Biochamber

1.0 Product description

The MD culture chamber[1] (Biochamber) is a stainless steel device designed for *in situ* culture and enrichment of microorganisms (Figure 1). The Biochamber is suitable for microbial prospecting, fouling and biofilm studies, assessment of biodiversity, metagenomics and gene expression studies. The device is robust and designed to be placed in a wide range of environments, including the sea, fresh water, for a period of days to months. It is reusable. The device can withstand extremes of temperature (from below 0°C to 120°C, i.e. all temperatures at which life is found). A particular feature of the Biochamber is that a central chamber connects with a porous membrane (on one surface) allowing microorganisms to be cultured on the external surface but with a connection to the inner chamber. A second external membrane has no such connection and acts as a control. By comparing culture on the two outer membranes an informed view can be made as to which microorganisms are enriched by the contents of the central chamber. Microorganisms or components (protein, DNA, RNA) can be recovered for further analyzed or can be imaged. The Biochamber does not include porous membranes required for use (Section 6.0).

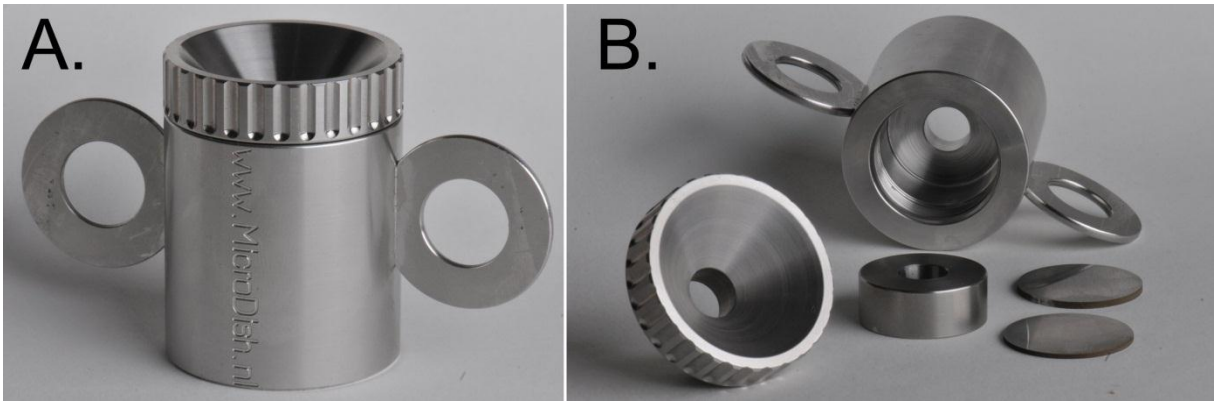

Figure 1. *Biochamber* VIEW ONE. A. Side view (diameter about 4 cm). B. Disassembled. Photo: Arno van der Kant.

[1] Patent pending

2.0 Why Enrich…? Why Culture *in situ…*?

Most microorganisms cannot currently be cultured. Further, it is important (for example in isolating microorganisms with biotechnology applications) to isolate microorganisms with desired phenotypes. Further, such microorganisms may initially be very rare in the environment and so direct high throughput screening is expensive and unlikely to succeed. Enrichment culture is a way of improving the chances of success. Microbial ecologists and commercial scientists use enrichment in the laboratory – for example growing microorganisms on prawn shells to enrich for chitin and other waste degrading bacteria. Enrichment in the natural environment is even more powerful. However, there are currently two major problems with the state of the art. The first is the lack of a commercially available and standardized device. The second is that it is difficult to tell how well an enrichment culture is working – the microorganisms recovered may or may not have the desired properties.

