

## **Aerobic TCE degradation by willows and three root colonizing strains of *B. cepacia***

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# Aerobic TCE degradation by willows and three root colonizing bacterial strains of *B. cepacia*

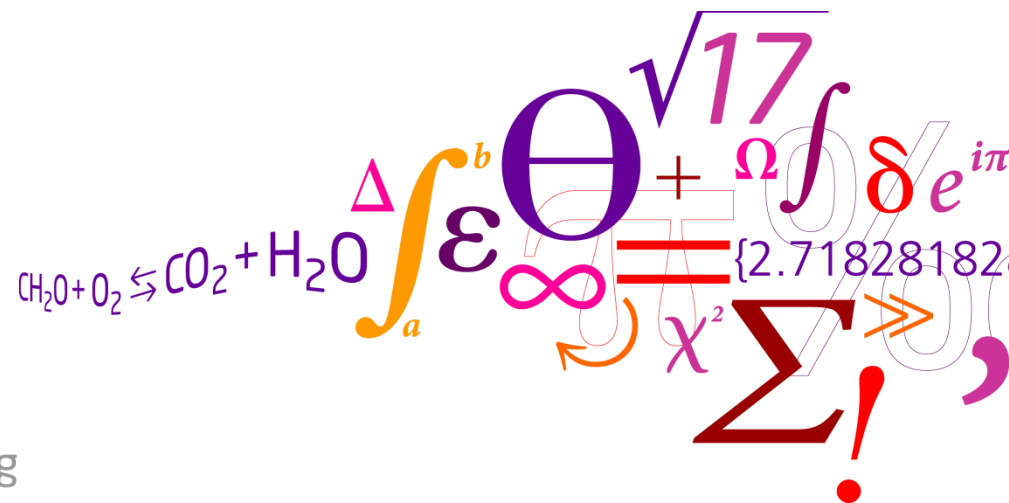
By

**Lauge Clausen**

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Mette Broholm

Stefan Trapp



DTU Environment

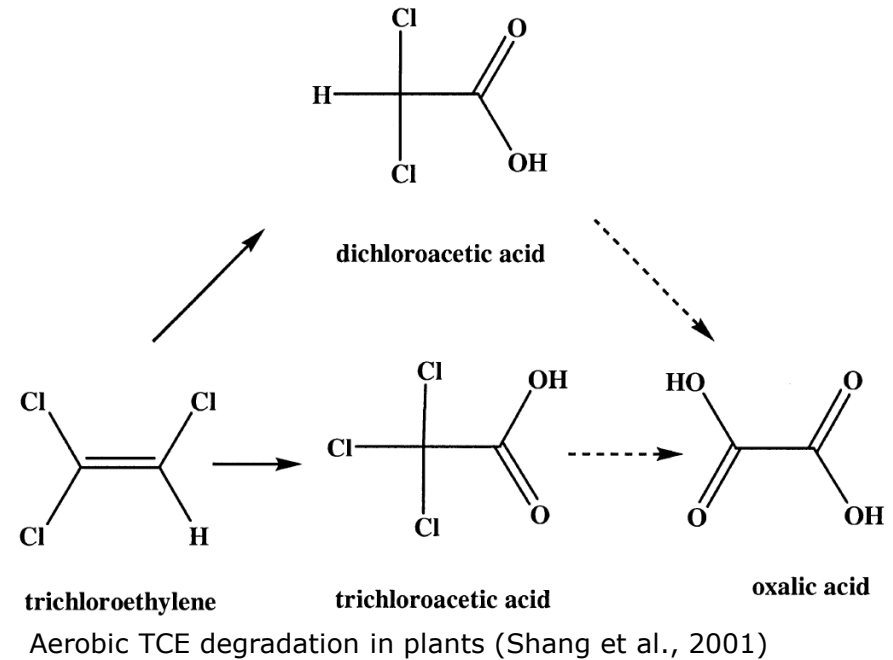
Department of Environmental Engineering

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# Introduction

Several studies indicate aerobic **Trichloroethylene (TCE)** degradation by plants.

Degradation pathway similar to that of mammals (monooxygenase):



TCE → **trichloroacetic acid** (TCAA) and **dichloroacetic acid** (DCAA) → oxalic acid

But we know very little about the rates...

# Test groups

| Group           | Description  | No. of replicates |
|-----------------|--|-------------------|
| Control with Cl | Standard Hoagland nutrient solution, no TCE                        | 5                 |
| Control no Cl   | Modified Hoagland with no Cl, no TCE                               | 5                 |
| Low exposure    | Start exposure 5 mg TCE/L, no added bacteria                       | 5                 |
| High exposure   | Start exposure 20 mg TCE/L, no added bacteria                      | 5                 |
| Dead            | 20 mg TCE/L with a dead willow stick, no refill, no added bacteria | 4                 |
| Control         | Start exposure 25 mg TCE/L, no added bacteria                      | 6                 |
| 301C            | Start exposure 25 mg TCE/L, <i>B. cepacia</i> 301C                 | 6                 |
| PRI-31          | Start exposure 25 mg TCE/L, <i>B. cepacia</i> PRI-31               | 6                 |
| pTOM            | Start exposure 25 mg TCE/L, <i>B. cepacia</i> pTOM                 | 6                 |
| c301C           | <i>B. cepacia</i> 301C, no TCE                                     | 3                 |

Temp. ~ 20°C; Light intensity ~ 5000 lux; Humidity ~ 50%

# Lab set-up





# ***Burkholderia cepacia*** (*Pseudomonas cepacia*)

Gram-negative

Rod-shaped

Size: 1.6- 3.2  $\mu\text{m}$

Degradation of chlorinated compounds (TCE, 2,4,5-trichlorophenoxyacetic acid and more)

(Folsom, 1990)

Colonize plant roots

(DMI, 2015)

*B. Cepacia* strains used:

