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# ANALYSIS OF MICROBIAL COMMUNITIES FOR THE IDENTIFICATION OF INOCULANTS FOR AN IN-SITU BIOREACTOR FOR TREATING HCH CONTAMINATION IN GROUNDWATER

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Gaiker
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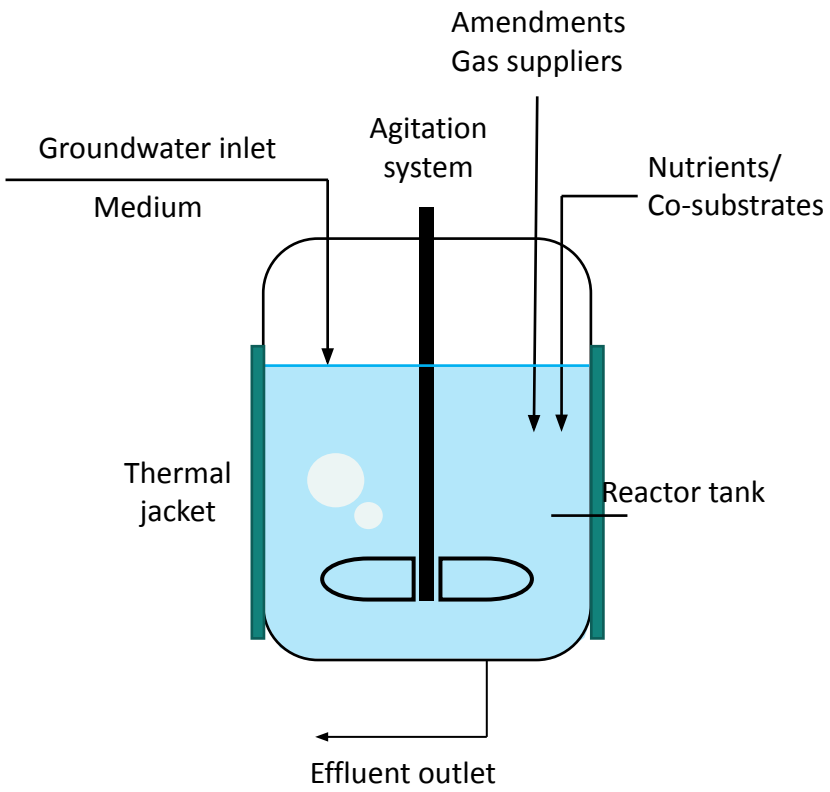
GOBIERNO DE ARAGON

# BIOREACTOR AS REMEDIATION SYSTEM

A bioreactor is a biotechnological device capable of producing a controlled and isolated environment that guarantees and maximizes the growth of a culture of microorganisms that carry out the degradation of the contaminants of interest in the aquifer



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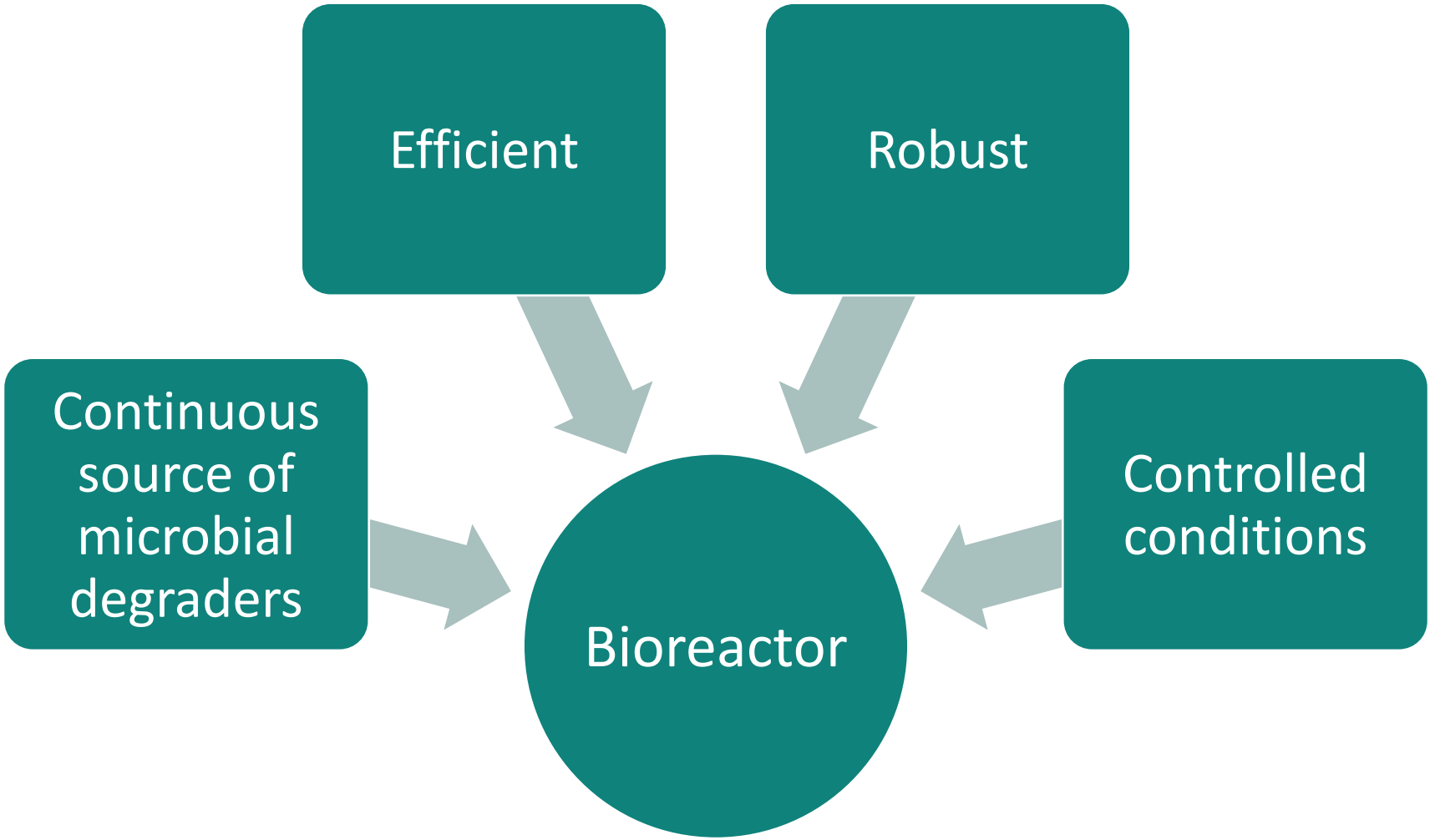
# *IN SITU* BIOREACTOR CONFIGURATION



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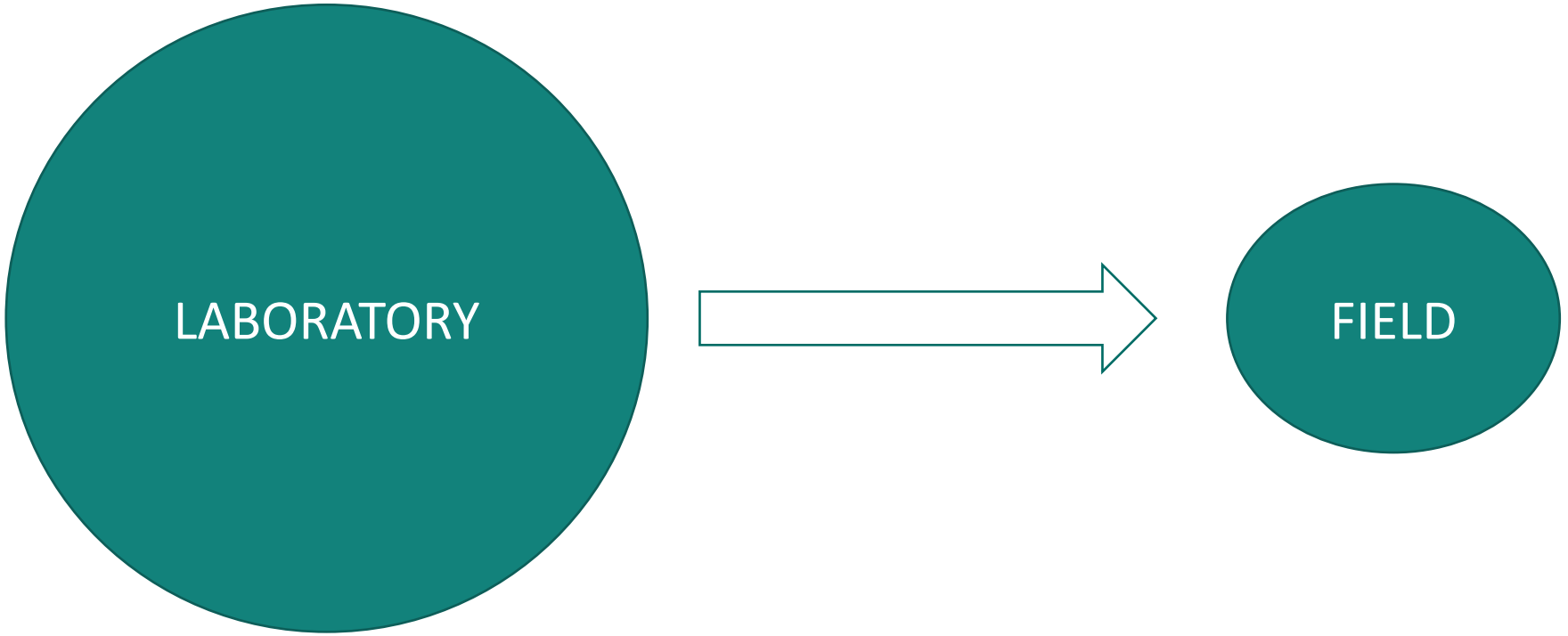
# SCALING PROCESS IN BIOREACTOR DEVELOPMENT



