MaCuMBA

Marine Microorganisms: Cultivation Methods for Improving their Biotechnological Applications

Project number: 311957
Start of the project (duration): 1st August 2012 (48 months)

Collaborative Project
Seventh Framework Programme
Cooperation, KBBE

Deliverable D8.4

Adaptation of a Microdish chip for in situ Cultivation

Organisation name of lead contractor: AquaTT

Due date of deliverable: M12
Actual submission date: M12 (via web site)

Revision: V.1

Project co-funded by the European Commission within the Seventh Framework Programme (2007-2013)	
Dissemination Level	
PU Public	
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	**x**
CO Confidential, only for members of the consortium (including the Commission Services)	

List of reviewers

Issue	Date	Implemented by
v.1	09/01/2014	MicroDish BV

Indicate any document related to this deliverable (report, website, ppt etc) and give file name: See MaCUMBA web site for documentation under D8.4

** Please attach deliverable documents and any additional material if needed.*

Table of Contents

Glossary

MDCC: MicroDish Culture Chip, a disposable cultivation device based around porous aluminium oxide.

In situ cultivation: Cultivation in the natural environment (e.g. the sea) with the aim of enriching one or more groups of microorganisms.

Culture Chamber: A reusable cultivation device used to place a MDCC in the natural environment, in this case the sea. This allows cultivation *in situ*. After recovery of this device the microorganisms can be analysed by molecular methods (e.g. DNA sequencing) or further cultivation in the laboratory. The culture chamber forms the basis of a **microbioreactor.**

PAO: Porous aluminium oxide, the base ceramic material for the MDCC.

Summary

Rationale: Microorganisms can be cultivated better when components of their natural environment are present, compared with laboratory medium and techniques. The MDCC and culture chamber are devices that use natural materials (sediment, sea water) as the basis of a growth medium.

Objective(s): To adapt the MDCC (see Glossary) for in situ cultivation by fabrication of a culture chamber that will accept MDCC and PAO to allow microcultivation in the natural environment. This task lies within Task 8.3: Development of microbioreactor systems for cell culture based on the Microdish Culture Chip. D8.4: Adaptation of Microdish chip for in situ cultivation.

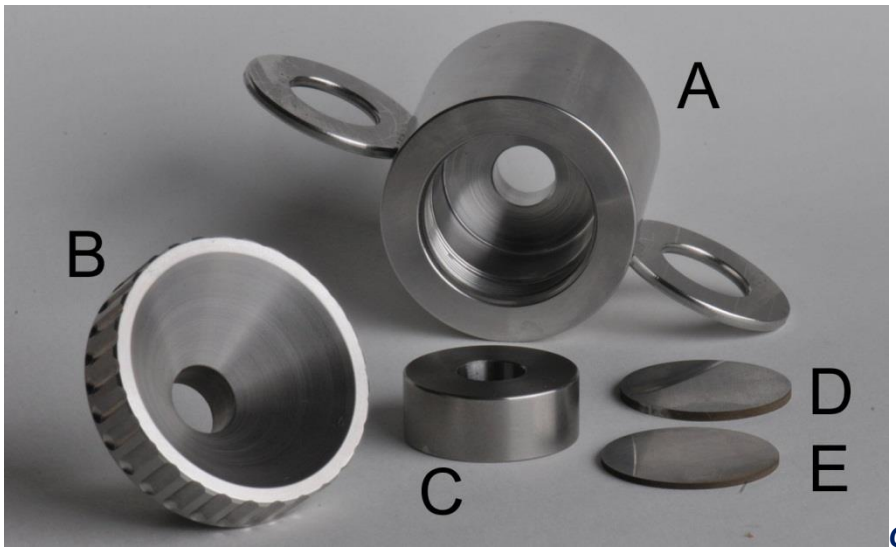
Partner(s) involved in Deliverable production: MD (6).

