# nielgenlab

## Objective

Study the implementation of MAD2Media's advanced optimization techniques on sorbitol production yields to improve the purity of sorbitol.

### Introduction

Sorbitol, initially commercialized as a sugar substitute, is a sugar alcohol with diverse applications within the food and pharmaceutical industries. As of late, sorbitol has been found to be synthesized from various sources, including cyanobacteria, a form of bacteria that receives energy via photosynthesis.

While A+ media is the most used form of media culture for work involving marine cyanobacteria, MAD2 and MMA (modified A+ media), which contain a greater amount of nitrogen and phosphorus compared to A+ media, have been found to enhance growth conditions of cyanobacteria *Synechococcus sp.* PCC 7002.

We propose the utilization of the cyanobacterial strain, PCC 7002, due to its distinguished track record as an industrially proficient microorganism in the context of biochemical production, which will ultimately demonstrate photosynthetic production from sunlight and carbon dioxide. By optimizing media growth conditions and fermentation parameters, we aim to maximize sorbitol production yields.

These advancements not only contribute to sustainability efforts by reducing environmental impact through more efficient production methods but also hold promise for future innovations.

Left: A+ MAD2, and MMA flasks on incubator within Caron Plant Growth Chamber Right: Extraction of A+, MAD2, and MMA media in Biosafety Lab Cabinet

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## Media Optimization to Improve Photosynthetic Sorbitol Production

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#### Methods

Prepare the three media types, A+, MAD2, and MMA (modified A+ media), using A+ media as the base, supplemented with vitamin B12 and antibiotics (KAN and G30) [A+ medias contain 11.8 mM and 0.37 mM of NaNO<sub>3</sub> and KH<sub>2</sub>PO<sub>4</sub>, respectively. Meanwhile, MAD2 media contains 192 mM & 2.4 mM of NaNO<sub>3</sub> & KH<sub>2</sub>PO<sub>4</sub>, respectively. The levels of nitrogen and phosphorus are the determining factors that modify the A+ media.].

Create A+, MAD2, and MMA medias using sterile A+ media, antibiotics (KAN and G30), and vitamin 12.

Grow PCC 7002 overnight on an incubator within the Caron Plant Growth Chamber (150.0  $\pm$  2.0  $\mu$ E).

Add 400  $\mu$ l of sterile DI H<sub>2</sub>O to A+ and MAD2 flasks as well as 400  $\mu$ l of sterile diluted KH<sub>2</sub>PO<sub>4</sub> to MMA flasks prior to extraction.

Extract 500 µl of media and measure OD730 of medias using Beckman Coulter DU 800 Spectrometer.

Filter out cells, label, and store samples every 24 hours for 10 days.

Measure sorbitol amount of all samples with a Hewlett-Packard Agilent 1100 HPLC System.

#### Results

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## References







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