

## Project Overview

### OBJECTIVE

Multiform Harvest uses industrial waste streams to produce fertilizer (struvite). Calcium inhibits this process, but can be precipitated out with addition of oxalic acid. Oxalic acid can be produced by *Aspergillus niger* (black mold) fed with lactose from whey permeate, a waste product from the dairy industry. This project develops a process for producing oxalic acid from whey permeate for wastewater treatment applications.

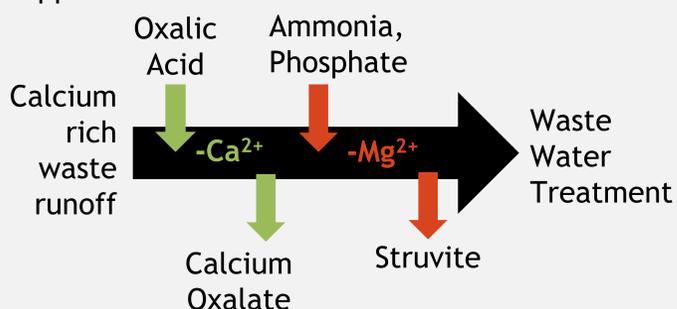


Fig.1: Oxalic acid facilitates formation of struvite from a waste stream, such as dairy waste or landfill leachate, by removing calcium ions.

### METHODS

Cell concentration can be measured by dry weight. Lactose concentration can be measured with a blood glucose monitor after hydrolysis by lactase. Oxalic acid can be quantified by precipitation with calcium chloride and subsequent titration with potassium permanganate.

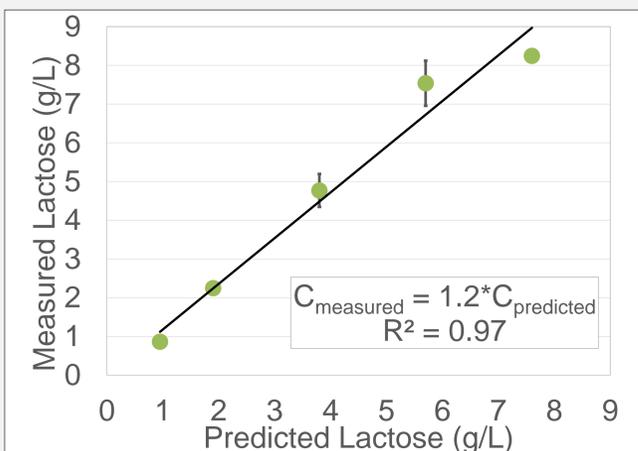


Fig.2: Calibration curve of lactose added versus lactose measured. Hydrolyzed lactose forms glucose that is measured by a ReliOn Ultima Blood Glucose sensor. Lactose is calculated from a stoichiometric conversion from the sensor reading. Error bars represent 90% confidence intervals. An ideal correlation would have a linear regression slope of one.

# PRODUCTION OF OXALIC ACID FROM SUGARY WASTES

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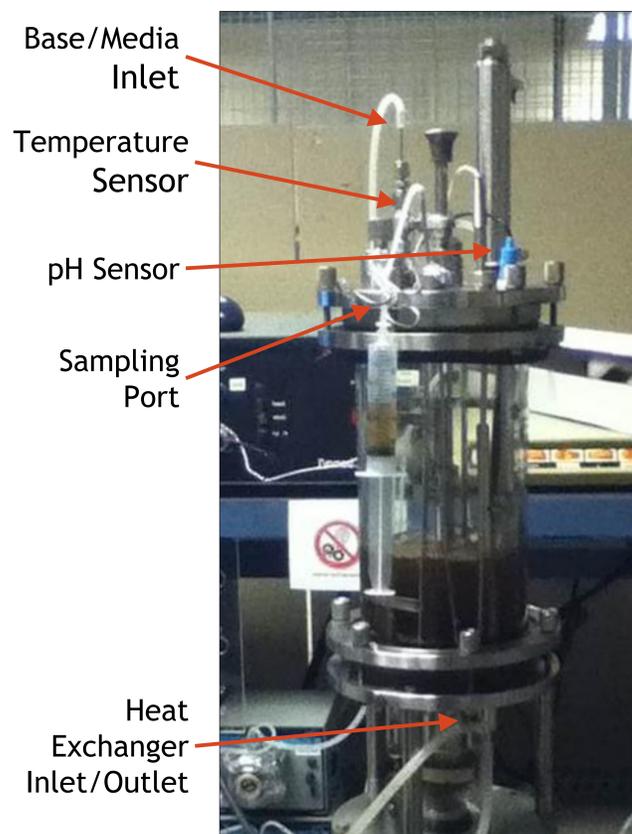


Fig. 3: The bioreactor uses a PID controller to control temperature with a heat exchanger and pH by adding acid or base from an attached source. Samples are taken with a syringe from the sampling port.



Fig. 4: Freeze dried *A. niger* from Shanghai Touchhealthy Biotechnology have been used in bioreactor trials.