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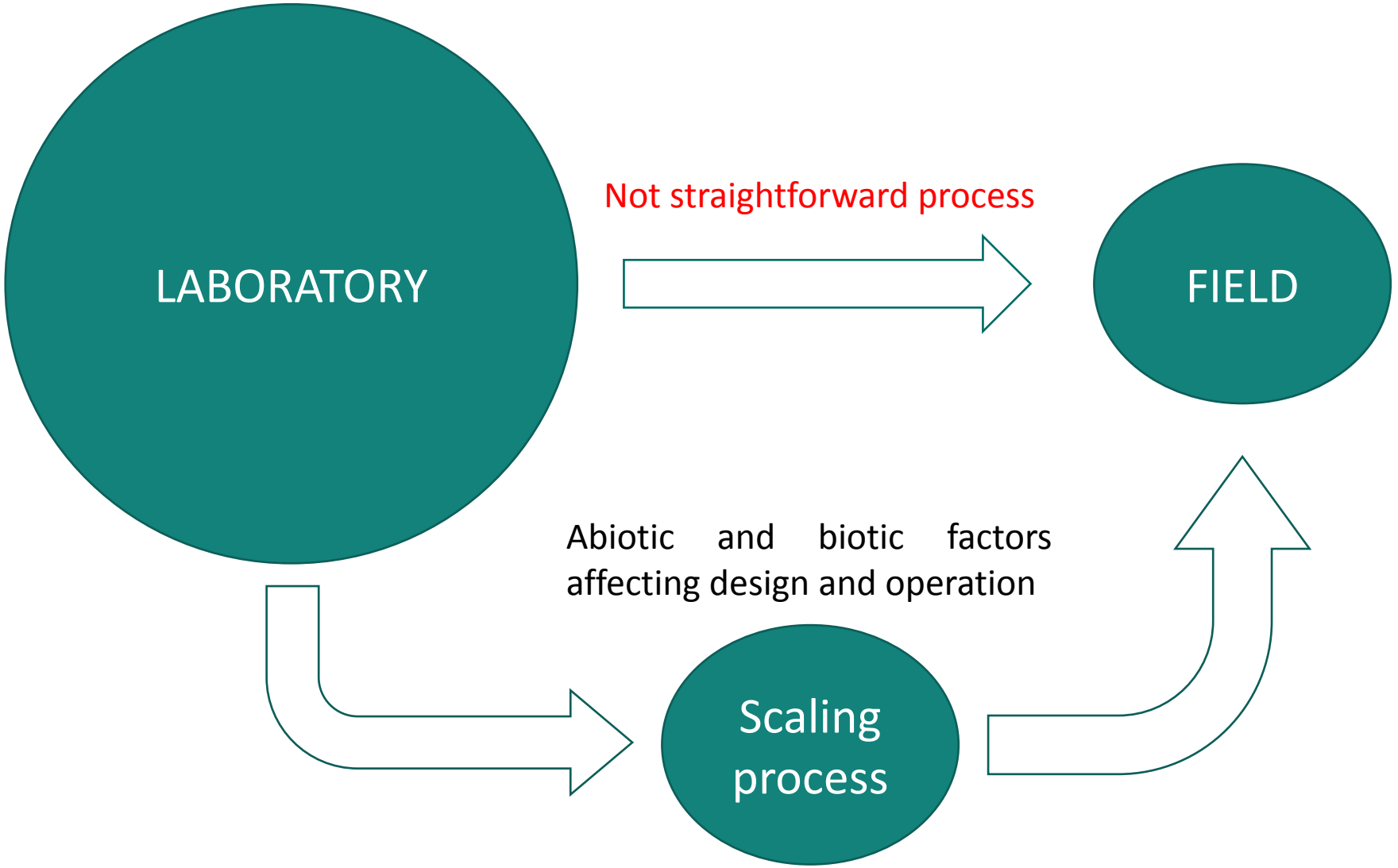

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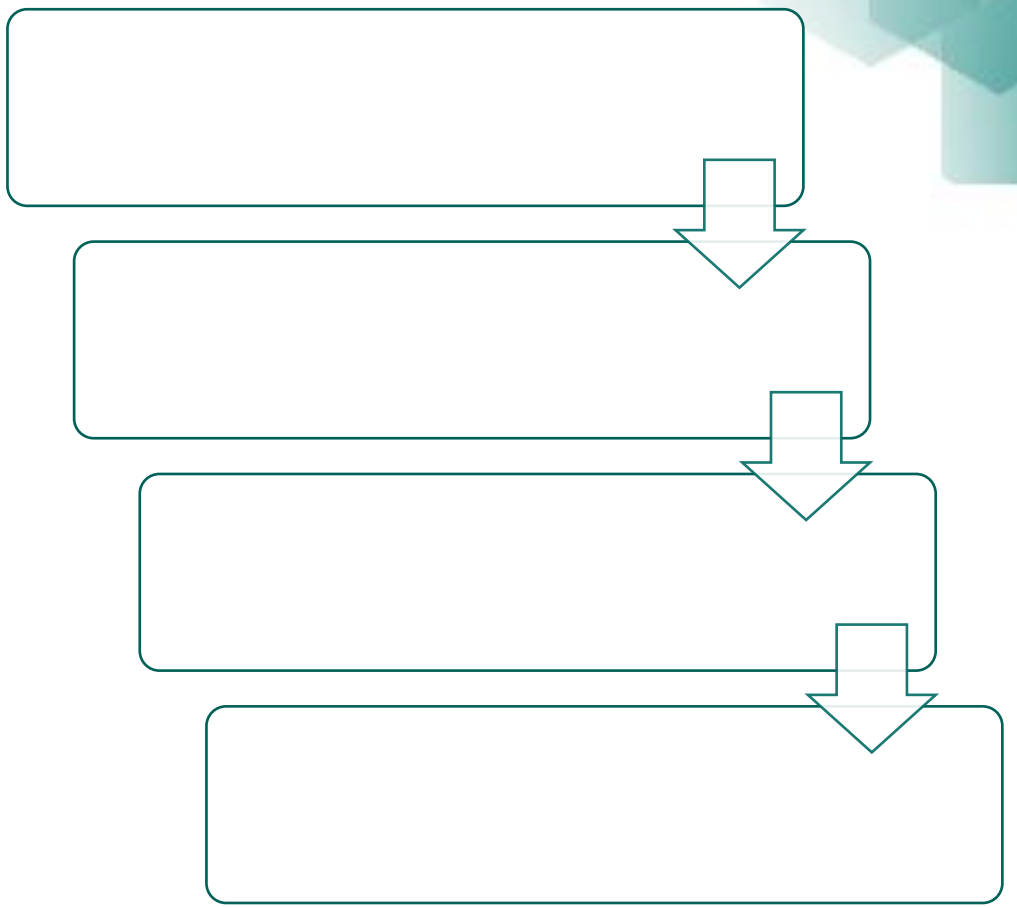
# THE IMPORTANCE OF A GOOD STARTING POINT



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# THE IMPORTANCE OF A GOOD STARTING POINT

The selection of a **good inoculum** is a key factor in order to obtain consortia of **adapted microorganisms** that can acclimatize more easily to the conditions of the environment, that are viable, versatile and with a **high capacity to degrade the contaminants of interest**.

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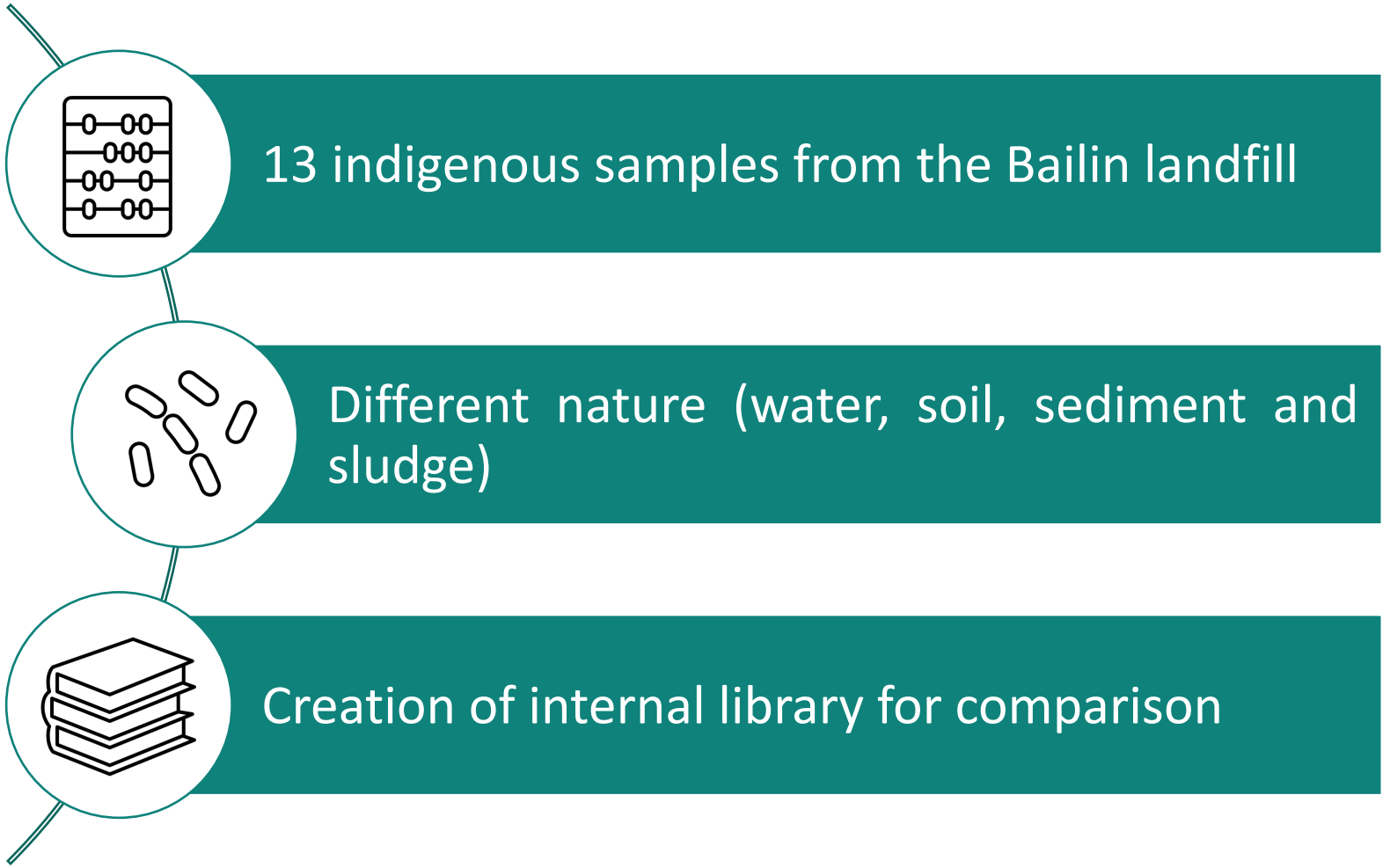
## OBJECTIVE

**Characterization of different samples impacted by HCH for its potential selection as inoculants for the development of an *in-situ* bioreactor**



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# INOCULANT IDENTIFICATION AND SELECTION