301C

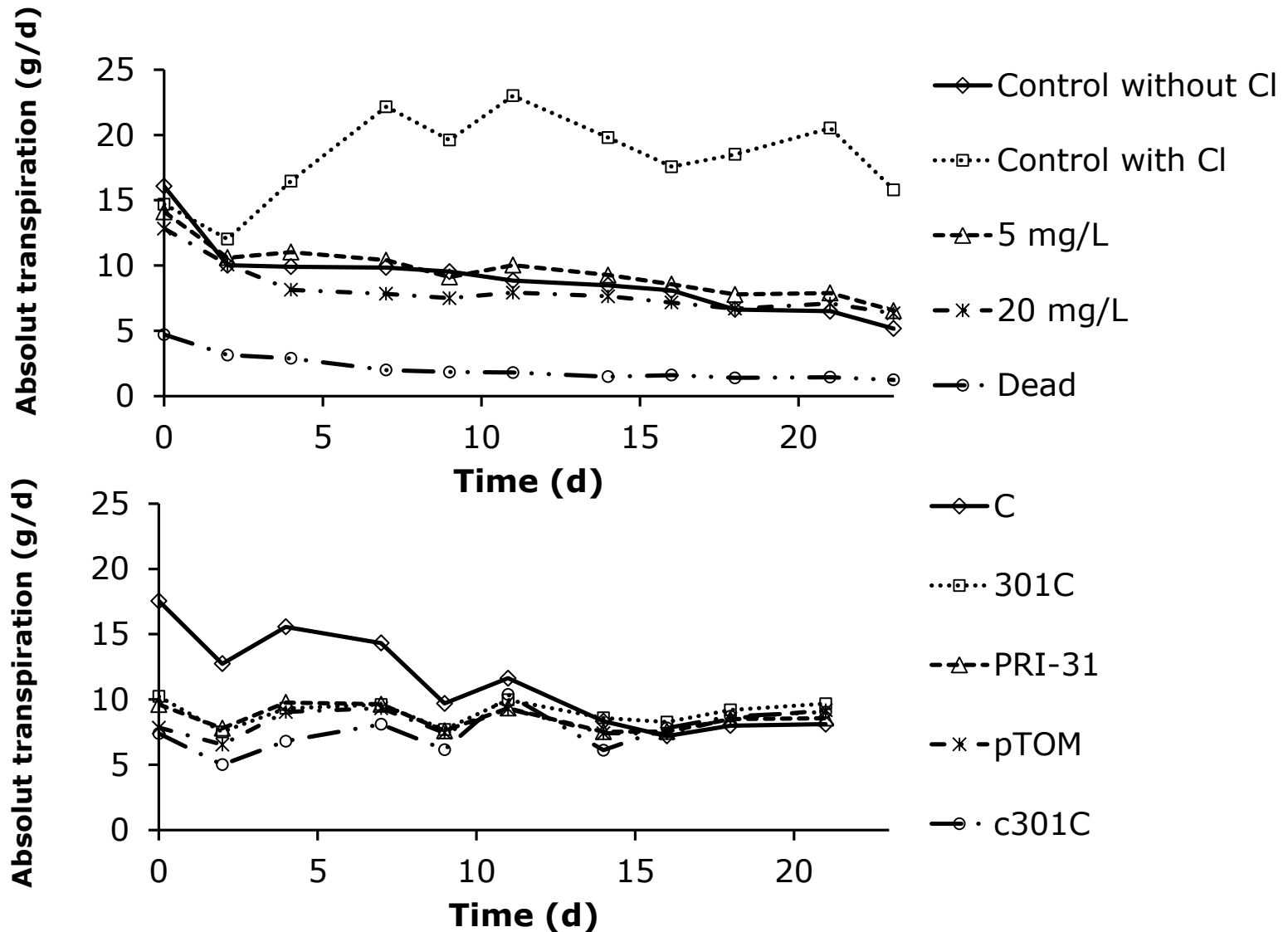
PRI-31

pTOM

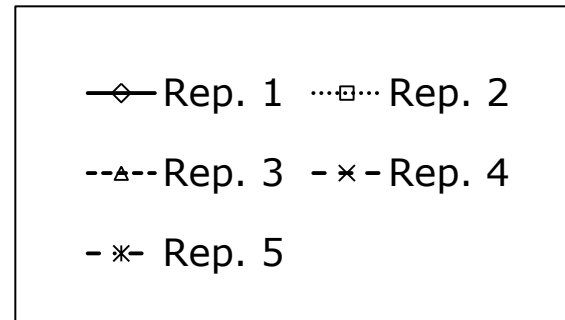
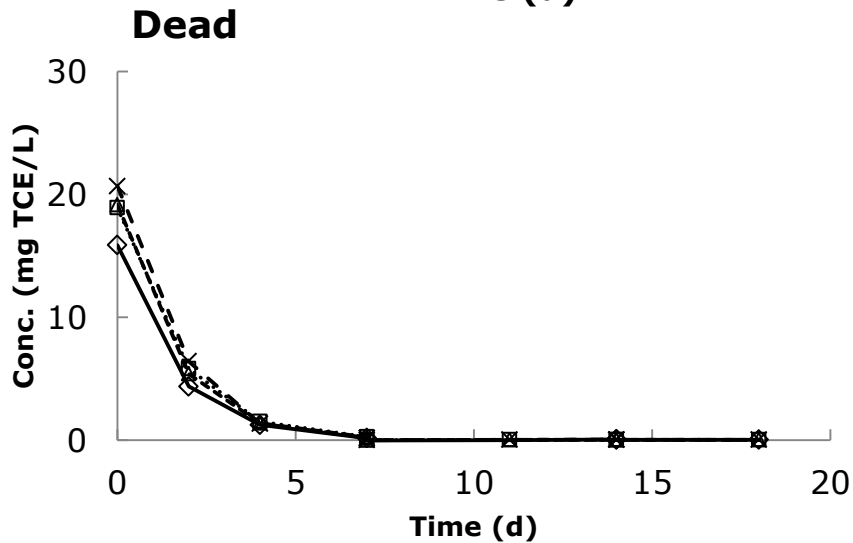
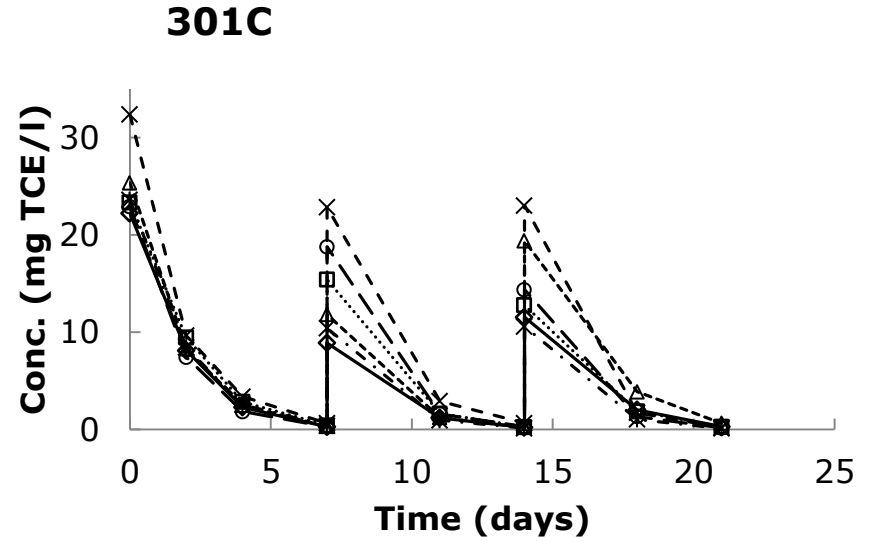
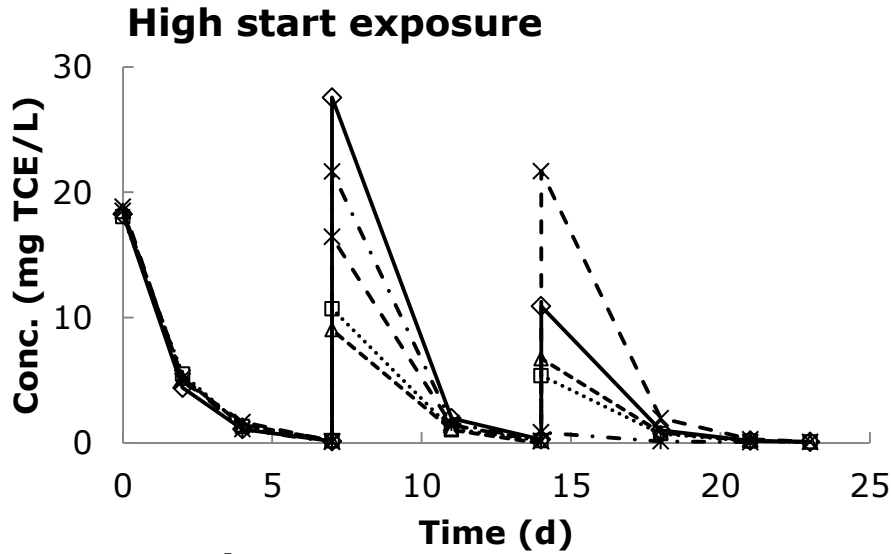


