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Agile BioFoundry

U.S. DEPARTMENT OF ENERGY | Energy Efficiency & Renewable Energy

BIOENERGY TECHNOLOGIES OFFICE

DOE Bioenergy Technologies Office (BETO)
2023 Project Peer Review

Application of Machine Learning to Improve Biobased Glucaric Acid Production

April 5, 2023
Technology Area Session: Agile BioFoundry

National Laboratory PI: Violeta Sànchez i Nogué, NREL
Kalion, Inc: Darcy Prather

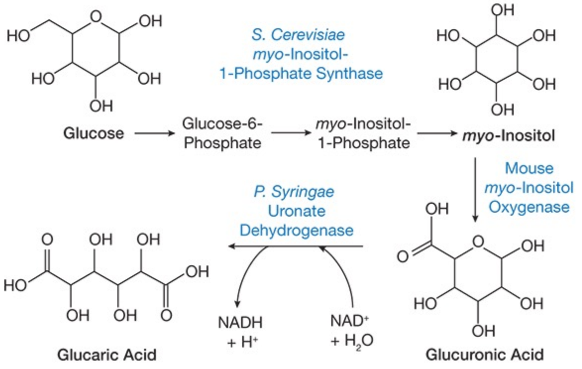
Project overview

Kalion, Inc. produces and delivers high-purity **glucaric acid** to companies to discover the potential of this chemical for industrial, materials, and pharmaceuticals markets.

- **Project Goal:** Apply machine learning in conjunction with high-throughput cultivations and metabolomics to understand and overcome two challenges:
  - **Improve glucaric acid productivity**. The rate of production slows considerably after 48 hours, which limits the overall productivity that can be achieved in the process.
  - **Decrease glucaric acid production costs.** Components from complex media are necessary to achieve robust production. Thus, identifying the specific components that generate that improvement can lead to a simplified and less costly medium formulation.

KALION, INC.

Bacterial pathway for glucaric acid production



https://www.kalioninc.com/articles

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Approach for project

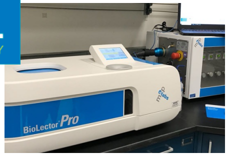
Conduct cultivations in bioreactors under different conditions, analyze metabolites via metabolomics, and use machine learning approaches to:

- Identify correlations between specific metabolites and glucaric acid production rate
- Define successive rounds of media simplification to drive down costs

Challenges

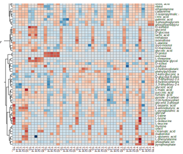
- Scaling down the cultivations to 2 mL-bioreactors for media screening could limit productivity and product profile resolution
- Limited number of experimental campaigns may limit the power of machine learning

NREL Transforming ENERGY



Microbial cultivations Analytics

Pacific Northwest NATIONAL LABORATORY



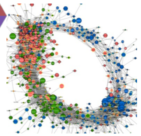
Metabolomics

BERKELEY LAB

art

Propose media compositions

Argonne NATIONAL LABORATORY



Analyze growth kinetics and genetic modifications

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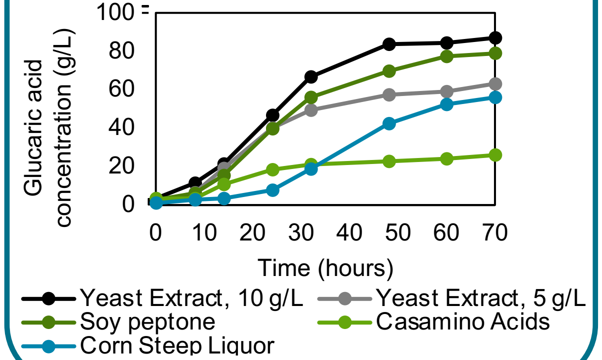
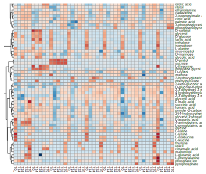
Progress and outcomes