1. Introduction – Why Design a Culture Chip for *in situ* Cultivation and Co-culture….?

Most microorganisms cannot currently be cultured. Further, it is important (for example in isolating microorganisms with biotechnology applications) to isolate microorganisms with desired phenotypes. Such microorganisms may initially be rare in the environment and thus direct high throughput screening is expensive and also 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 may be 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. Therefore, MD has adapted the MDCC culture chip for deployment in the natural environment for co-culture and enrichment. This is designed to allow incubation of MDCC in new situations and to extend the range of applications of the culture chips, including the move towards higher throughput.

2. Culture Chamber Construction

The culture chamber was manufactured from stainless steel with the components noted in Figure 1. It is designed to accommodate two MDCC or pieces of PAO (porous aluminium oxide) described below. The chamber is slightly over 3 cm in diameter (excluding the handles on section A) with a sample volume (inside chamber formed within C) of 0.5 ml. We have currently manufactured 10.



C

Figure 1 ***Culture Chamber Disassembled.*** *A. Main body. B. Screw cap. C. Ring forming inner chamber. D. Spacer plate 1. E. Spacer plate 2.*

3. Adapting MDCC to Culture Chamber

The standard MDCC (8 x 36 mm) was replaced by 25 mm diameter circles of PAO. In POP experiments these were used. We have designed a shadow mask that works with this size of PAO substrate allowing production of MDCC in this format. Currently this is limited to small numbers using an in house photolithographic process but this allows the division of the surface of the PAO into many compartments facilitating recovery of individual microcolonies.

4. Set up and Loading

1. Place a sterile 25 mm MDCC or PAO 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 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. Sample Recovery for DNA extraction or Imaging MDCC

Recovery from Outer Surface of MDCC - Method 1: With chamber assembled repeatedly pipette desired extraction solution (100 microlitres to 1 ml) within well to remove organisms from outside of MDCC (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 MDCC - Method 2: Remove membrane from Biochamber and soak out or vortex in 2 ml extraction solution (see above).

Recovery from Chamber - Method 3: Dissemble chamber, remove MDCC 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. Example of Use

The central chamber was loaded with sea weed (a red algae) and associated microorganisms and sealed with two 25 mm diameter ceramic membranes (PAO) as shown in Figure 2. The biochamber was incubated in sea water without nutrient addition for 8 days. After this time the microorganisms on the outside of PAO substrates, A and B were stained with the dyes Syto9 and Hexidium Iodide (reference 3) and imaged. 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/and or associated microbiota in the central culture chamber and therefore a successful enrichment.

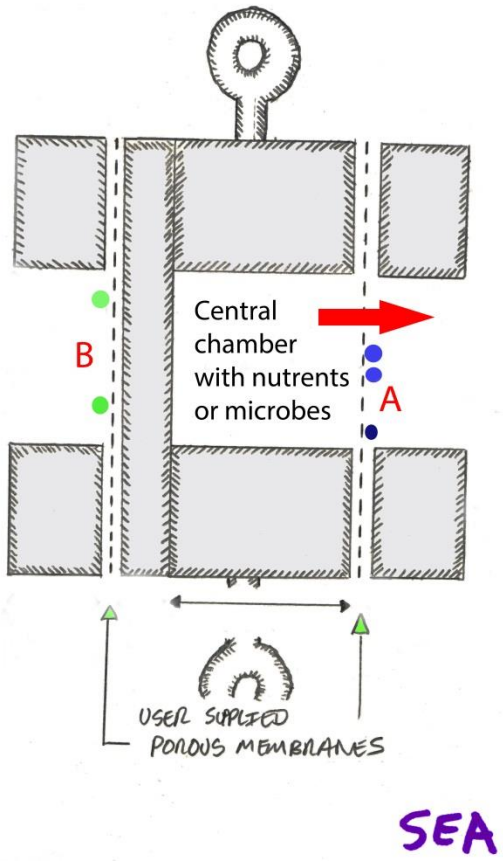


Figure 2*. Biochamber Cross section, not to scale.* A: PAO/MDCC surface connected to central chamber. B: PAO/MDCC.

7. References

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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