*Burkholderia cepacia*, (PHIL, 2015)

# Transpiration of the willows



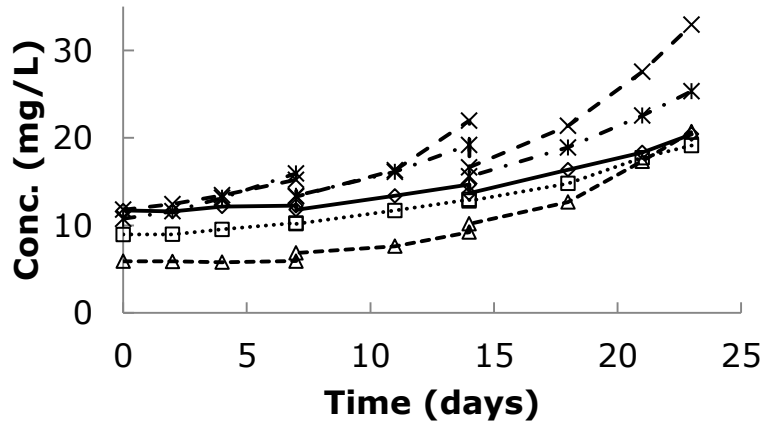
# TCE in solution



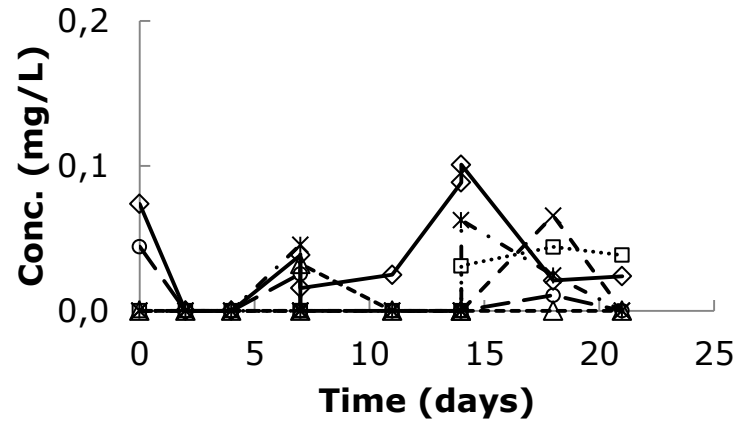


# Cl in solution

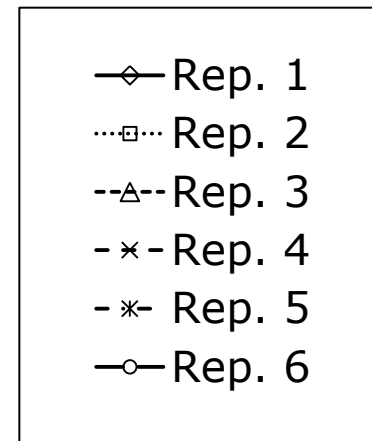
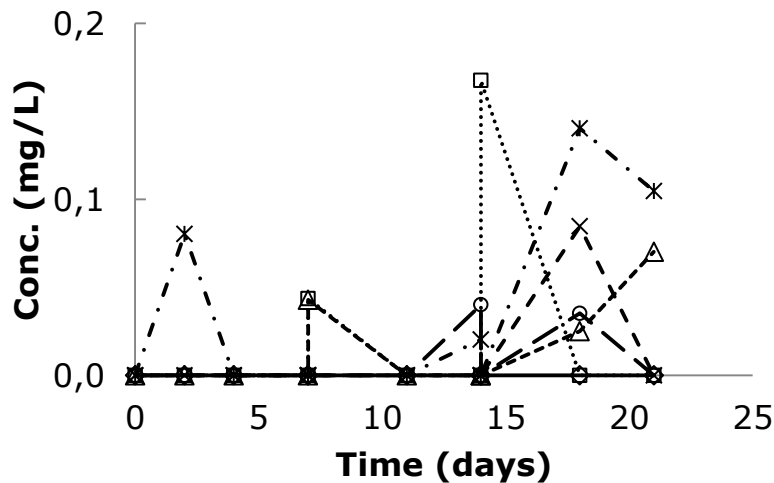
**Control (with Cl)**



