to each autoclaving cycle. Replacement filters can be obtained easily from laboratory suppliers (e.g. Whatman Cellulose filter, round, Sort 1, 185 mm, 1*100 ST, VWR Art. No. 512-1007).

For most cultivations, however, use of the gas filter is not strictly necessary. The top lid with uncovered holes alone will provide sufficient protection to reduce evaporation and prevent contaminations. Removal of the gas filter results in a gas transfer, that is 10 times faster compared to use with gas filter. For this reason, it is recommended to use the box without gas filter for microbial high cell density cultivations.

b) Without active humidification

Incubation of 96 round well plates inside the box for microtitre plates but without active humidification will suffer evaporation losses between 6% and 12% within 24 h, depending on the brand of the plate:

Brand of plate	TPP	No name
Evaporation loss in 24 h	5.9%	11.4%

(Data from Infors AG, Dr. D. Bruecher).

As a means of passive humidification one layer (= 6 pieces) of open 6 well plates filled with water can be added to the box. This results in a 50% reduction of evaporation losses and may provide a good enough solution if active humidification is not available:

Brand of plate	TPP	No name
Evaporation loss in 24 h with passive humidification	2.6%	5.0%

(Data from Infors AG, Dr. D. Bruecher).

5. Microbial growth in 96 standard round well plates

Comparison of *E. coli* growth in 96 standard round well plates incubated inside the box for microtitre plates showed, that there is no difference in growth no matter if the microtitre plates were left uncovered, covered with plastic lids or covered with a gas permeable membrane (Fig. 5).

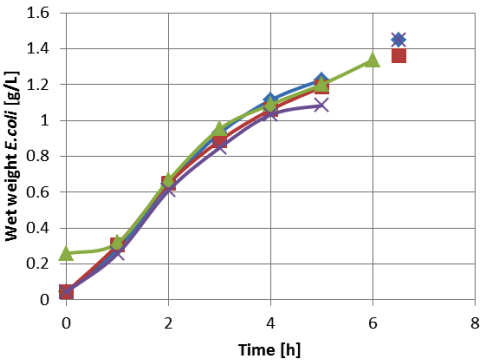

Fig. 5: Growth curve *E. Coli* over 6 hours

- Open: microtitre plates uncovered
- GPM (A): covered with gas permeable membrane, total incubation time 48 h
- GPM (B): covered with gas permeable membrane, total incubation time 6.5 h
- Plastic: covered with plastic lid

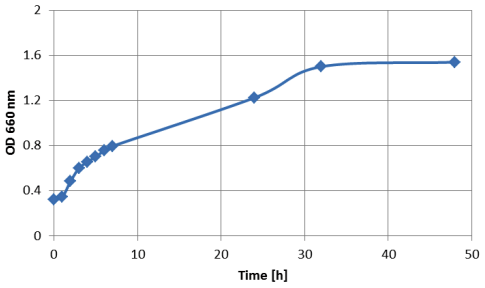

Fig. 6: Growth curve *E. coli* with gas permeable membrane over 48 hours

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