### BIOREACTOR TRIALS

Both artificial whey permeate and whey permeate from Darigold have been used as growth media in a batch bioreactor.

Artificial Whey Permeate Recipe:

- 10 g/L peptone
- 10 g/L yeast extract
- 50 g/L lactose

Bioreactor Operating Conditions:

- pH = 6
- Temperature = 30 °C
- Agitation Rate = 250 rpm

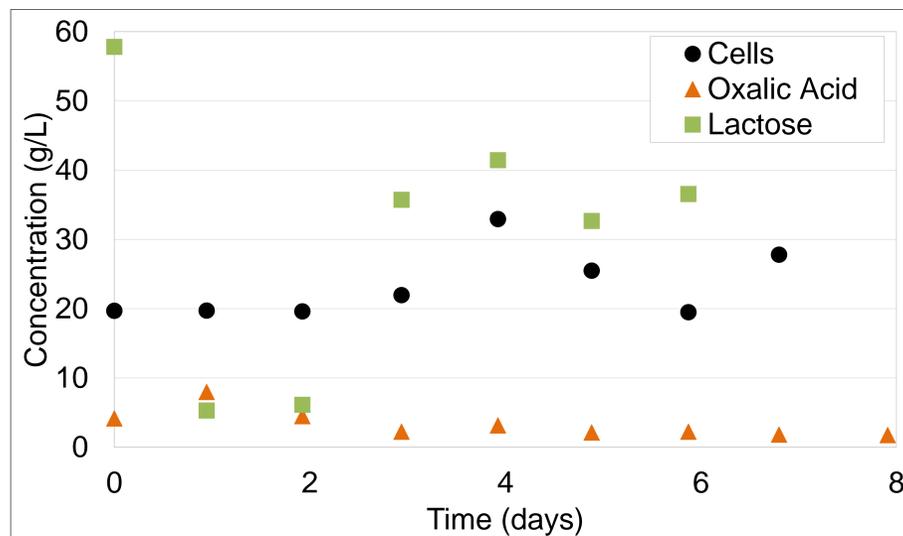


Fig. 5: Concentration of cells, oxalic acid, and lactose in the bioreactor were measured over an 8 day run. Lactose decreased dramatically over the first few days, while oxalic acid increased. However, cell mass and lactose rose while oxalic acid production fell on the third and fourth days, likely due to contamination in samples (see Fig 6). Issues in control equipment led to inconsistent pH (pH may have dropped below 6 periodically) and could have caused inconsistency in results.

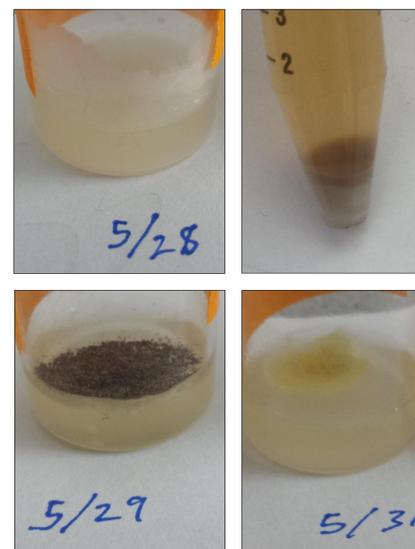


Fig. 6: Contamination of samples from trial three caused discrepancies in results. The *A. niger* continued to grow in the lactose and oxalic acid assay samples after being autoclaved and centrifuged for 30 minutes each.

## Future Work and Bioreactor Scale-Up



Fig. 7: USDA cell line was cultured and used in trial 3.

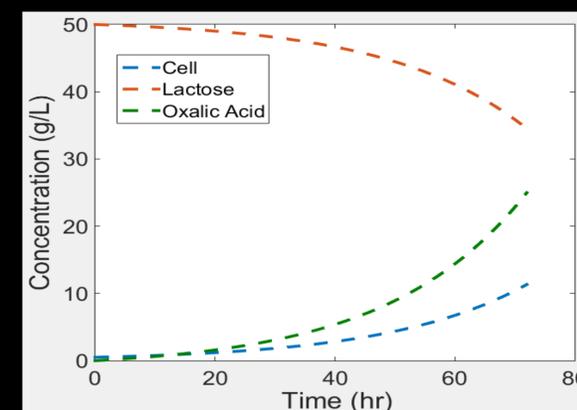


Fig. 8: Growth kinetics (modeled in MATLAB) predict cell, lactose, and oxalic acid concentrations which are used for assessing scale-up properties.

### SCALE-UP CONSIDERATIONS

A scaled-up process will be designed to treat 100,000 gal/day waste stream containing 300 ppm Ca<sup>2+</sup> and will consider:

- Growth kinetics from bioreactor trials
- Adequate oxygen and nutrient transport (well mixed)
- Configuration (continuous flow) and whey/waste stream flow rates

### ACKNOWLEDGEMENTS

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