**Control (TCE, no added bac.)**

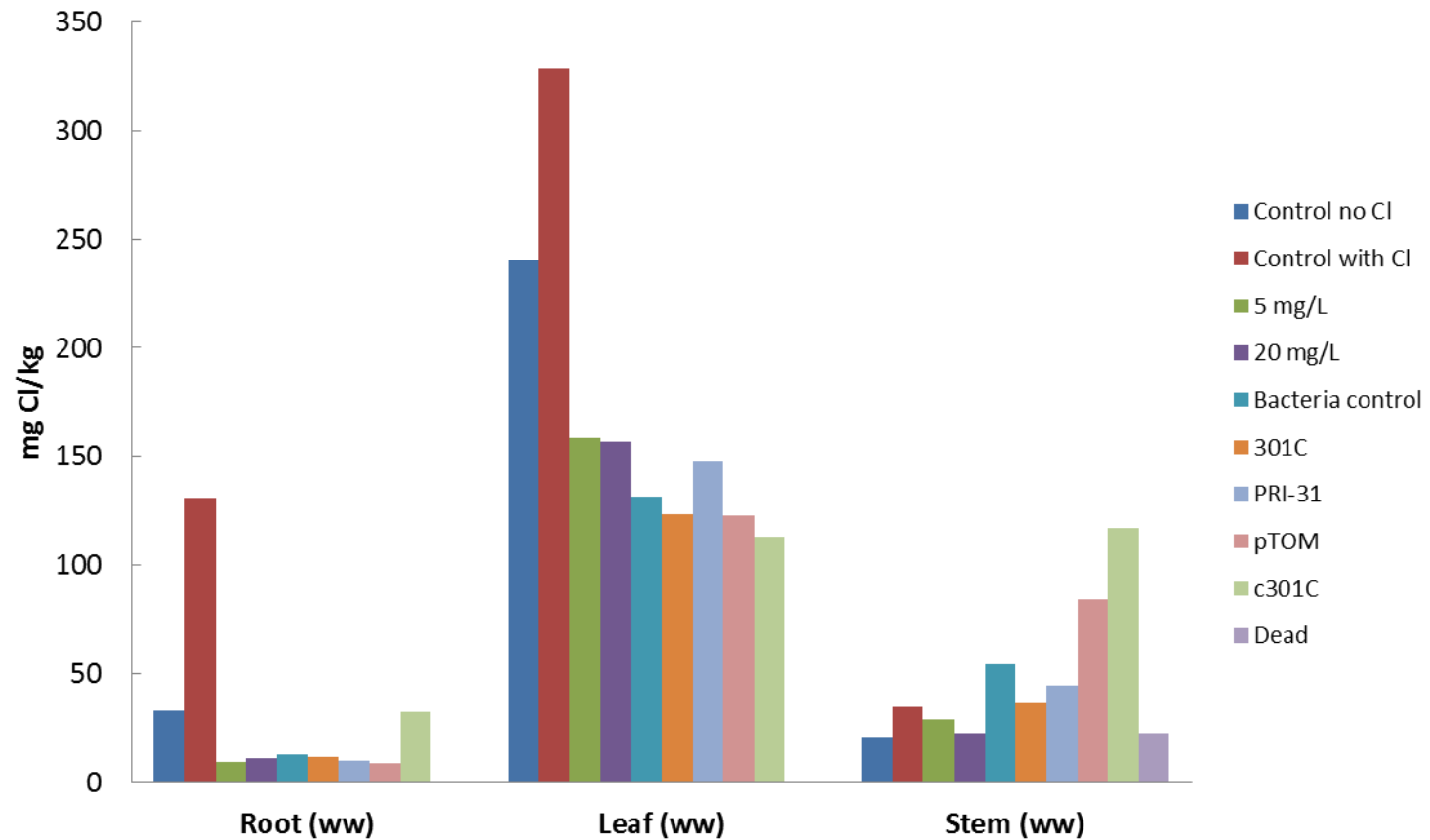


**301C**



# Cl in plant tissue

Average plant tissue Cl conc. (ww) at termination



# Cl mass

Mass of Cl (mg) added to the test-systems as TCE, total mass of Cl (mg) in the plants, Cl mass (mg) in solution, total Cl mass present and % distribution of Cl mass in the plant compartments: Root, Leaf and stem, at experiment termination.

| <b>Group</b>                  | <b>Cl added (mg)</b> | <b>Cl in plant (mg)</b> | <b>Cl in sol. (mg)</b> | <b>Tot. Cl (mg)</b> |
|-------------------------------|----------------------|-------------------------|------------------------|---------------------|
| <b>Control no Cl</b>          | 0.0 (0.0)            | 1.9 (0.9)               | 0.0 (0.0)              | 1.9 (0.9)           |
| <b>Control with Cl</b>        | 89.1 (5.7)           | 4.5 (0.9)               | 5.6 (1.2)              | 10.1 (2.1)          |
| <b>5 mg TCE/L</b>             | 18.9 (6.5)           | 1.9 (0.9)               | 0.0 (0.0)              | 1.9 (0.9)           |
| <b>20 mg TCE/L</b>            | 20.7 (5.2)           | 1.3 (0.3)               | 0.1 (0.1)              | 1.4 (0.4)           |
| <b>Dead</b>                   | 6.3 (0.7)            | 0.8 (0.2)               | 0.0 (0.0)              | 0.8 (0.2)           |
| <b>Control (TCE, no bac.)</b> | 20.9 (3.0)           | 2.4 (1.0)               | 0.0 (0.0)              | 2.4 (1.0)           |
| <b>301C</b>                   | 19.2 (3.4)           | 1.6 (0.7)               | 0.0 (0.0)              | 1.6 (0.7)           |
| <b>PR1-31</b>                 | 18.4 (1.4)           | 1.8 (1.1)               | 0.0 (0.0)              | 1.8 (1.1)           |
| <b>pTOM</b>                   | 17.0 (1.7)           | 2.5 (2.8)               | 0.0 (0.0)              | 2.5 (2.8)           |
| <b>301C, no TCE</b>           | 0.0 (0.0)            | 4.9 (6.6)               | 0.0 (0.0)              | 4.9 (6.6)           |

# TCE removal rates

First order removal rates (1/day)

| <b>Group</b>                  | <b>rate <i>k</i></b><br>(week 1) | <b>rate <i>k</i></b><br>(week 2) | <b>rate <i>k</i></b><br>(week 3) |
|-------------------------------|----------------------------------|----------------------------------|----------------------------------|
| <b>5 mg TCE/L</b>             | 0.77 (0.07)                      | 0.62 (0.06)                      | 0.56 (0.06)                      |
| <b>20 mg TCE/L</b>            | 0.70 (0.05)                      | 0.63 (0.06)                      | 0.55 (0.05)                      |
| <b>Dead</b>                   | 0.65 (0.03)                      | -                                | -                                |
| <b>Control (TCE, no bac.)</b> | 0.65 (0.06)                      | 0.58 (0.04)                      | 0.62 (0.06)                      |
| <b>301C</b>                   | 0.61 (0.03)                      | 0.58 (0.04)                      | 0.60 (0.07)                      |
| <b>PR1-31</b>                 | 0.66 (0.07)                      | 0.60 (0.08)                      | 0.64 (0.09)                      |
| <b>pTOM</b>                   | 0.64 (0.05)                      | 0.53 (0.03)                      | 0.59 (0.05)                      |

Values are average of the replicates. Numbers in brackets indicate +/- 95% confidence intervals.

