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SCOPE: Evaluation of auto-decontaminating efficiency.

Deliverables: a report describing the assays and the results

Partial deliverables corresponding to :

- experiment n°4 bis corresponding to part 2 of the file « Protocoles pour Proteus_2010_07_29 » sent to Proteus by Email on july 30th 2010 and amended by Email (October 14th 2010).

1- Description of the assay

The material to be evaluated is made of treated ceramic macro-particles named CARDPool 1. The assay is a dynamic assay: the microorganisms suspension flows through a column containing the particles.

2- Calibration

The evaluated strain is a microalgae *Anabaena constricta.*

The initial concentration of the microorganisms' suspension is, as described in the protocol provided by the Client, 300 cfu / mL of suspension. In a first attempt, it is necessary to evaluate the adsorption of the microorganisms to the untreated particles (negative control) in order to adjust the initial concentration if appropriate.

In addition, in order to improve the sensitivity of the assay, it may be profitable to centrifuge the sample. However, in some cases, the centrifugation may have a negative impact on the viability of the microorganisms.

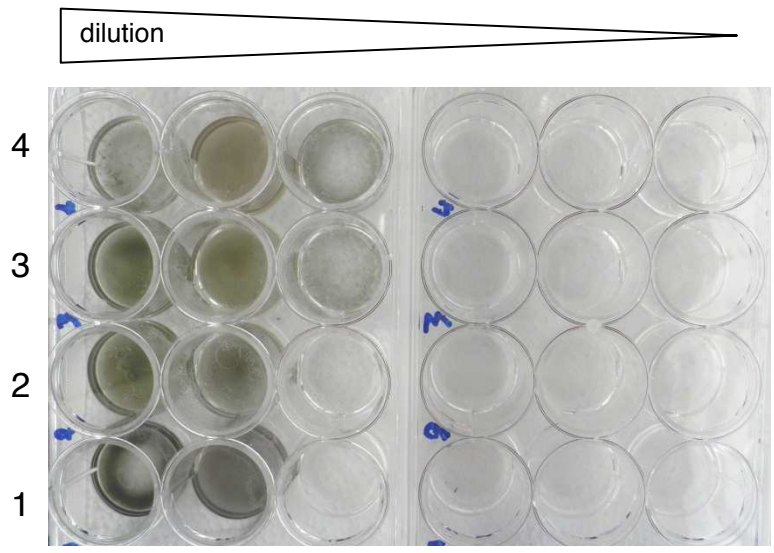
The following experiment has been performed to evaluate the adsorption of the microorganisms onto the untreated particles and to evaluate the interest of centrifugation.

A suspension at 300 cfu / mL has been prepared. From this suspension, four samples have been prepared:

1. the initial suspension at 300 cfu / mL with no additional treatment
2. the initial suspension at 300 cfu / mL concentrated 100 times by centrifugation
3. suspension passed through 3 g of the untreated particles (outflow of 3.5 mL/s)
4. suspension passed through 3 g of the untreated particles (outflow of 3.5 mL/s) and concentrated 100 times by centrifugation

The recovered microorganisms have been quantified in these samples by culture of serial 1/3 dilutions of the samples.

In each well, growth has been detected by eye due to the green colour of the algae.



The results show that the adsorption by the untreated ceramic is not significant (compare lanes 1 and 3) and that centrifugation does not improve sensitivity (compare lanes 3 and 4). Centrifugation has not been performed during the auto-decontamination efficiency assays.

3- Evaluation of the auto-decontamination efficiency

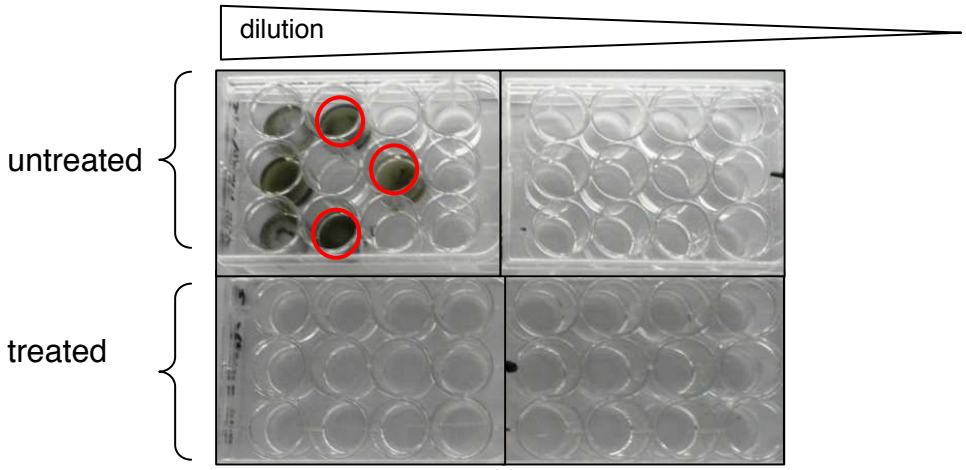
3.1- Protocol

The dynamic assay has been performed as follows:

- 3g of ceramic particles have been placed in a burette.
- 50mL of an *A. constricta* cell suspension at 300 cells/mL have been loaded onto this column
- the tap has been opened to obtain an outflow of 3.5 mL/s
- the flow through has been collected
- the recovered micro-organisms have been quantified in the sample by culture of 1/3 serial dilutions of the samples.

For each condition, both treated and untreated ceramic particles have been tested in parallel, each of them in triplicate

3.2- Results



2.3 Efficiency

The efficiency has been calculated as follows : when growth is detected in one well less than in controls, this means that viability is reduced by 1/3 ; when growth is detected in two wells less than in controls, this means that viability is reduced by 1/9…

For all the assays that are described here, the efficiency is superior to the measurable efficiency: no microbial growth is detected even in the undiluted sample (first well of the serial dilutions). The measurable efficiency varies from 3^2 to 3^3, depending on the concentration of the microorganisms in the negative controls (untreated ceramic); this corresponds to 88.89% to 96.30%.

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