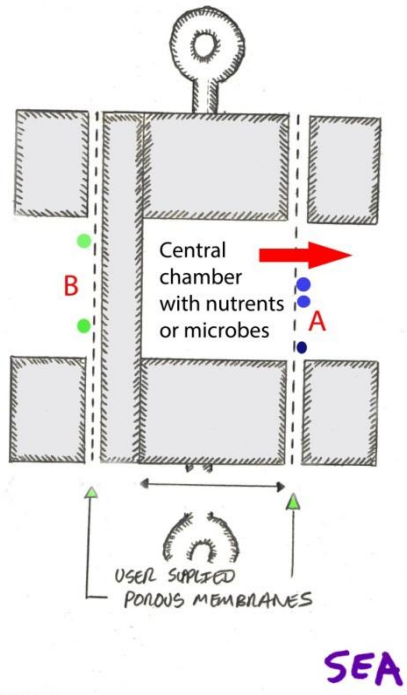

Figure 2. *Biochamber* VIEW TWO. Cross section, not to scale. A: membrane surface connected to central chamber. B: Control membrane.

3.0 Sterility and Cleaning

Cleaning can be performed with a mild detergent then water. Sterilization is generally unnecessary for environmental applications, though if required is best performed with the chamber disassembled. Autoclaving (15 min at 110°C), dry heat (200°C, 2 h) are possible. However, generally ethanol washing and drying in a laminar flow hood is sufficient. The membranes should generally be new for each experiment.

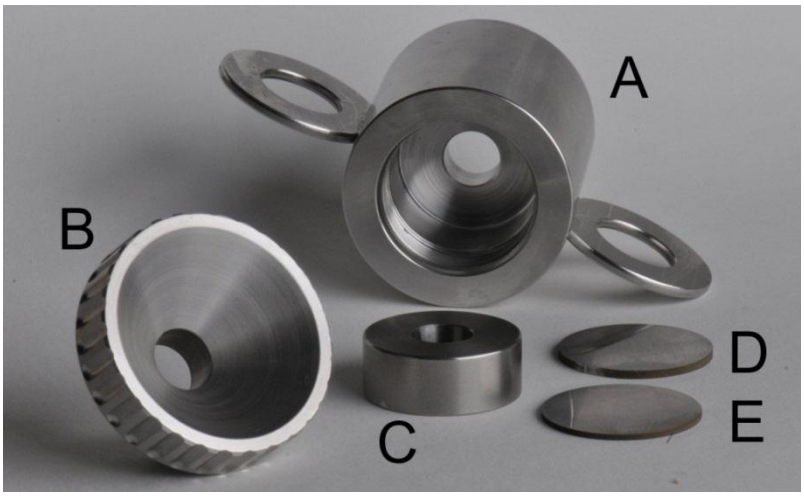

Figure 3. *Biochamber* VIEW THREE. Disassembled. A. Main body. B. Screw cap. C. Ring forming inner chamber. D. Spacer plate 1. E. Spacer plate 2.

4.0 Set Up and Loading

1. Place a sterile 25 mm membrane in chamber (Membrane B in Figure 2).
2. Add both spacer plates (D and E). OPTIONAL: Remove one spacer plate to facilitate inserting a thicker membrane or stack of membranes or filter paper or other material to slow the rate of release of a soluble compound from the central chamber.
3. Add heavy metal ring (C).
4. Load central chamber with material that forms the basis of enrichment (water, polymers, sediment, plant material) so chamber just full. OPTIONAL: Homogenize the material for optimum dispersal. OPTIONAL: Immobilize the material in a gel or polymer to decrease the release rate.
5. Add second membrane (membrane A in Figure 2).
6. Clamp down lid (B) until finger tight. OPTIONAL: A dry graphite lubricant may be applied to the screw thread to facilitate taking the item apart if it will be subjected to a corrosive or extreme environment (e.g. saline or high temperature).
7. Suspend in water/enrichment site anchoring by one or both of external rings.

