Improvements in *Microcystis* Quantification Using Sonication and Flow Cytometry

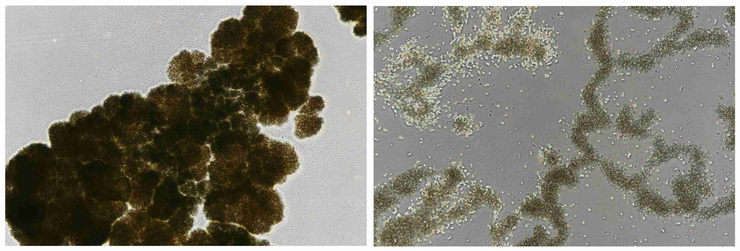
Nicole Stockley and Malcolm McFarland

Harbor Branch Oceanographic Institute at Florida Atlantic University, Fort Pierce, FL

Introduction

The Indian River Lagoon has been significantly impacted by blooms of the toxic cyanobacteria *Microcystis* in recent years. Accurate measurement of algal biomass is critical to examining the both the causes and effects of these recurrent blooms. This includes the role of water quality parameters such as temperature and nutrients in initiating and prolonging blooms and the potential health effects for people and animals in contact with impacted waterways. However, *Microcystis* tends to form amorphous colonies that can range from dozens to thousands of cells and can have varying cell densities, complicating traditional microscopy cell count methods. Flow cytometry is a viable alternative to microscopy as single cells are within the detectable size range, but colonies are still an issue as they are generally too large to be measured and rapidly float to the surface of the sample tube and are not able to be effectively measured.

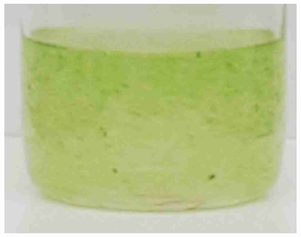
Microscopy images of Microcystis *colonies: dense (right) and lacey (far right).*

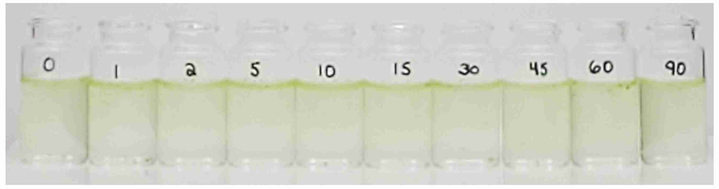


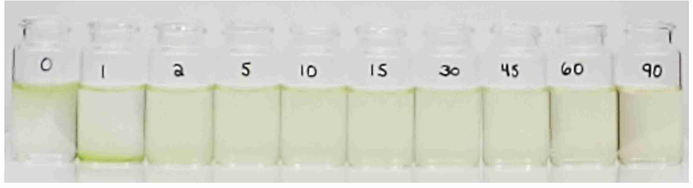
Sonication for Disaggregation

In order to accurately quantify *Microcystis* using flow cytometry, the large colonies should be completely broken up into individual cells. Sonication is a method that can achieve this disaggregation, but too little sonication may not be sufficient and too much will cause cell lysis, resulting in uncountable cell debris. The goals of this work is to identify the sonication time that maximizes the number of single *Microcystis* cells quantified by the flow cytometer. To start, 15 mL of each culture or field sample was dispensed into individual 20 mL scintillation vials. Each vial was then subjected to sonication for varying lengths of time (Fisher FB120, 120 W).

Culture (LOMC16) in media bottle before experiment (below). Culture dispensed into vials before (right, top) and after (right, bottom) sonication.

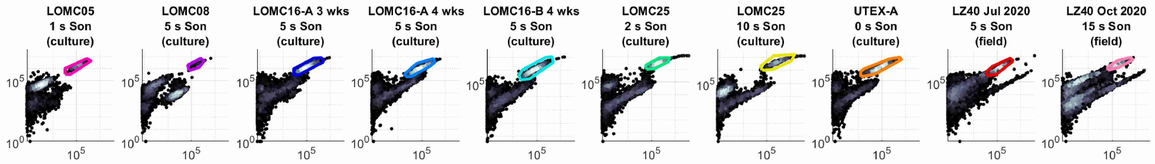






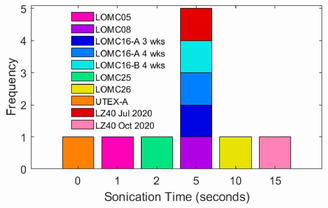
Flow Cytometry Analysis

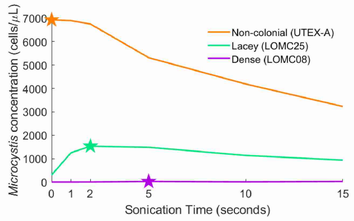
Following sonication, each sample was processed using the flow cytometer. Using the non-colonial UTEX-A culture as a reference, the likely cluster of individual *Microcystis* cells was identified using a manual gating routine in Matlab. While the general location of the cluster was consistent, the specific ratio of phycocyanin to chlorophyll-a for each sample varied slightly, so unique gates were used for each while remaining constant across the sonication times.

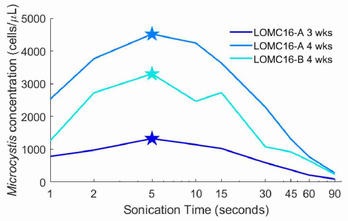


Results

Most of the samples tested shows optimal results with 5 seconds of sonication (below, left). Differences in the peak concentration of individual *Microcystis* cells were observed when comparing cultures of different colony morphologies, with denser colonies requiring more sonication time (below, center). No difference was seen between the same culture tested at varying ages (3 & 4 weeks of the same inoculation and 4 weeks of a 2nd inoculation, below right).







Conclusions

While the results of this analysis seem clear, it is important to note that cultures of *Microcystis* often exhibit different colony morphology than field samples and may be more readily disaggregated. Field samples can also exhibit different colony morphologies, especially with seasonal variations. Physiologic state may also play a role in the ease with which colonies may be disaggregated. More work must be done using "fresh caught" field samples, ideally encompassing an entire bloom period, in order to confirm the wide applicability of these findings. Additionally, the method of determining the gates is subject to human discretion and small changes can significantly change the outcome of the calculated results. An automated routine using a trained neural network to identify the single cell *Microcystis* population would improve the repeatability of this experiment.

Acknowledgements

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