OECD 201 – Comparison of Algae Growth Inhibition Reference Studies Conducted in a Conventional and Closed System

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Background

Some of the substances which have to be registered under REACh are volatile. The design of ecotoxicological studies such as the fish toxicity test (OECD 203), Daphnia immobilisation test (OECD 202) and Algae growth inhibition test (OECD 201) is different from standard cases as indicated in the OECD 23 and ISO 14442 Guidance documents on aquatic toxicity testing of difficult substances and mixtures. Especially for the algae growth inhibition test following the OECD 201 the differences are obvious: without the CO_2 – supply from the surrounding air the algae cannot grow. Therefore these algae need other carbon sources, e.g. $NaHCO_3$. Additionally to keep the pH of the test media as constant as possible, HEPES-buffer has to be added to the test water. The consequences of these special conditions for the algal growth have to be checked by performing controls. Not much literature is available which compares the growth of algae under open and closed conditions or compares the possible differences in sensitivity of the algae to substances between algae which grow under normal condition and in a closed system. To establish a reliable data set, we conducted studies with the same algae strain under conventional and closed system conditions to investigate these differences.

Material & Methods

We followed the methodology descripted in the OECD 201.

- Test species: *Pseudokirchneriella subcapitata*
- Medium: AAP
- 3 replicates/concentration + 6 replicates/control
- Start: 5’000 cells/mL
- Static design
- Duration 72h
- Test flasks shaked in a temperature (23°C) controlled incubator
- Illumination by LEDs (continuously and uniformly)
- 5-6 concentrations tested (Potassium Dichromate)
- Cell density determined by cell counter (Casy TT), algal biomass by fluorescence measurement (SpectraMax)

Hypothesis

There is no difference in terms of toxicity (NOEC, ECx) between the conventional and the closed OECD 201 design.

Results & Discussion

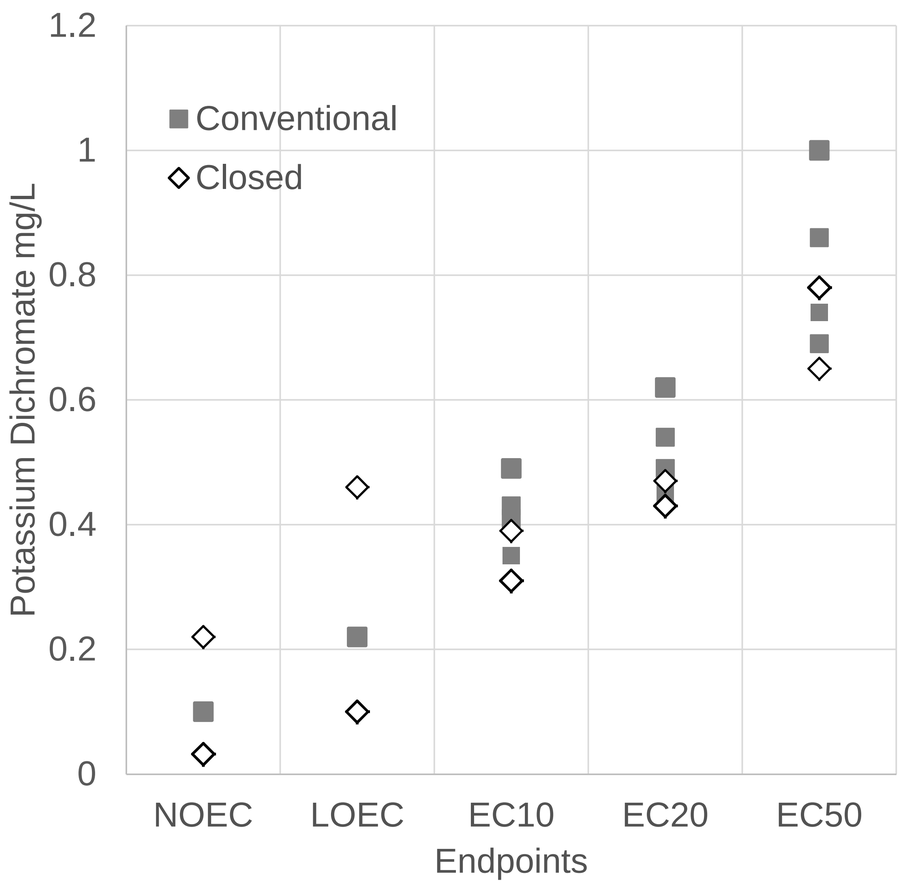

Figure 1: **Growth Rate** for the Conventional and Closed System : No Significant Difference