5.0 How to Recover or Image Samples

Recovery from Outer Surface of Membrane - Method 1: With chamber assembled repeatedly pipette desired extraction solution (100 µl to 1 ml) within well to remove organisms from outside of membrane (A or B). The solution may be designed for nucleic acids preparation or RNA stabilization/preservation, organism recovery (containing a mild detergent such as Tween 20 if desired). Extremes of pH (acid below pH 3 or alkaline above pH 9) are not recommended.

Recovery from Membrane - Method 2: Remove membrane from Biochamber and soak out or vortex in 2 ml extraction solution.

Recovery from Chamber - Method 3: Dissemble chamber, remove membrane A and extract contents of central chamber.

Imaging *in situ* – Method 4: Alternatively microcolonies and biofilms may be stained by placing the membrane, microorganisms up, on a microscope slide containing fluorogenic dyes or fixed for electron microscopy (reference 3).

6.0 Which membranes should I use and how many….?

Most membranes (25 mm) diameter are possible including polycarbonate, nylon, ceramic. A pore size of 0.22 or 0.45 microns are recommended for confining microorganisms but larger pore sizes are possible. The membrane porosity is one way of fine tuning how microorganisms and other materials (such as macromolecules) are contained, or changing the release rate of macromolecules or other compounds from the central chamber. See also 4.0 (step 2) for fine tuning porosity.

7.0 Example of Use

The central chamber was loaded with sea weed and sealed with two 25 mm diameter ceramic membranes. The biochamber was incubated in sea water without nutrient addition for 8 days. After this time the microorganisms on the outside of membranes A and B were stained with Syto9 and Hexidium Iodide (reference 3) and imaged Figure 4. The microbial population on membrane A was > 20 fold more abundant than on membrane B (judged by fluorogenic stain and fluorescence microscopy) indicating a dependence (direct or indirect) on the sea weed in the central culture chamber and therefore a successful enrichment.

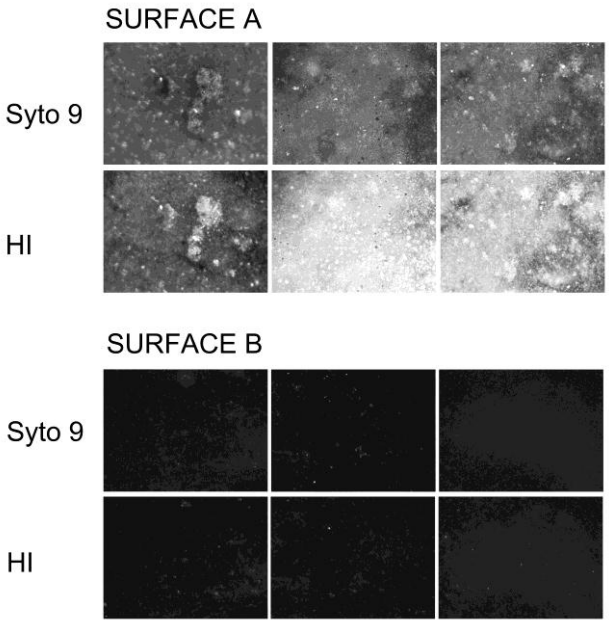

Figure 4. Imaging surface of the biochamber after 8 days. Imaging was of 4 x 2.8 mm areas of the PAO surfaces using an Olympus BX41 microscope equipped with a x4 objective lens. Syto 9 and hexidium iodide staining both indicate that there is substantially more growth on the unblocked PAO surface (A) than the blocked (B).

8.0 References – *in situ*

1. Kaeberlein *et al.,* Isolating "Uncultivable" Microorganisms in Pure Culture in a Simulated Natural Environment. Science 296 (2002) 1127-1129.

2. http://en.wikipedia.org/wiki/Enrichment_culture

3. Ingham,CJ, van den Ende M, Pijnenburg, PC, Wever PC and Schneeberger PM (2005) Growth of microorganisms in a multiplexed format on a highly porous inorganic membrane (Anopore) App Environ Micro 71:8978-8981.

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