# Conclusion

1. Cl conc. in solution increases as the willows take up water
2. No or very little TCE was mineralized to Cl
3. We cannot see significant difference between the TCE removal rates  $k_{vol}$ ,  $k_{vol} + k_{tree}$  and  $k_{vol} + k_{tree} + k_{bac}$ .

## To do...

To improve the study use labelled TCE.

Analysis which will be done:

TCAA (samples stored at -80 °C)

Microbial community of the plant roots

# Questions?

# References

Department of Microbiology and Immunology (DMI), 2015, College of Medicine, University of Illinois; "Genes for 2,4,5- Trichlorophenoxyacetic Acid Metabolism in *B. cepacia* AC1100".

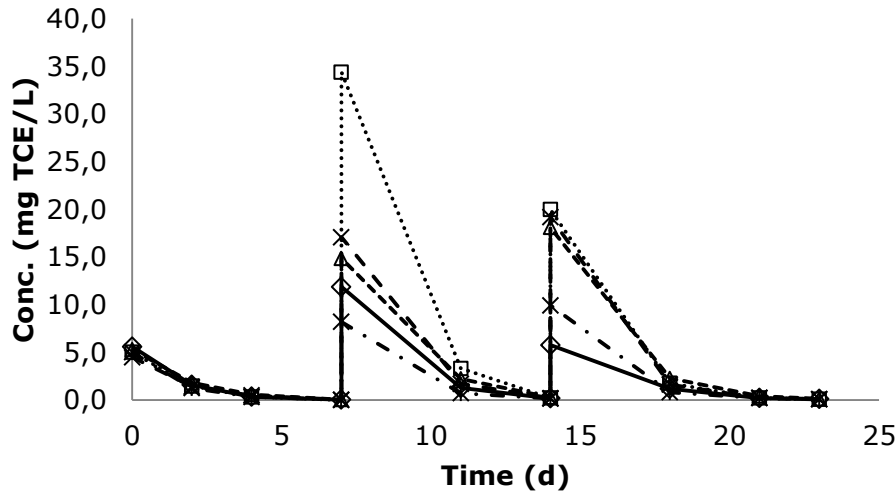
Folsom, B.R., Chapman, P.J. and Pritchard P.H., 1990, Phenol and trichloroethylene degradation by *Pseudomonas cepacia* G4: Kinetics and interactions between substrates. *Appl. Environ. Microbiol.* 56 (5): 1279-1285.

Public Health Image Library (PHIL), 2015, Center for disease control and prevention, link: <http://phil.cdc.gov/phil/details.asp?pid=255> – accessed 15/09/2015

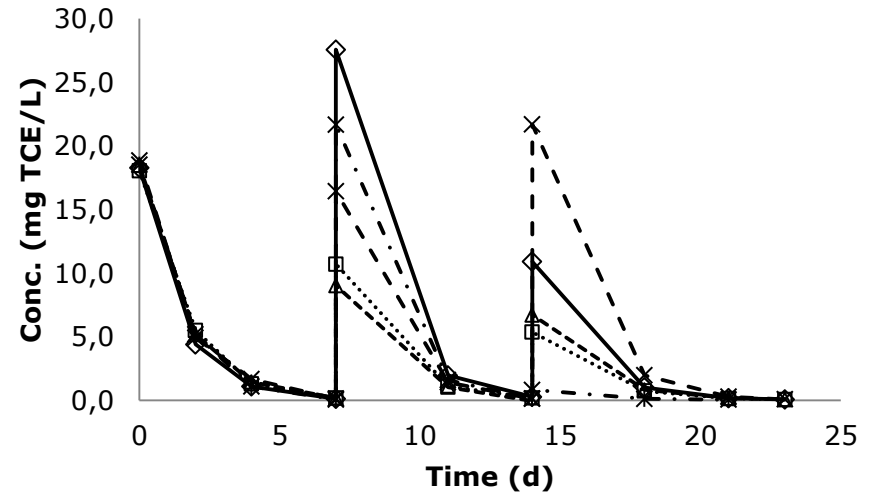


# TCE in solution

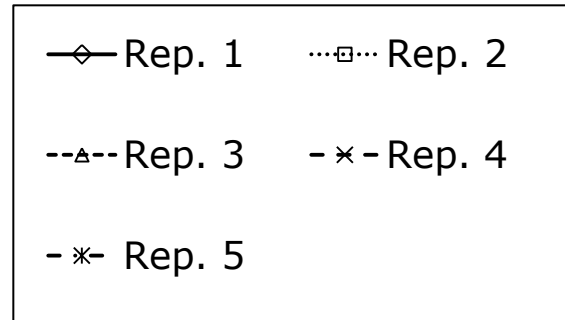
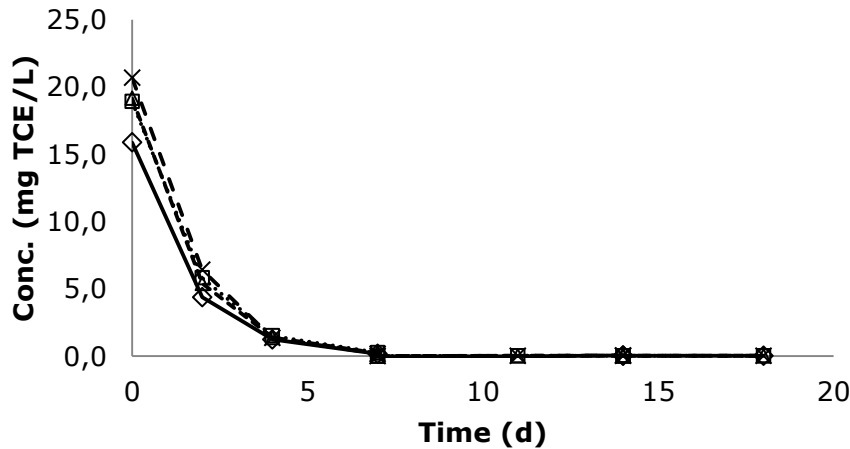
Start exposure 5 mg TCE/L