Water

Soil

Sediment

Sludge

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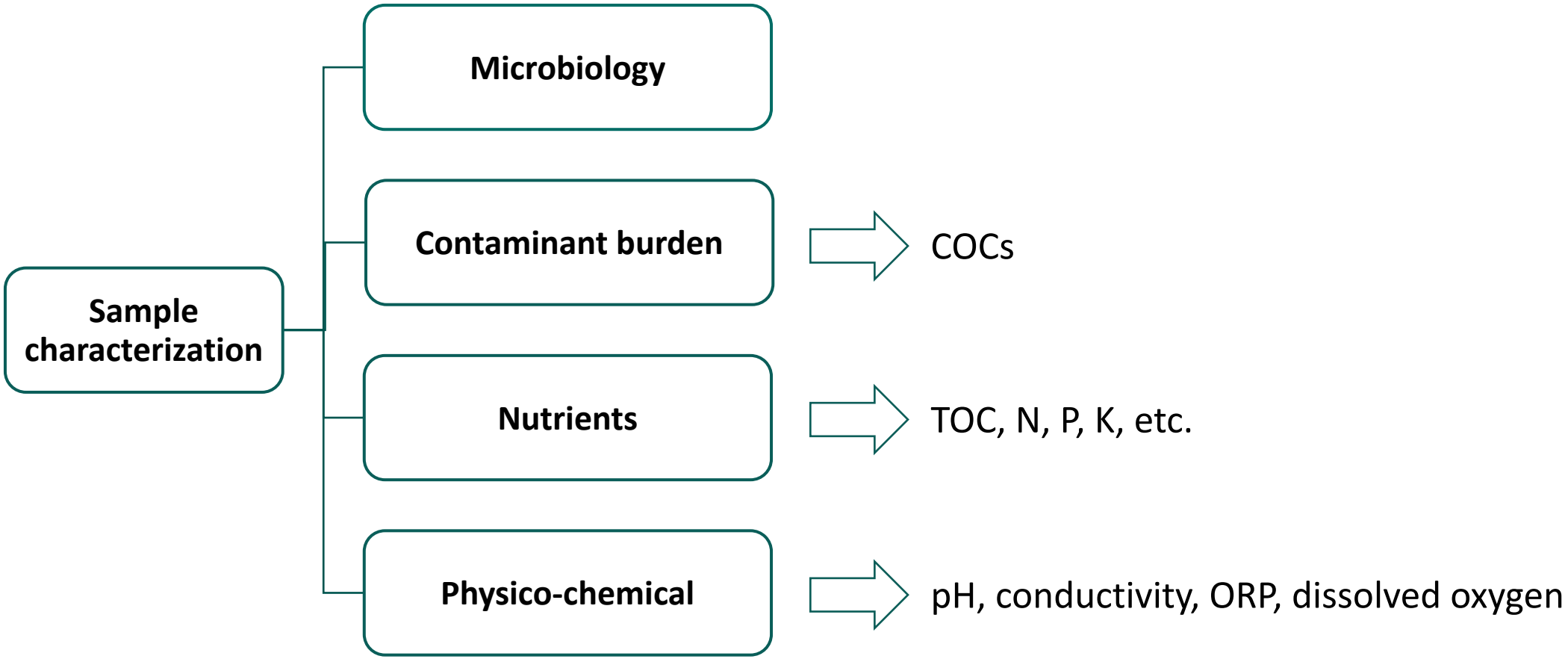
# INOCULANT CHARACTERIZATION



# INOCULANT CHARACTERIZATION

**Sample characterization**

- **Microbiology**
- Contaminant burden
- Nutrients
- Physico-chemical

## Attributes

I) Viable and cultivable biomass in generic medium

***Plate count Generic medium***

II) Viable and cultivable biomass in minimal medium (HCH as carbon source)

***Plate count Minimal medium***

III) Microbial activity and diversity

***Biolog Ecoplate microplate***

IV) Tolerance to different [contaminant] (inhibitory effect)

***Biolog MT2 microplate***

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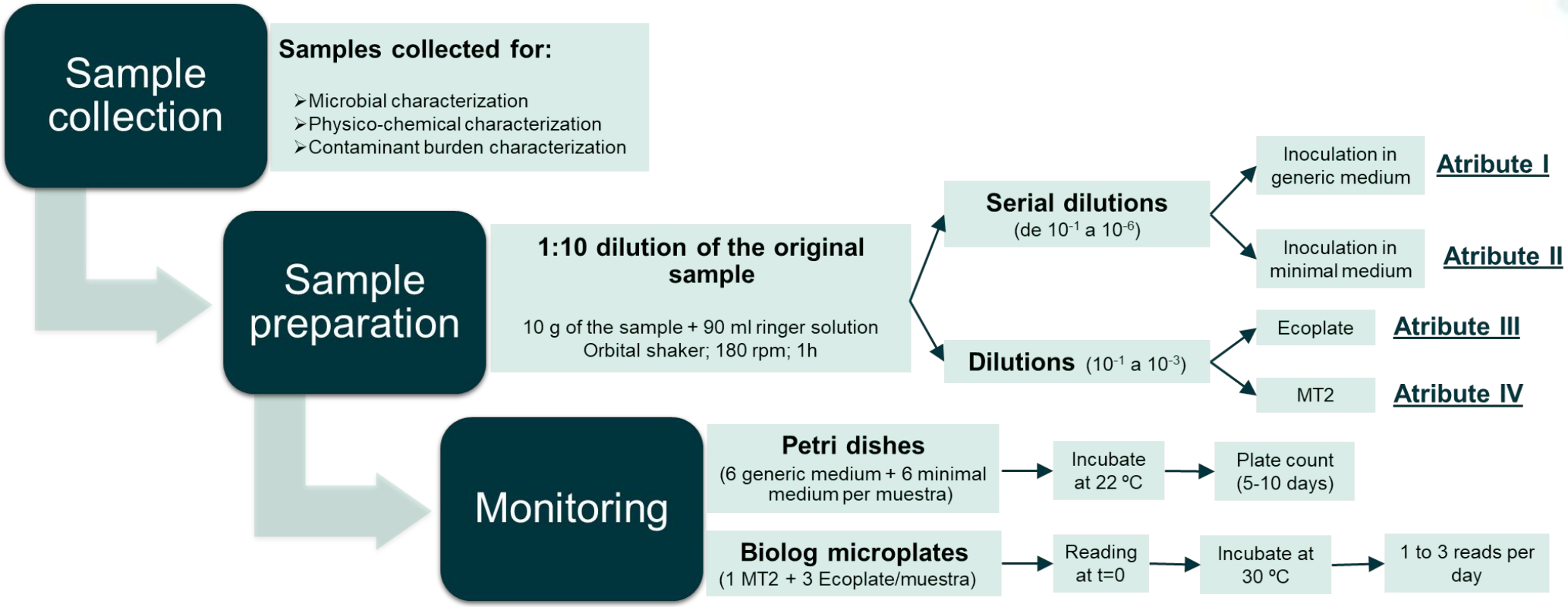
# CHARACTERIZATION WORKFLOW




3 week-period

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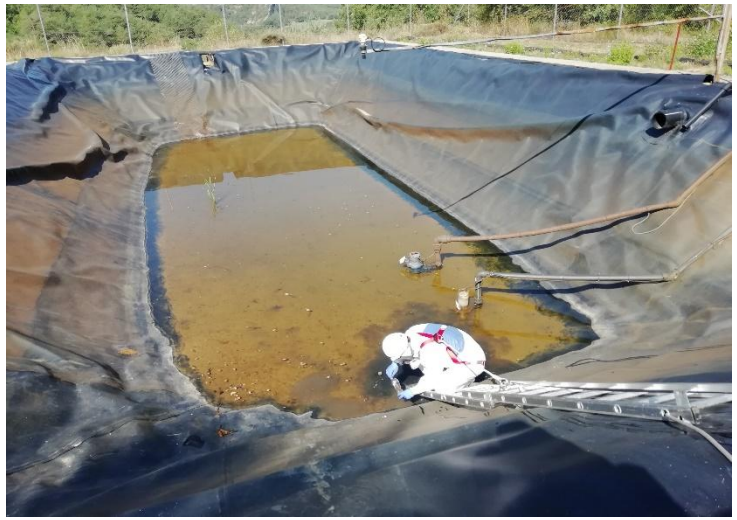
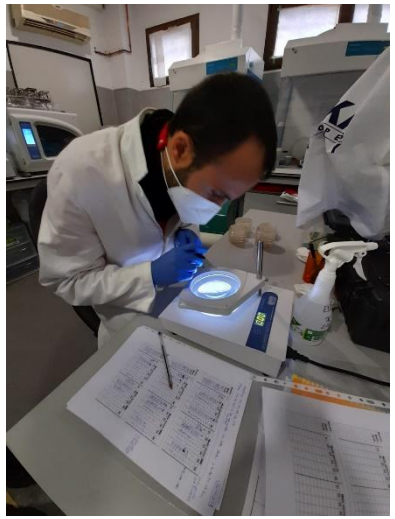
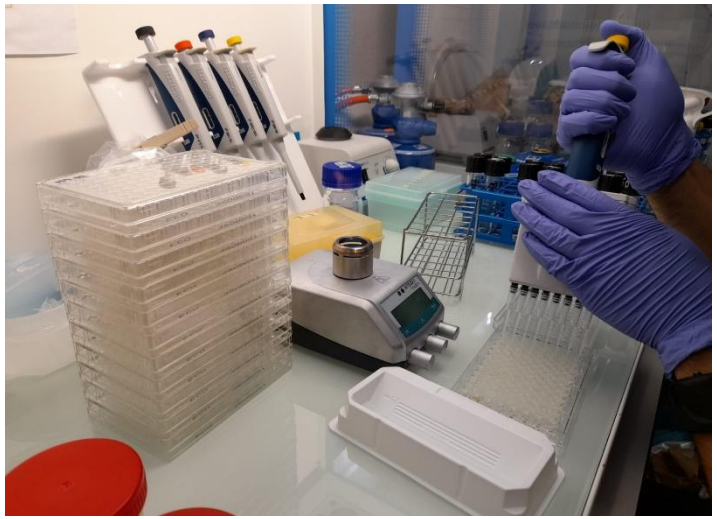
# FIELD WORKS