Glucaric acid production evaluated in different rich media

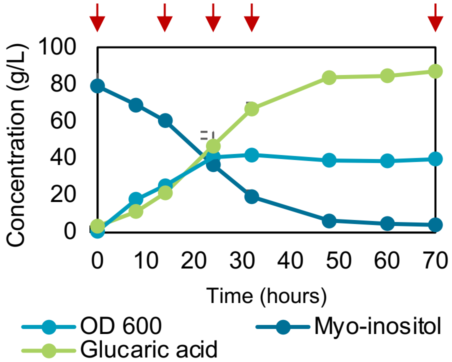
Evaluation in 0.5 L bioreactors



Metabolomics and metal analysis during cultivations



93 identified metabolites + unidentifiable 99 peaks detected



Abundance of 14 metabolites significantly decreased during the cultivation

Group	Compound
1	Glycerol-3-phosphate Glucose-6-phosphate
2a	**D-mannose** **Trehalose** **D-sorbitol**
2b	Isomaltose 1,3,5-pentanetriol
3	Tyrosol Tyramine
4a	Succinic acid 2-keto-gluconic acid Glyceric acid
4b	2,3-dihydroxy-2-methylbutanoic acid 2,3-dihdroxy-2-methylpropionic acid

- 14 metabolites from rich media were identified to be potentially the cause for performance differences
- Sugars were the main metabolites that significantly decreased during the bacterial cultivation

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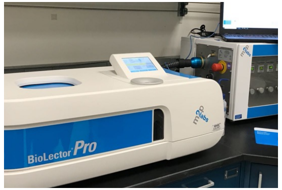
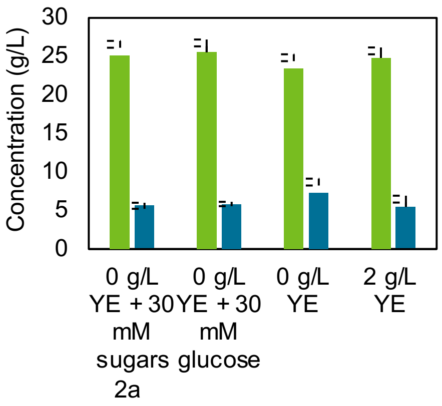
Progress and outcomes

The titer was improved only with the sugars group 2a

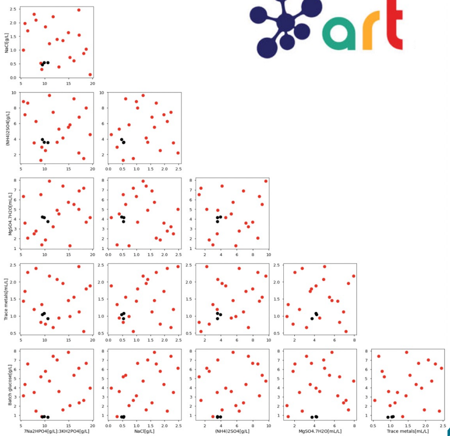




YE=yeast extract

A training set was generated for a learn-friendly experimental campaign to enable training of machine learning models with predictive capability

Components	Starting concentrations	Range to Test
Na_2HPO_4 : KH_2PO_4	7.0 g/L : 3.0 g/L	5– 20 g/L
NaCl	0.5 g/L	0.1 – 2.5 g/L
$(NH_4)_2SO_4$	3.7 g/L	1 – 10 g/L
$MgSO_4{\cdot}7H_20$	4.0 mL/L	1 – 8 mL/L
Trace metals solution	1.0 mL/L	0.5 – 2.5 mL/L
Batch glucose	**0.8 g/L**	**0.8 – 8 g/L**



- Supplemental sugars enhance the overall performance, but the improvement is not sugar specific
- Glucose was selected as carbon source for further bioprocess optimization through ART
- The concentration of base media components was also evaluated to test effects on bacterial performance

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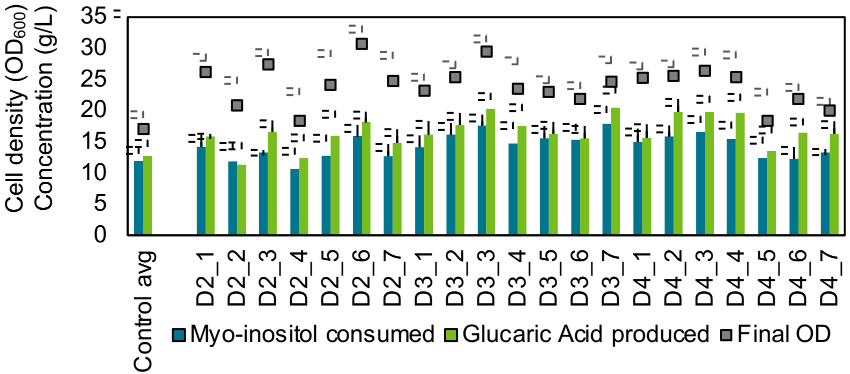
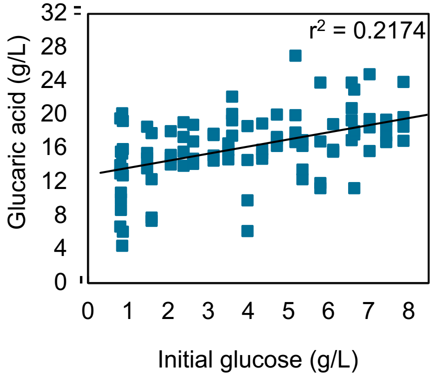
Progress and outcomes