Start exposure 20 mg TCE/L

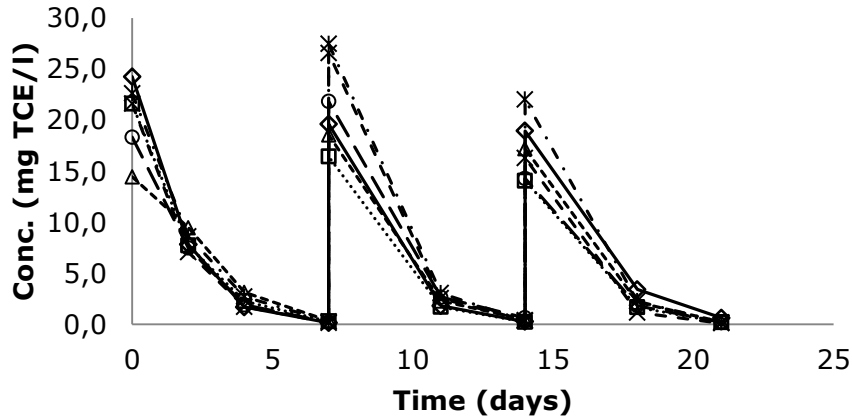


TCE evaporation (Start exposure 20 mg TCE/L)

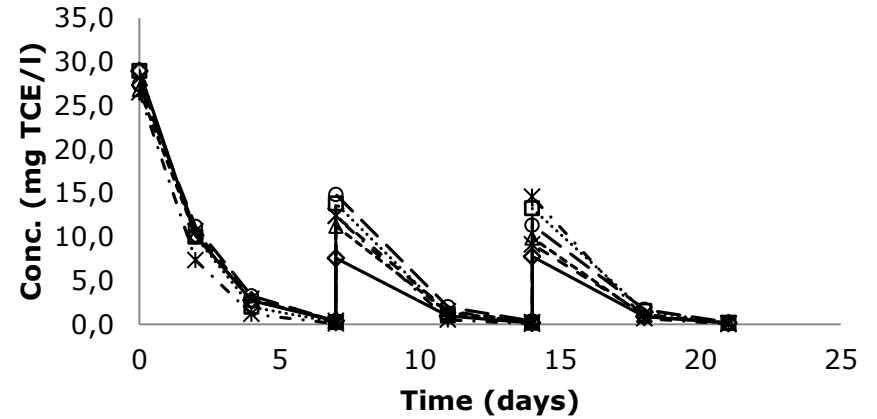


# TCE in solution

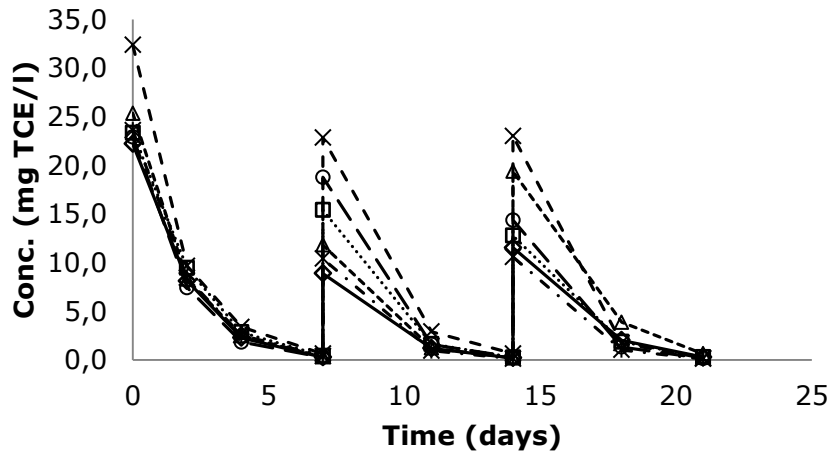
## Control (TCE, no added bacteria)



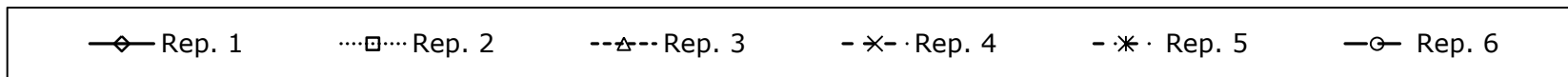
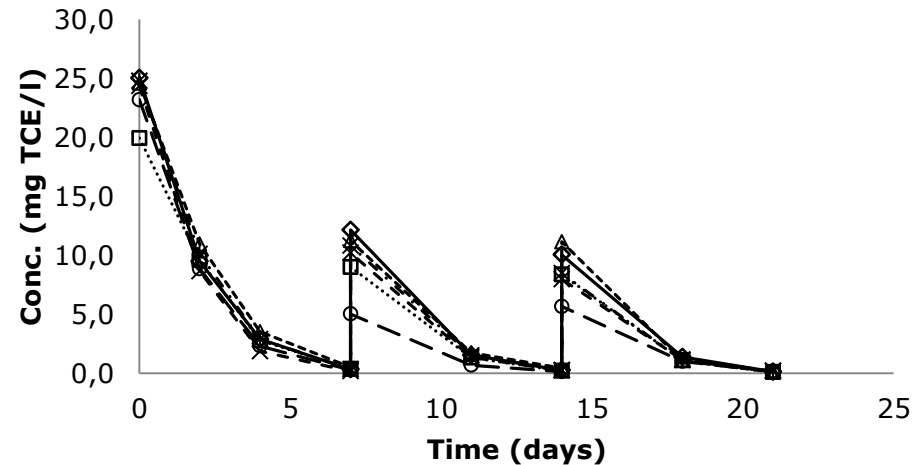
## PRI-31