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# ATTRIBUTES I AND II. PLATE COUNT EXPERIMENTS

I — *Plate count Generic medium*

II — *Plate count Minimal medium*

III — *Biolog Ecoplate microplate*

IV — *Biolog MT2 microplate*

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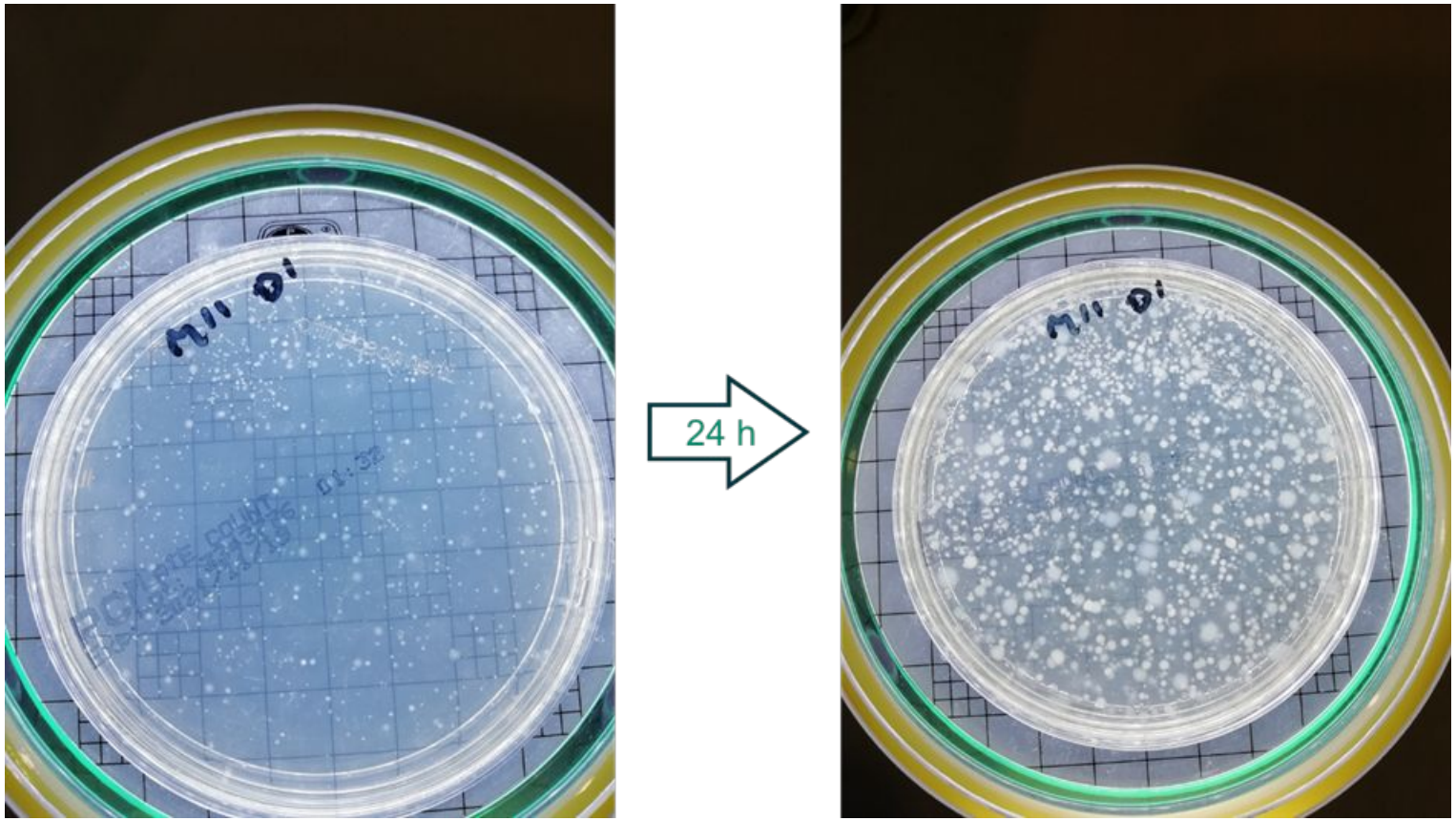


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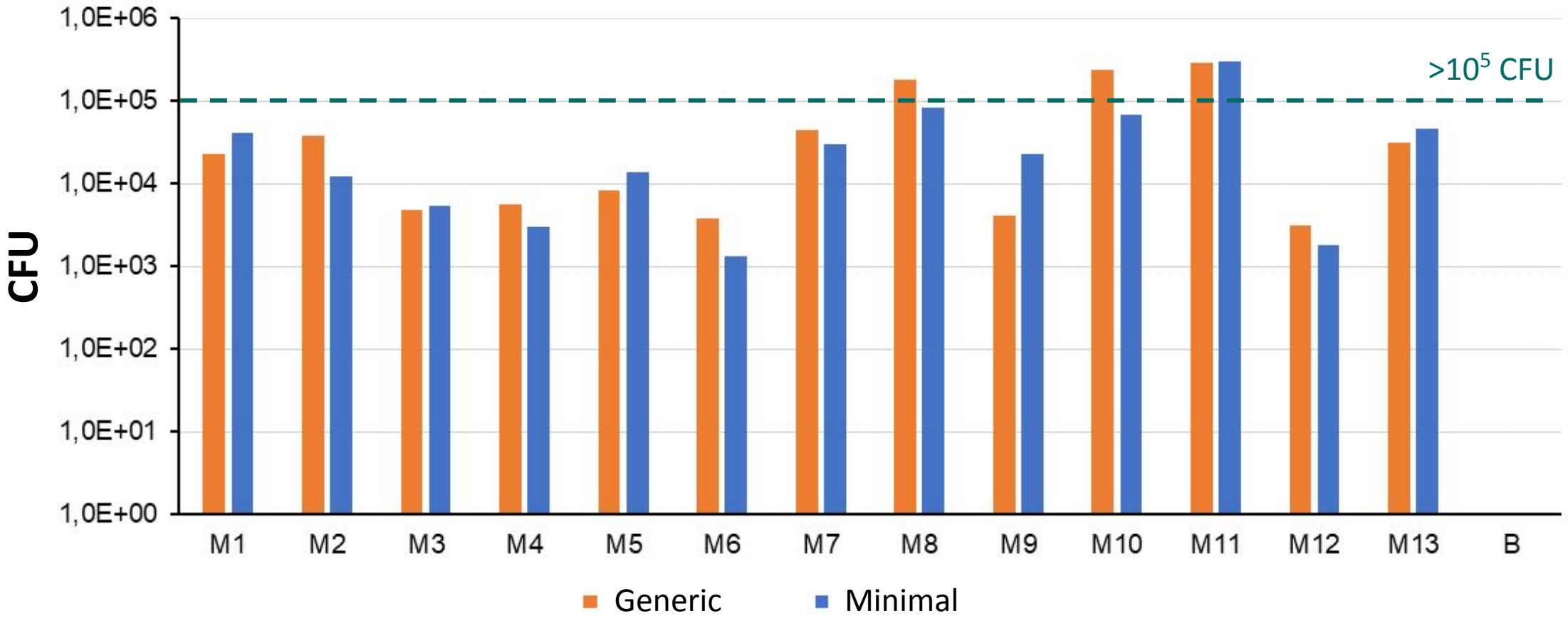

# ATTRIBUTES III AND IV. MICROPLATE EXPERIMENTS

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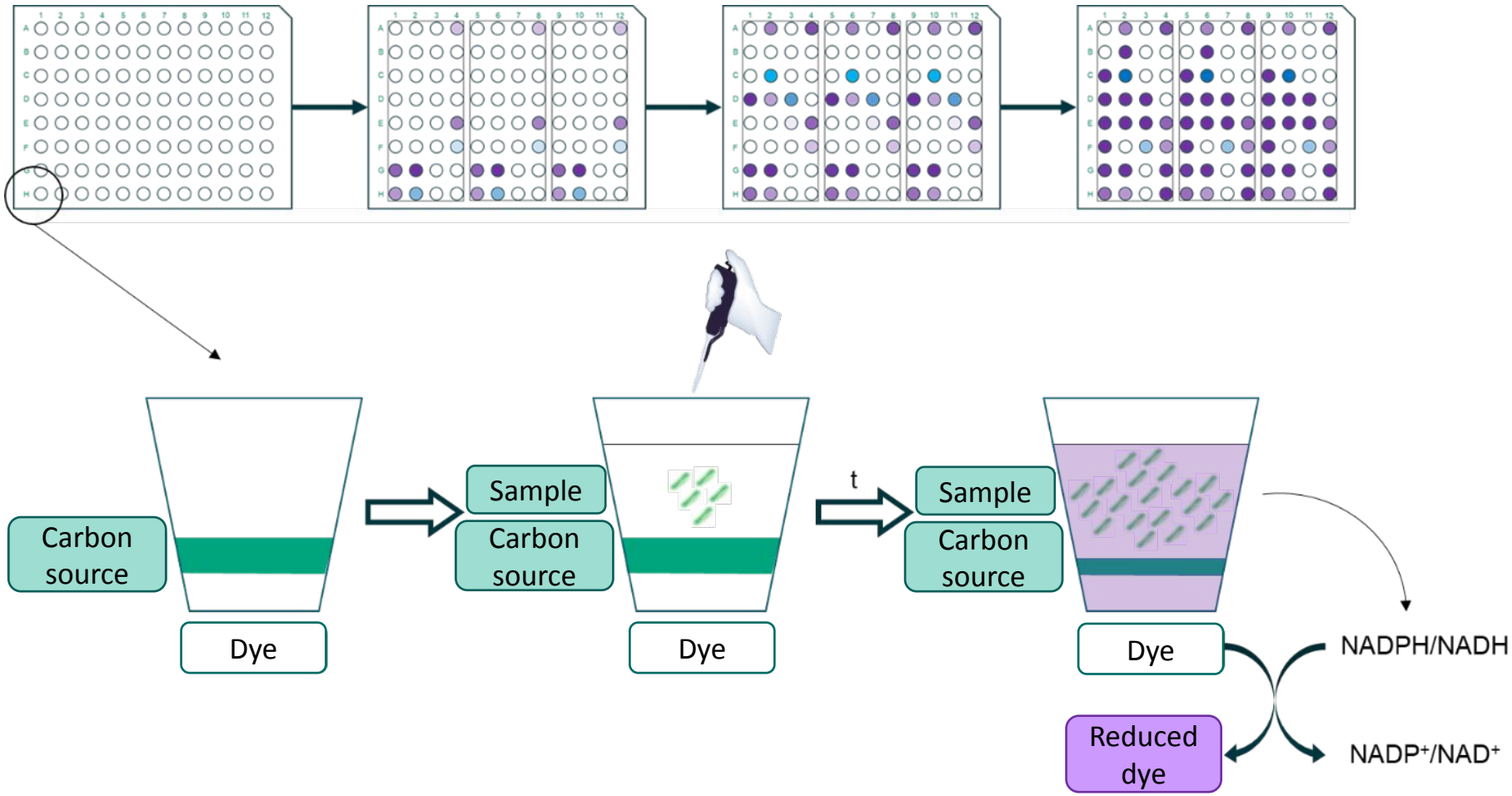
IV **Biolog MT2 microplate**



