

Breaking up is hard to do: Investigating the importance of breaking up algal chains in reducing the mean %CoV for section-by-section specific growth rate in *Anabaena flos-aquae* Toxicity Testing

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Abstract

The OECD 201 Guideline details culturing and testing techniques recommended for performing environmental testing with freshwater algal species. However, meeting the validity criteria specified in this guideline can be difficult when testing with the cyanobacterium *Anabaena flos-aquae*, especially when compared to the more commonly tested algae species *Pseudokirchneriella subcapitata*. In particular, the mean coefficient of variation (%CoV) for section-by-section specific growth rate is often higher for *A.flos-aquae* than the 35% recommended in the guideline. Guidance is provided specifically for *A.flos-aquae* that includes testing at a lower light intensities (40-60 $\mu\text{E m}^{-2}\text{s}^{-1}$) than other algal species and placing test vessels on an orbital shaker table at 150 rpm. Specific guidance on inoculation density is also given. The document highlights the tendency for *A.flos-aquae* to develop “aggregates of nested chains of cells”. Therefore, an important strategy for reducing variability in cell counts may be to break up these aggregates of nested chains. The objective of this investigation was to present data from a series of control replicates which, prior to counting, had undergone a series of techniques designed to break up cell aggregates and reduce overall chain length. Preliminary data indicated that reducing overall chain length may provide a more even distribution of *A.flos-aquae* cells across the counting chamber, thus reducing overall cell count variability and lowering the mean %CofV of section-by-section specific growth rate to acceptable levels.

Methods

Experimental Design

Experimental Procedures were based on those described in OECD 201 Guideline, with 6 Control Replicates per study initiated under the following conditions:

- Growth Medium; AAP
- Initial *A.flos-aquae* starting density; 2×10^4
- Temperature range; 21-24°C
- Continuous illumination in the range; 40-60 $\mu\text{E m}^{-2}\text{s}^{-1}$
- Continuous shaking rate; 150 rpm

Techniques used to break up chain length

The following additional steps were taken for each replicate at each 24 hour counting interval:

- The replicate was vigorously pipetted using a 25 mL pipette and bulb to ensure a homogenous distribution of cells, and a 1-2 mL subsample was removed.
- The subsample was sonicated for approximately 30 seconds.
- The subsample was subjected to further vigorous pipetting with a 10 mL pipette and bulb to allow further break-up of chains.
- Counts were performed using a haemocytometer.

Additional Data Collected

- Comparison between average chain lengths of *A.flos-aquae* before and after above treatment.

A Comparison Between *A.flos-aquae* Chain Length before and after Break-up

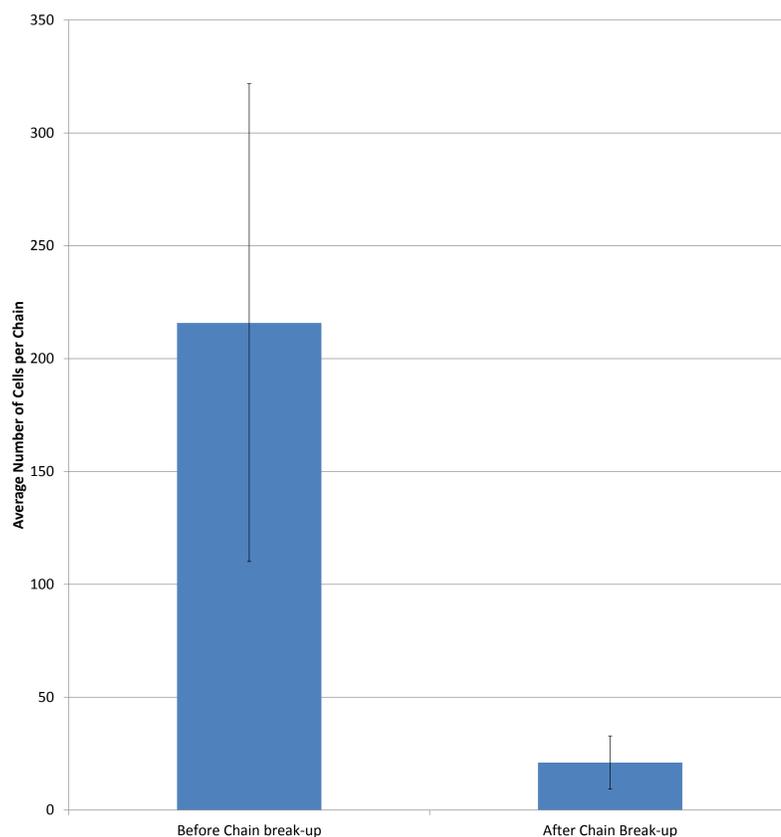


Figure 1: Error bars display standard deviation based on mean chain length before and after chain break-up

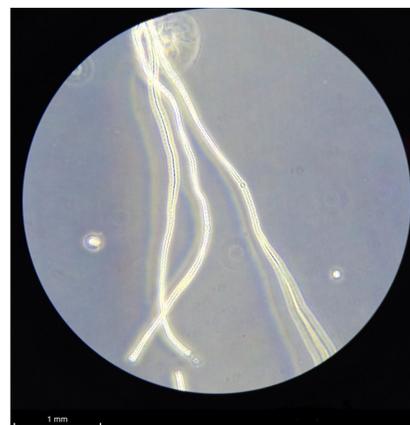


Figure 2: *A.flos-aquae* before chain break-up

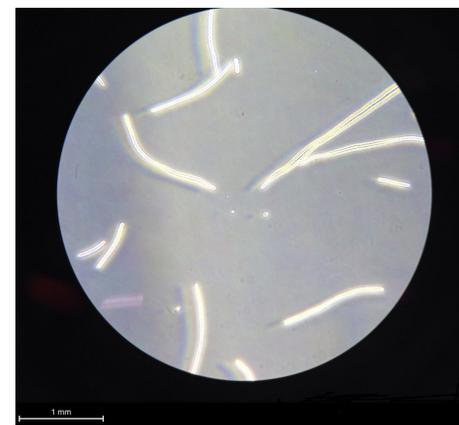


Figure 3: *A.flos-aquae* after chain break-up

Preliminary Conclusions

- A Reduced light intensity, increased agitation using an orbital shaker and a higher initial inoculation density when compared to the more commonly tested *Pseudokirchneriella subcapitata* positively impacts on *A.flos-aquae* growth.
- The “breaking-up” techniques described significantly reduces chain length and the likelihood of chain aggregates. This can lead to a more even distribution of cells across the haemocytometer.
- Following the procedures described, all 3 growth criteria were met for 50% of the studies conducted.
- Despite chain length reductions, the Section-by-Section Growth Rate %CofV is the most common Growth Criteria that is not achieved in *A.flos-aquae* testing.
- Further research is required to determine techniques to lower the Section-by-Section Growth Rate %CofV, and increase the likelihood of passing this difficult Growth Criteria.

References

OECD, 2006. OECD Guideline for Testing of Chemicals. Freshwater Alga and Cyanobacteria, Growth Inhibition Test. Guideline #201. Adopted 23 March 2006.

	Specific Growth Rate/day	Section-by-Section Growth Rate (%CofV)	Average Specific Growth Rate (%CofV)
Mean ^a	1.250	39.22	3.97
Range	0.965-1.817	27-79-51.70	0.79-6.66
%Passed	100	50	100

^aN=8