## 301C

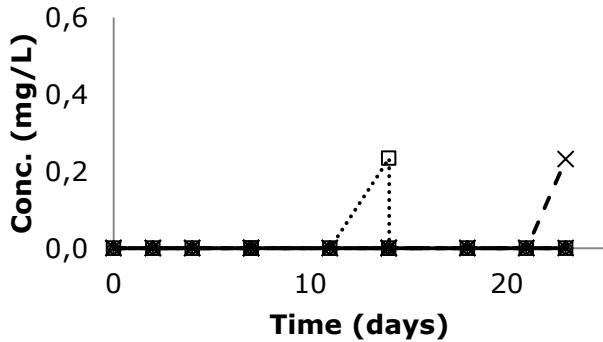


## pTOM

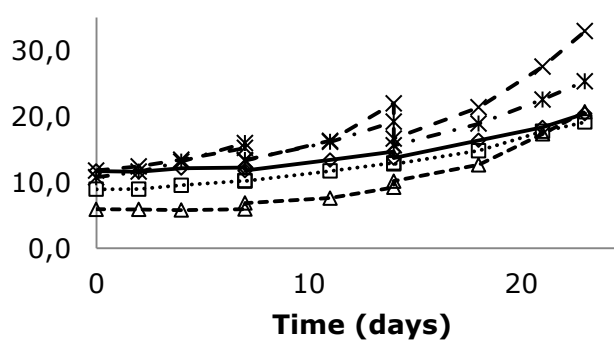


# Cl in solution

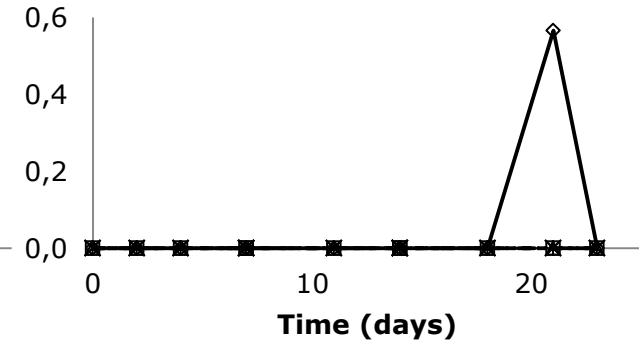
**Control (no Cl)**



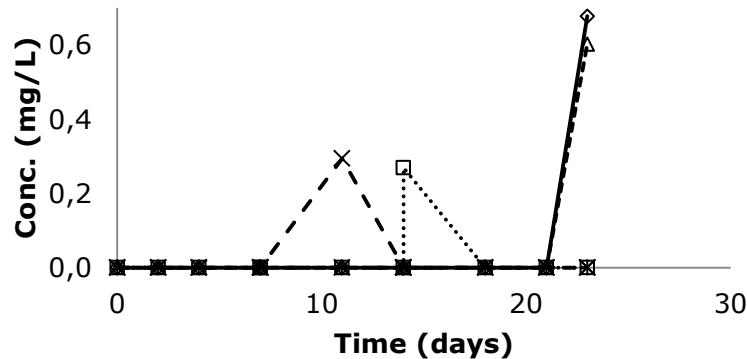
**Control (with Cl)**



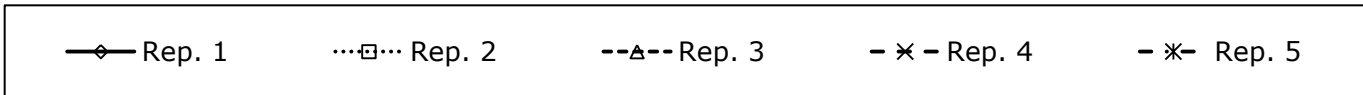
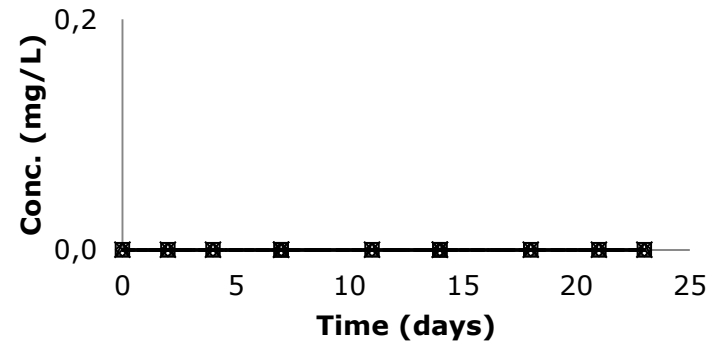
**Exposure 5 mg TCE/L**



**Exposure 20 mg TCE/L**

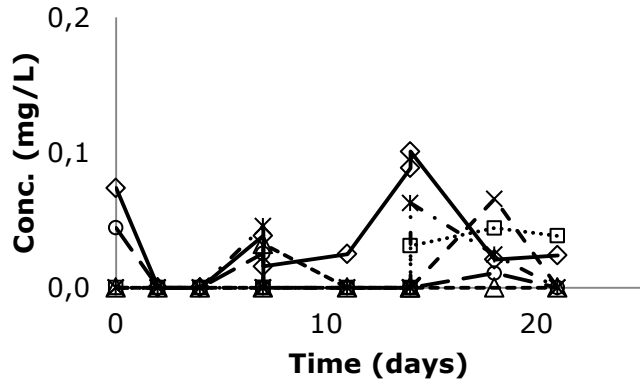


**Dead tree sticks**

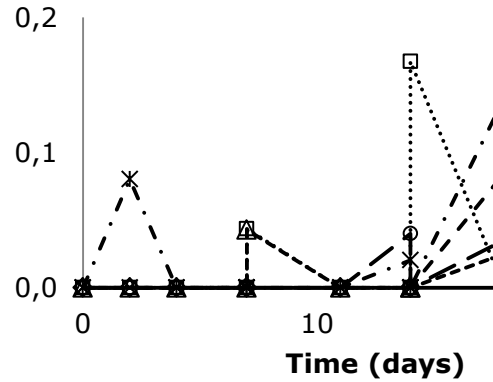


# Cl in solution

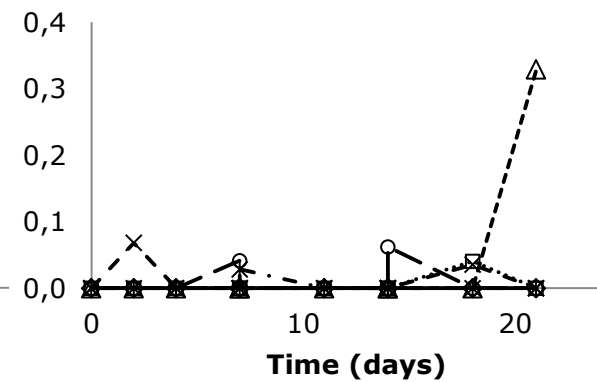
**Control** (TCE, no added bac.)