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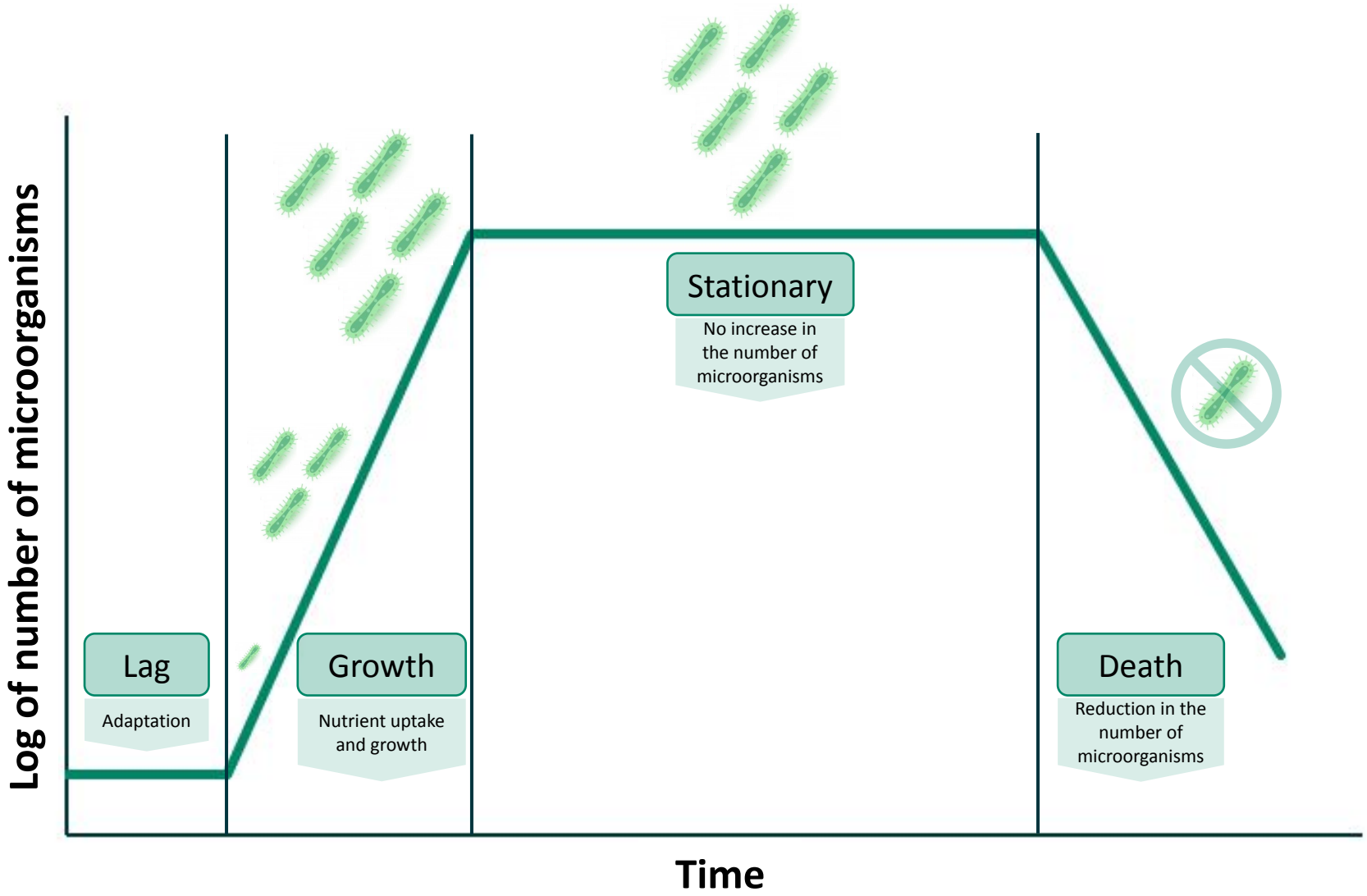

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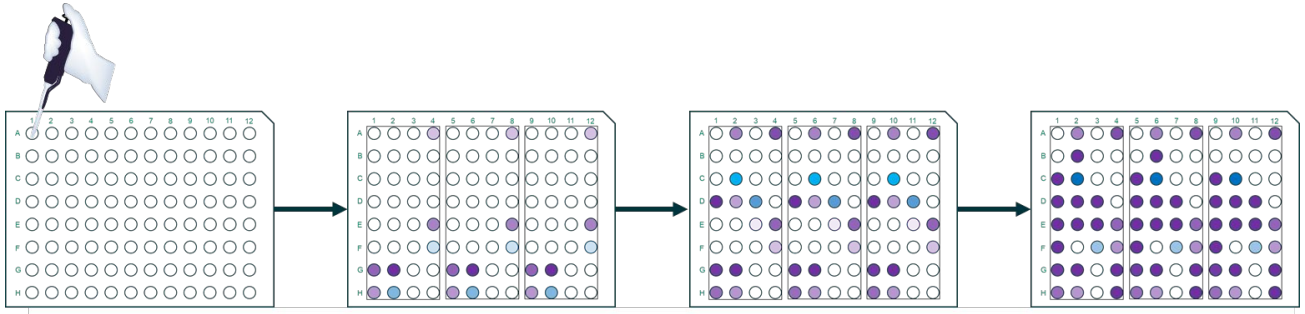

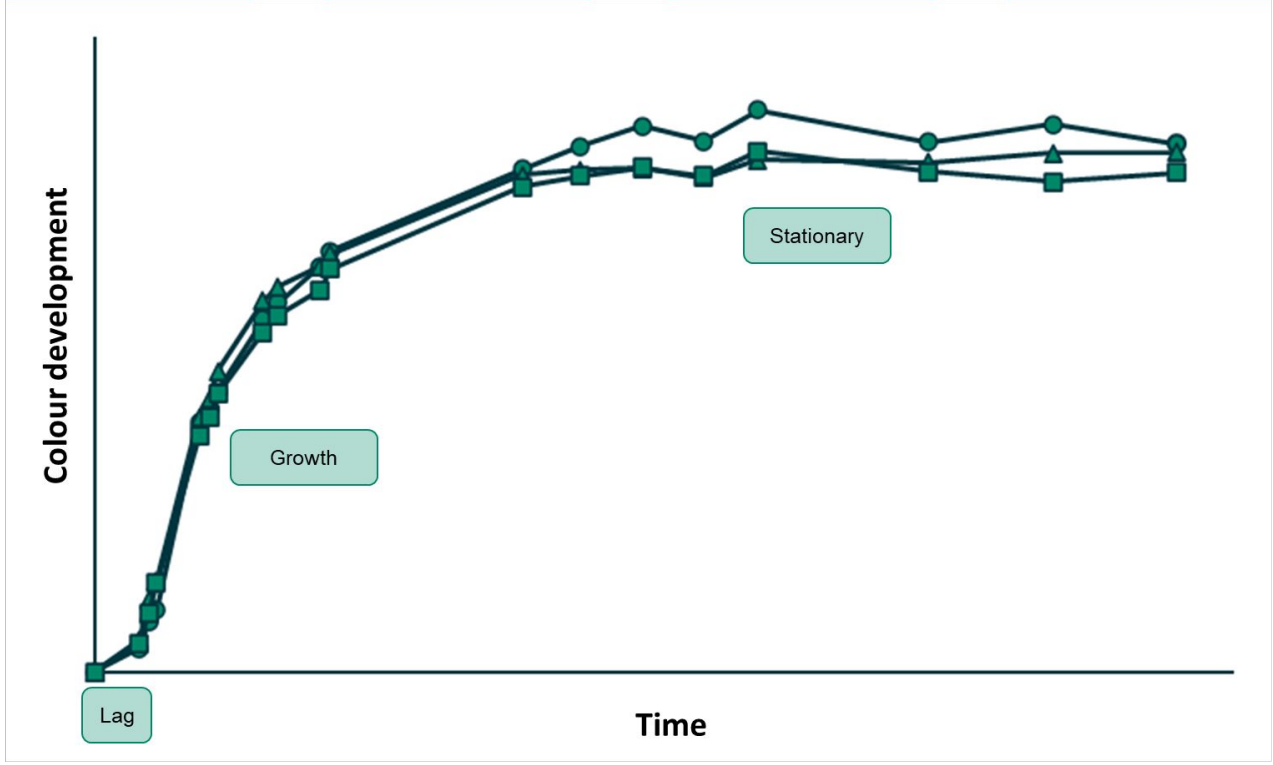


## Ecoplate

- Metabolic activity
- Biodiversity

## MT2

- Tolerable concentration of contaminants

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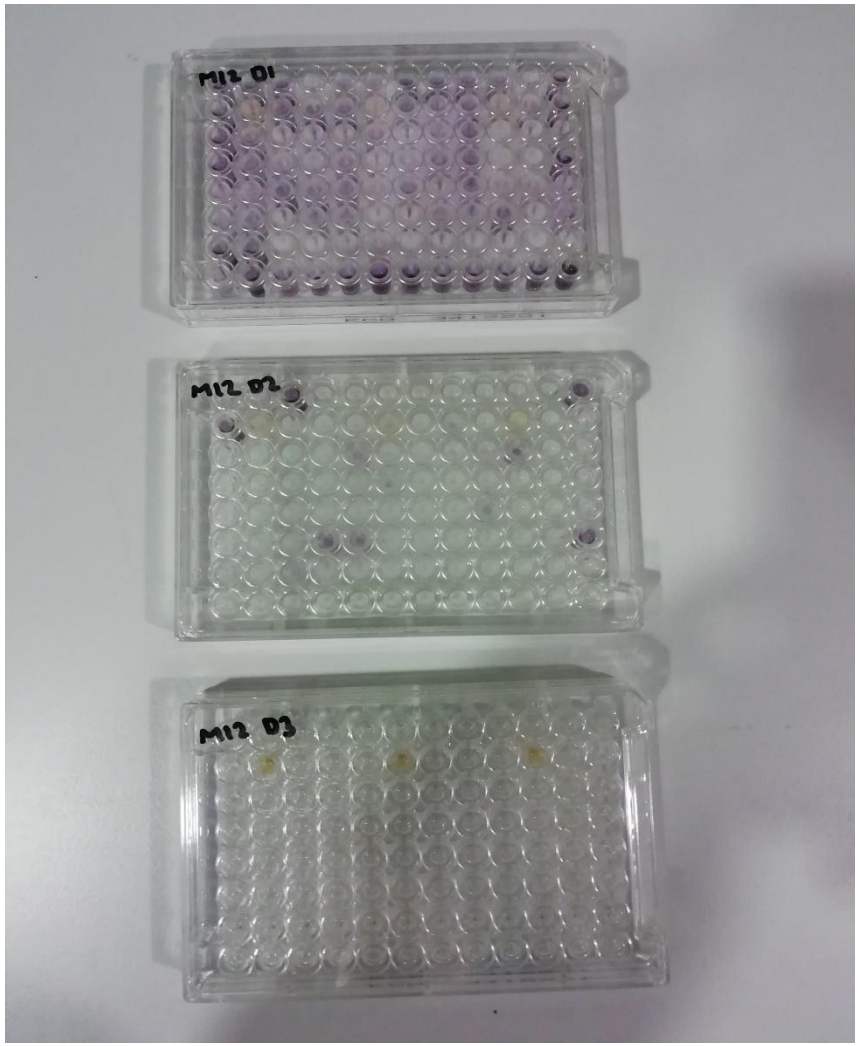