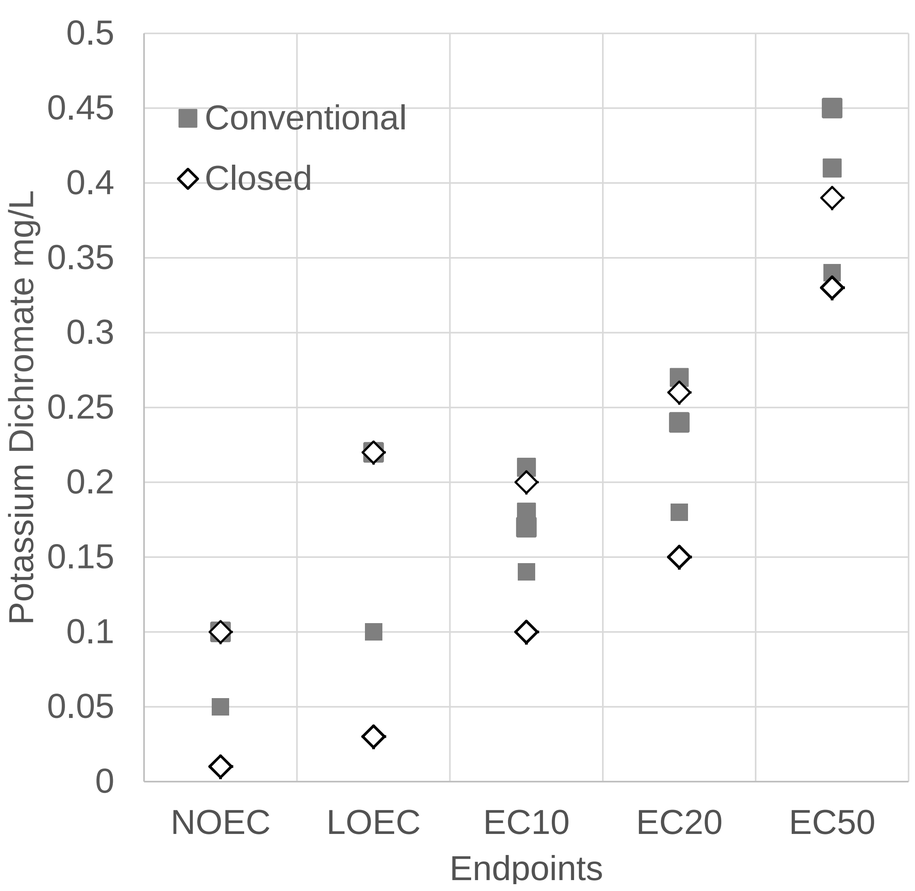

Figure 2: **Yield** for the Conventional and Closed System: No Significant Difference

Parameter	VC	Conventional				Closed	
Biomass increase [factor of]	≥16	134	116	197	99	107	150
Daily growth rates CV [%]	≤35	20	10	19	9.5	22	4
Average specific growth rate CV [%]	≤7	1.2	0.7	1.1	1.7	1.0	0.6

Table 2: Validity Criteria (VC): No Difference

Results & Discussion

- No significant differences for growth rate and yield between conventional and closed system (Figure 1,2)
- Maybe slightly higher toxicity for the closed system compared to conventional system (Figure 1,2)
- Validity criteria can be met with both systems (Table 1)
- In case of testing volatile substances it is recommended to perform reference test in closed system as well or compare to historical data of closed system data
- As there is a tendency that the algae in the closed system are slightly more sensitive, maybe a low compensation factor can be included in case a triggering endpoint is close.

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