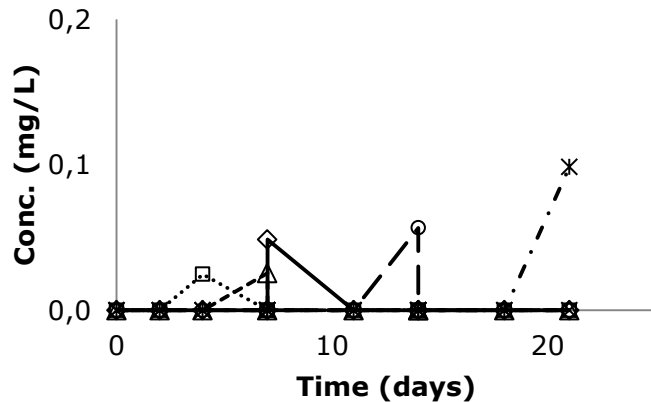
**301C**



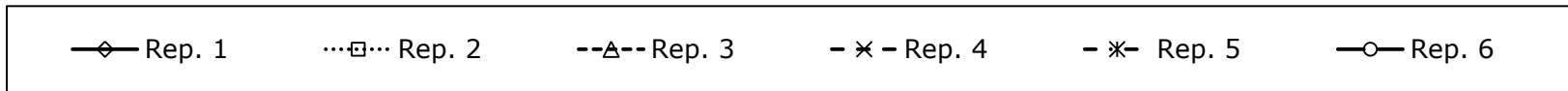
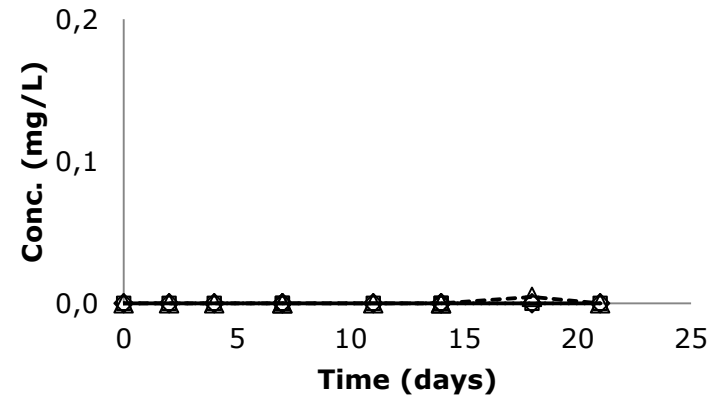
**PRI-31**



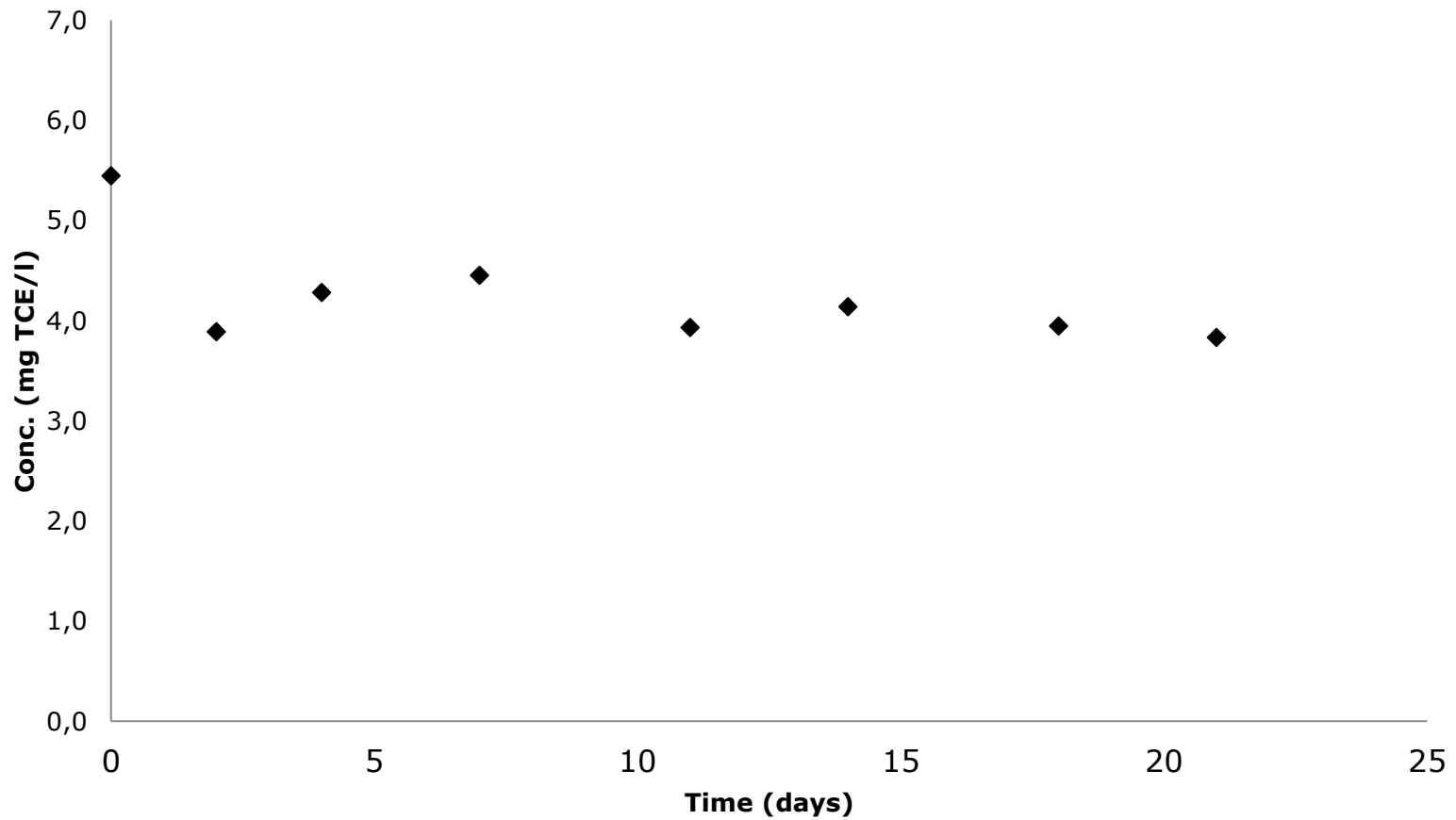
**pTOM**



**301C (no TCE)**



# TCE in vials with 301C (no trees)



# Cl in vials with 301C (no trees)