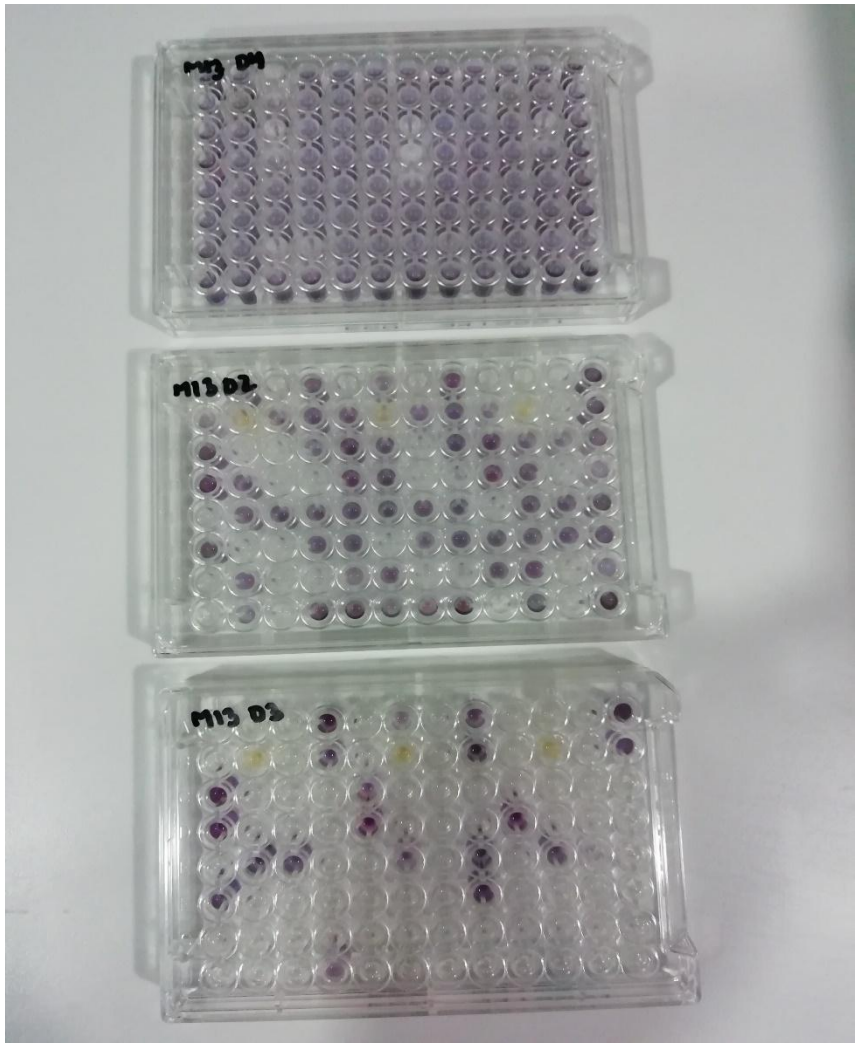


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# ATTRIBUTE III. MICROBIAL ACTIVITY AND DIVERSITY

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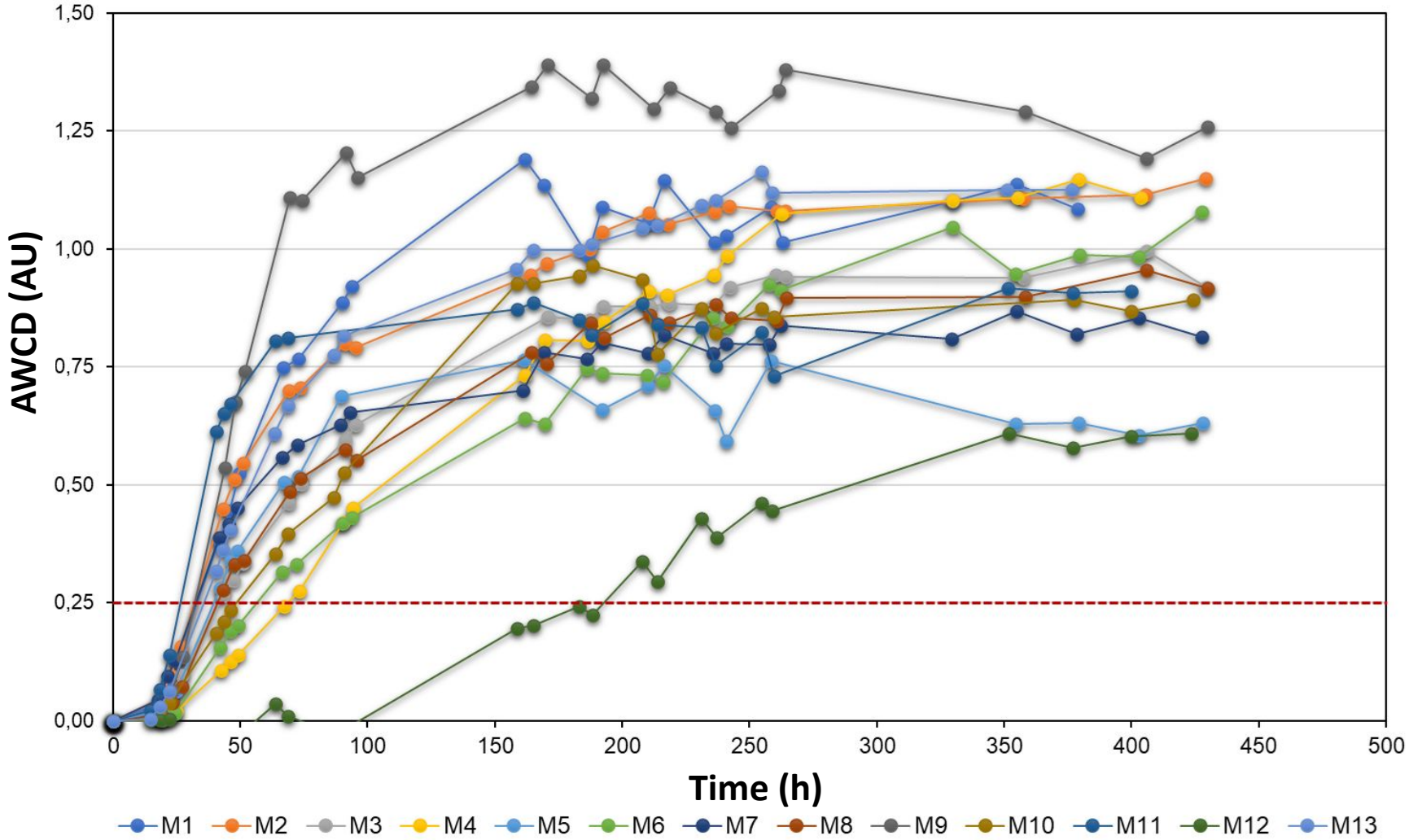

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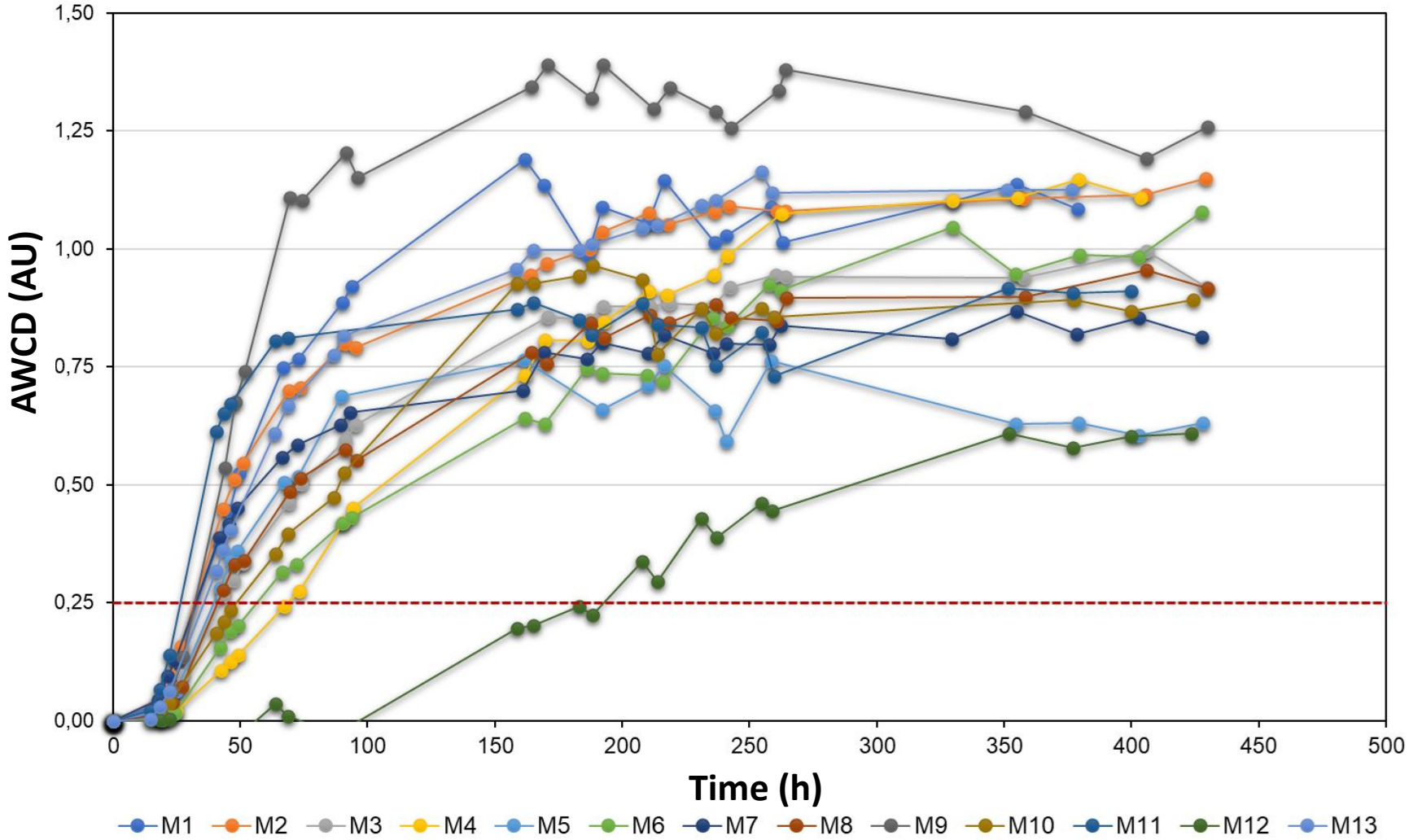


- **Lag phase duration**
- **t1/2**
- **AWCD**
- **NUS**
- **Diversity indexes**
  - Shannon > 3
  - Simpson > 0.9