Performance in 24 media compositions was assessed via final glucaric acid titer



High-through put bioreactor cultivations generate significant variability, which is not desirable as machine learning input. Optimized microfluidic plates are currently being tested for decreased intra-plate variability

Regression analyses were conducted between glucaric acid titer and initial metabolite concentration



- Glucose concentration showed the strongest correlation with glucaric acid titer
- Glucose concentration during the cultivation can be optimized to improve bacterial performance

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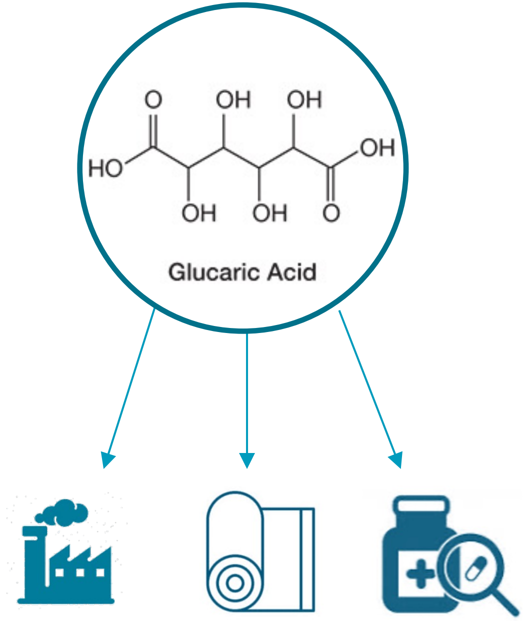
Impact

Scientific

- Production of biobased glucaric acid and suggestions for production improvement



Industry

- Working with Kalion Inc and partners to improve production process technologies
- Advance infrastructure and workflows (for machine learning in conjunction with high-throughput culturing and metabolomics) to improve more rapid and comprehensive analyses

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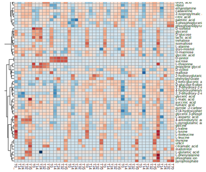
Summary



Overview

- Enhancing glucaric acid productivity and reducing media costs are necessary to improve process economics

Approach

- Identify metabolites from rich media that improve performance using metabolomics, high throughput cultivations in bioreactors, and machine learning-friendly experimental designs

Progress and outcomes

- The substrate feeding strategy is the potential major driver to enhance bacterial performance

Quad chart overview

Timeline
- Project Start: 3/1/2021
- Project End: 2/28/2023

	FY22 costed	Total Award (FY21-23)
DOE Funding	$255,000 ANL – $40,000 LBNL – $65,000 NREL – $75,000 PNNL – $75,000	$425,000 ANL – $110,000 LBNL – $75,000 NREL – $130,000 PNNL – $110,000
Cost Share (Kalion)		

Project Partners
ABF Labs: ANL, LBNL, NREL, PNNL

Industry Partner: Kalion, Inc.

Project Goal
Apply machine learning in conjunction with high-throughput cultivations and metabolomics to improve glucaric acid productivity and decrease production costs.

End of Project Milestone
Based on ART results, report the new media composition without yeast extract that confers a glucaric acid production at least 80% of the one obtained when using media supplemented with 5g/L yeast extract.

Funding Mechanism
FY20 ABF Directed Funding Opportunity

TRL at Project Start: 2

TRL at Project End: 3

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Acknowledgements:
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Project Contributors:
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LBNL: Tijana Radivojevic, Hector Garcia Martin

PNNL: Brenton Poirier, Tom Wietsma, Marija Velickovic, Young-Mo Kim

Kalion, Inc: Steven van Dien, Andrew Borchert, Tyler Smith, Darcy Prather