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# ATTRIBUTE IV. TOLERANCE TO DIFFERENT CONTAMINANT CONCENTRATIONS

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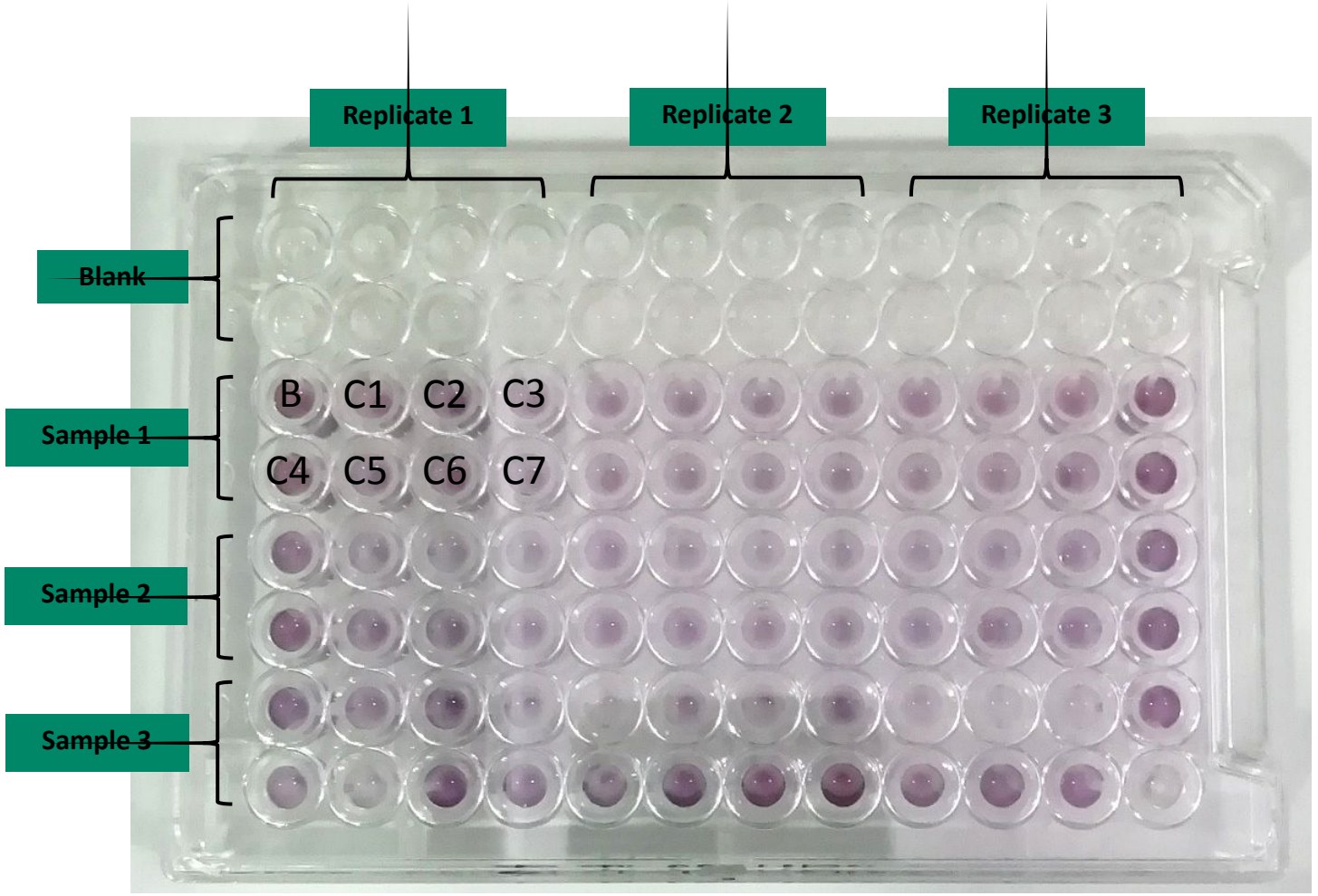
IV **Biolog MT2 microplate**

*7 different COC concentrations*

*C1 < C2 < … < C7*

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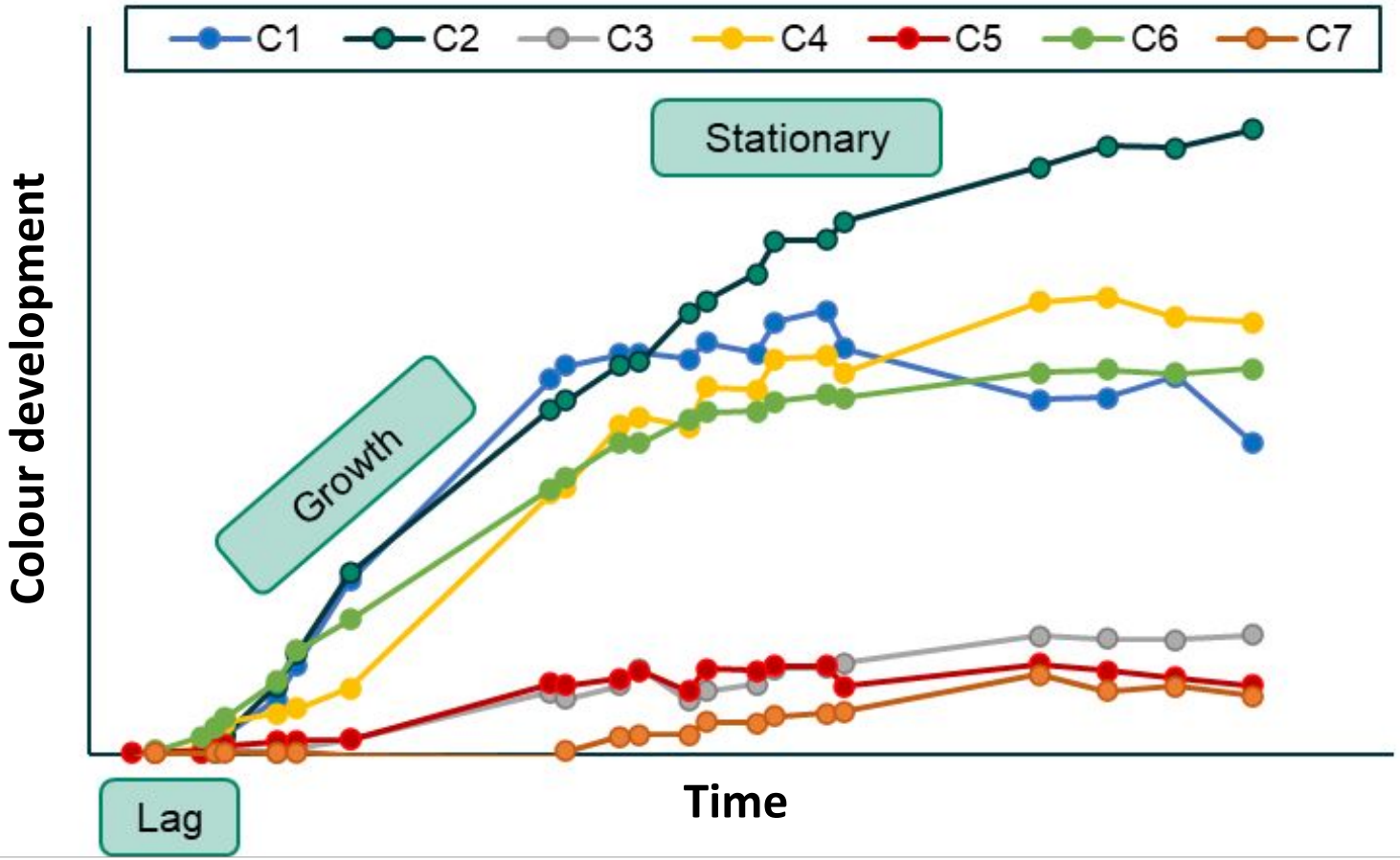


*C1 < C2 < … < C7*

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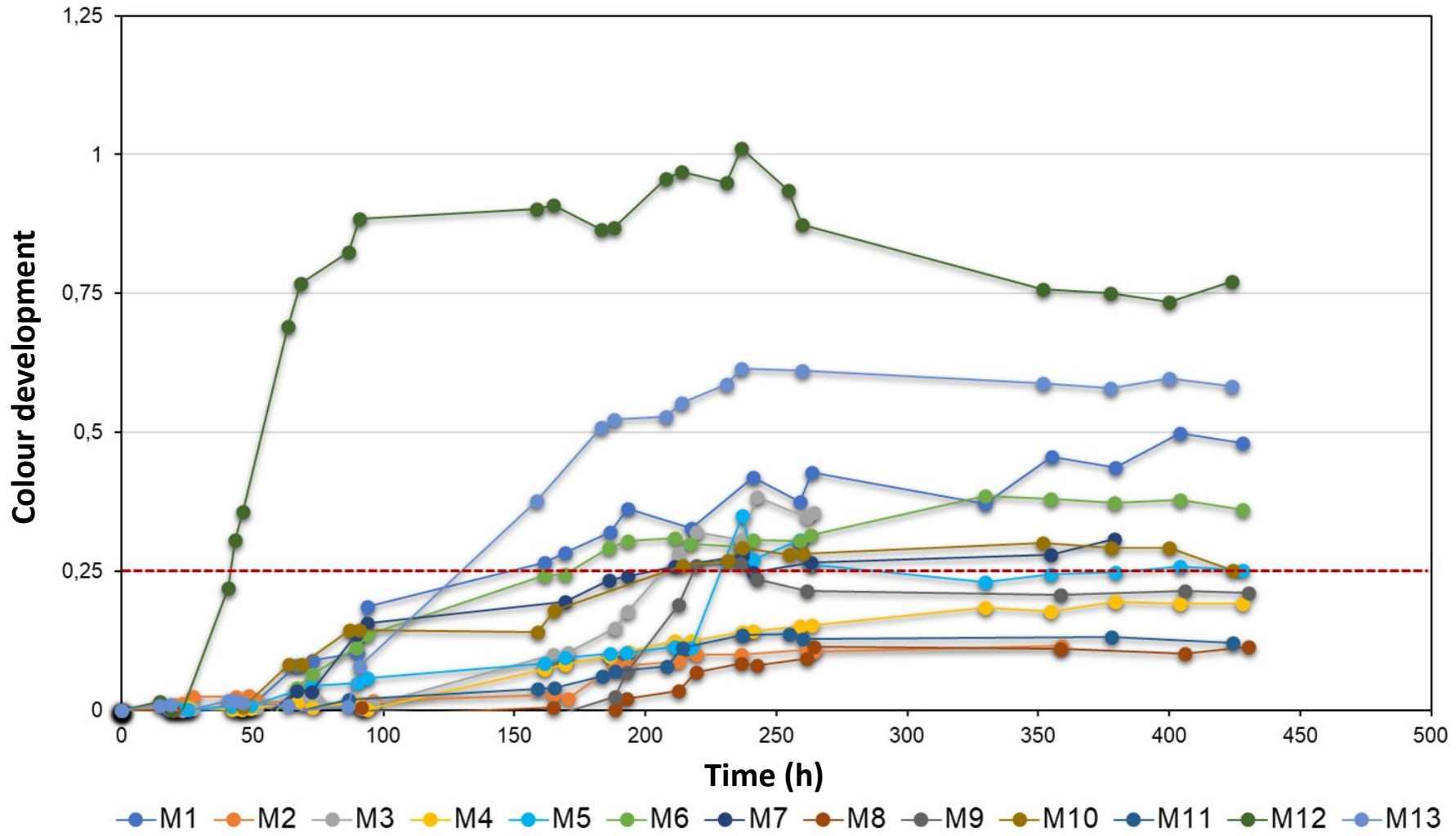


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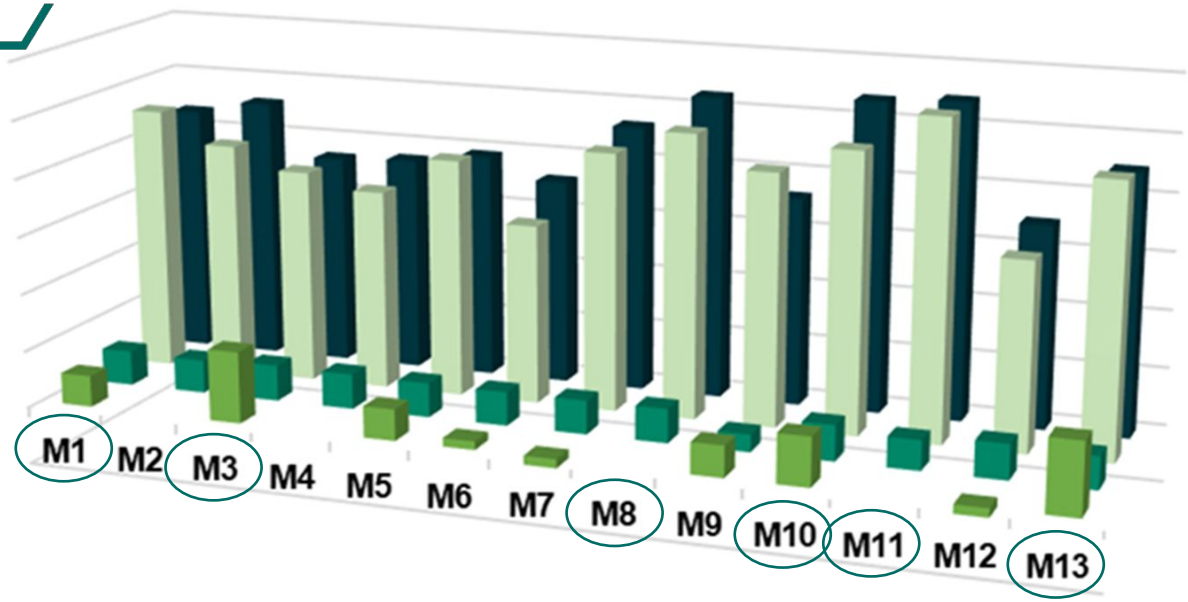
# CONCLUSIONS




- Viable and cultivable biomass in generic medium
- Viable and cultivable biomass in minimal medium (HCH as carbon source)
- Diversity (Shannon index)
- Tolerance (HCH concentration)

## Sample characterization

✔ Candidates for their potential use as microbial starters have been identified in the preliminary screening based on their CFU population (>$10^5$ CFU), microbial community diversity (Shannon indices above 3) and tolerance to concentrations of HCH between 1 to 35 ppm

## Candidate selection

✔ The most promising samples corresponded to matrices from areas of the site historically affected by HCH and with relatively homogeneous conditions over time: M1, M3, M8, M10, M11 y M13

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# THANK YOU FOR YOUR ATTENTION

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# INOCULANT IDENTIFICATION AND SELECTION

| No. | Matrix | Type | Description |
|---|---|---|---|
| M1 | Soil | Soil on the way to the new landfill | Soils impacted during the dismantling of the former landfill and transportation of residues to the new landfill. |
| M2 | Sludge | Downstream upwelling | An upwelling located downstream and in the lower part of the old landfill basin. It receives leachates from the former landfill surface and contributions from contaminated upwellings. Possibility to collect water and sludge at the same location |
| M3 | Water | Downstream upwelling | |
| M4 | Soil | Soil upstream the former landfill | Soils upstream of the former landfill. Impacted first during the exploitation of the old landfill and. later. while dismantling and transferring residues to the new landfill. |
| M5 | Sludge | Eastern shotcrete pond | Shotcrete pond located at the former landfill. Accumulates sediments dragged from the surface of the landfill. basically carbonate silts and some clays. with the presence of particulate HCH. Pond practically clogged and with some vegetation growing. |
| M6 | Sludge | Western shotcrete pond | Shotcrete pond located at the former landfill. Accumulates sediments dragged from the surface of the landfill basin. basically carbonate silts and some clays. with the presence of particulate HCH. The pond floods in storms and has not developed vegetation. Sampling at two levels to evaluate aerobic and anaerobic zones. |
| M7 | Sludge | Western shotcrete pond | |
| M8 | Groundwater | Piezometer | Piezometer with low concentrations of contaminants of concern. Mainly drilled in limestone. Low fracture density and limited water renewal. |
| M9 | Groundwater | Piezometer | Piezometer with high concentrations of contaminants of concern. Mainly drilled in sandstone and located just downstream the former landfill |
| M10 | Sediment | BT2 storm pond | Storm pond that accumulates sediments dragged from the former landfill and residues from other locations and current activities carried out at the site. It historically received a high pollutant load. It accumulates water and also has an area with solid and consolidated material. |
| M11 | Soil | BT2 storm pond | |
| M12 | Sediment | BT4 storm pond | Storm pond that accumulates sediments dragged from the former landfill. It has not received high contaminant loads over time |
| M13 | Sediment | BV1 discharge pond | Discharge pond at the outlet of the water treatment plant. Low HCH concentrations. |

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# ATTRIBUTE III. MICROBIAL ACTIVITY AND DIVERSITY

| Sample | Sample dilution | $t_{1/2}$ (h) | Lag phase (h) | Slope max (100.UA/h) | Area below curve $t_{1/2}$ (UA.h) | AWCD $t_{1/2}$ | $AWCD_{max}$ | NUS $t_{1/2}$ (n) | $NUS_{max}$ (n) | Shannon index[1] | Simpson index[2.*] | Shannon eveness index[3] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| M1 | $10^{-1}$ | 47 | 17.9 | 2.17 | 7.44 | 0.47 | 1.21 | 18 | 30 | 3.994 | 0.931 | 0.963 |
| M2 | $10^{-1}$ | 42 | 18.9 | 1.76 | 6.16 | 0.42 | 1.06 | 15 | 28 | 3.772 | 0.919 | 0.967 |
| M3 | $10^{-1}$ | 74 | 18.8 | 1.12 | 15.75 | 0.49 | 0.99 | 20 | 27 | 4.214 | 0.941 | 0.968 |
| M4 | $10^{-1}$ | 133 | 37.8 | 1.16 | 37.40 | 0.61 | 1.15 | 19 | 24 | 4.064 | 0.933 | 0.956 |
| M5 | $10^{-1}$ | 57 | 19.3 | 1.63 | 8.66 | 0.41 | 0.79 | 17 | 23 | 3.930 | 0.927 | 0.957 |
| M6 | $10^{-1}$ | 94 | 24.3 | 0.79 | 17.41 | 0.43 | 1.08 | 16 | 24 | 3.840 | 0.924 | 0.960 |
| M7 | $10^{-1}$ | 50 | 17.9 | 1.72 | 9.01 | 0.46 | 0.87 | 16 | 26 | 3.836 | 0.924 | 0.959 |
| M8 | $10^{-1}$ | 59 | 19.2 | 1.47 | 9.02 | 0.40 | 0.96 | 16 | 27 | 3.876 | 0.926 | 0.964 |
| M9 | $10^{-1}$ | 28 | 19.7 | 3.34 | 0.88 | 0.16 | 1.41 | 5 | 30 | 2.010 | 0.713 | 0.936 |
| M10 | $10^{-2}$ | 86 | 18.6 | 1.29 | 17.63 | 0.47 | 0.96 | 19 | 29 | 4.122 | 0.938 | 0.972 |
| M11 | $10^{-2}$ | 30 | 14.8 | 2.60 | 3.44 | 0.33 | 0.90 | 13 | 30 | 3.273 | 0.870 | 0.975 |
| M12 | $10^{-1}$ | 248 | 90.0 | 0.77 | 32.02 | 0.43 | 0.61 | 19 | 24 | 4.071 | 0.935 | 0.963 |
| M13 | $10^{-2}$ | 51 | 14.8 | 1.71 | 8.09 | 0.46 | 1.10 | 15 | 27 | 3.659 | 0.912 | 0.952 |

[1]Shannon index (H'): Value between 0.5 and 5. Values below 2 are considered low in diversity and above 3 are considered high in species diversity.

[2]Simpson index (D): Value between 0 and 1. The higher the value. the greater the diversity of the sample.

[3]Shannon evenness index (J'): Value between 0 and 1. The higher the value. the greater the biodiversity of the sample.

*Reciprocal value of Simpson's index: D-1

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# ATTRIBUTE IV. TOLERANCE TO DIFFERENT CONTAMINANT CONCENTRATIONS

| Sample | Sample dilution | COC stock solution dilution | [HCH] (ppm)* | Lag phase (h) | $t_i$ stationary pase (h) | $Abs_i$ stationary phase | t $Abs_{max}$ (h) | $Abs_{max}$ |
|---|---|---|---|---|---|---|---|---|
| M1 | $10^{-3}$ | 1/10 | 3.5 | 26 | 237 | 0.455 | 404 | 0.499 |
| M2 | $10^{-1}$ | 1 | 35 | 171 | 188 | 0.081 | 406 | 0.116 |
| M3 | $10^{-1}$ | 1/2 | 17.5 | 96 | 242 | 0.383 | 242 | 0.383 |
| M4 | $10^{-2}$ | 1/10 | 3.5 | 94 | 330 | 0.185 | 379 | 0.196 |
| M5 | $10^{-2}$ | 1/10 | 3.5 | 49 | 237 | 0.349 | 237 | 0.349 |
| M6 | $10^{-2}$ | 1/25 | 1.4 | 49 | 193 | 0.304 | 330 | 0.386 |
| M7 | $10^{-3}$ | 1/50 | 0.7 | 49 | 162 | 0.253 | 379 | 0.308 |
| M8 | $10^{-3}$ | 1 | 35 | 165 | 262 | 0.114 | 430 | 0.128 |
| M9 | $10^{-3}$ | 1/10 | 3.5 | 165 | 219 | 0.261 | 236 | 0.264 |
| M10 | $10^{-3}$ | 1/5 | 7 | 46 | 237 | 0.292 | 352 | 0.300 |
| M11 | $10^{-3}$ | 1/5 | 7 | 44 | 237 | 0.135 | 255 | 0.136 |
| M12 | $10^{-1}$ | 1/50 | 0.7 | 24 | 159 | 0.902 | 237 | 1.012 |
| M13 | $10^{-3}$ | 1/2 | 17.5 | 87 | 183 | 0.509 | 237 | 0.614 |

*Concentration estimated from laboratory analysis of the COC stock solution prior to preparation of MT2 microplates.

Red: VAbsorbance value <0.25. Not enough color development to consider a good growth rate and substrate utilization.