

Enhancing Cyanobacterial L-Alanine Production Via Nutritional and Process Optimization

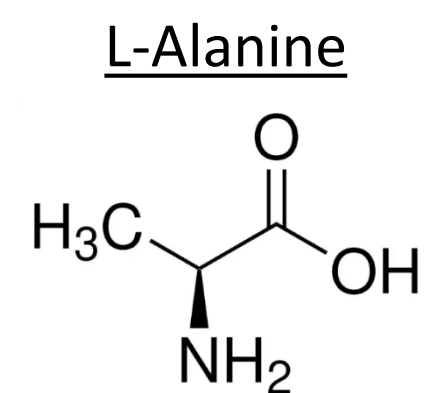
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Background

The cyanobacterium *Synechococcus* sp. PCC 7002 has been metabolically engineered to produce L-alanine from CO₂ and light, while also using different nitrogen sources.



L-alanine is a nutritional supplement

Nitrogen Sources:

1. Ammonium Sulfate



2. Sodium Nitrate



3. Urea



Genetic Modifications

Gene	Source	Integration site
<i>alaE</i>	<i>E. coli</i>	<i>aqu1</i>
<i>alaD</i>	<i>Cladophora feredayi</i> UTEX 2873	A2542

Analysis Methods

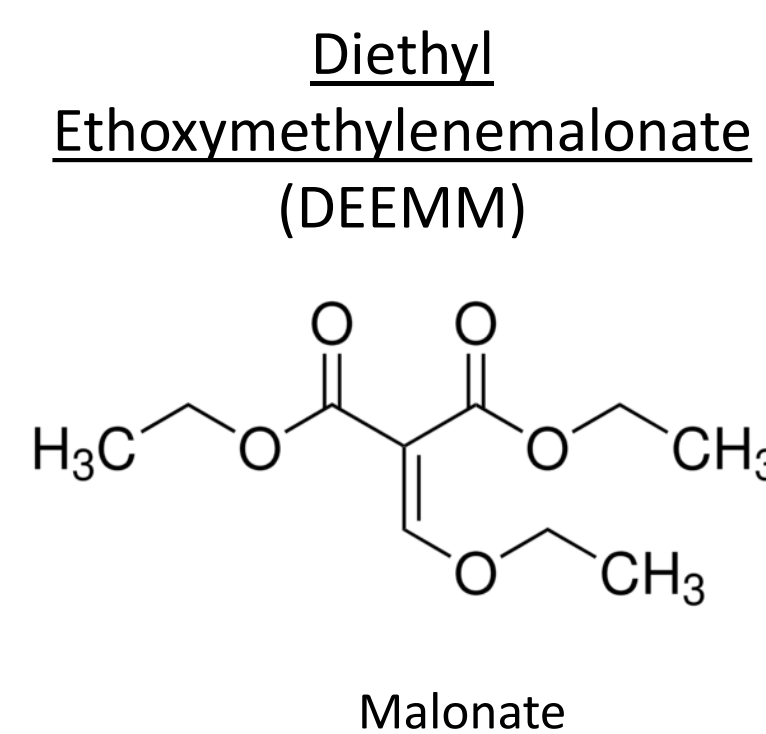
L-Alanine is naturally excreted into the extracellular media, from which is sampled.

Growth Rate:

- Characterized by optical density at 730 nm (OD₇₃₀)

DEEMM Derivatization and HPLC:

- L-Alanine in media reacted with DEEM to form N-alkylmalonate
- Malonate group is more UV-active than L-alanine
- Diode Array Detector (DAD) analyzes absorbance at varying wavelengths

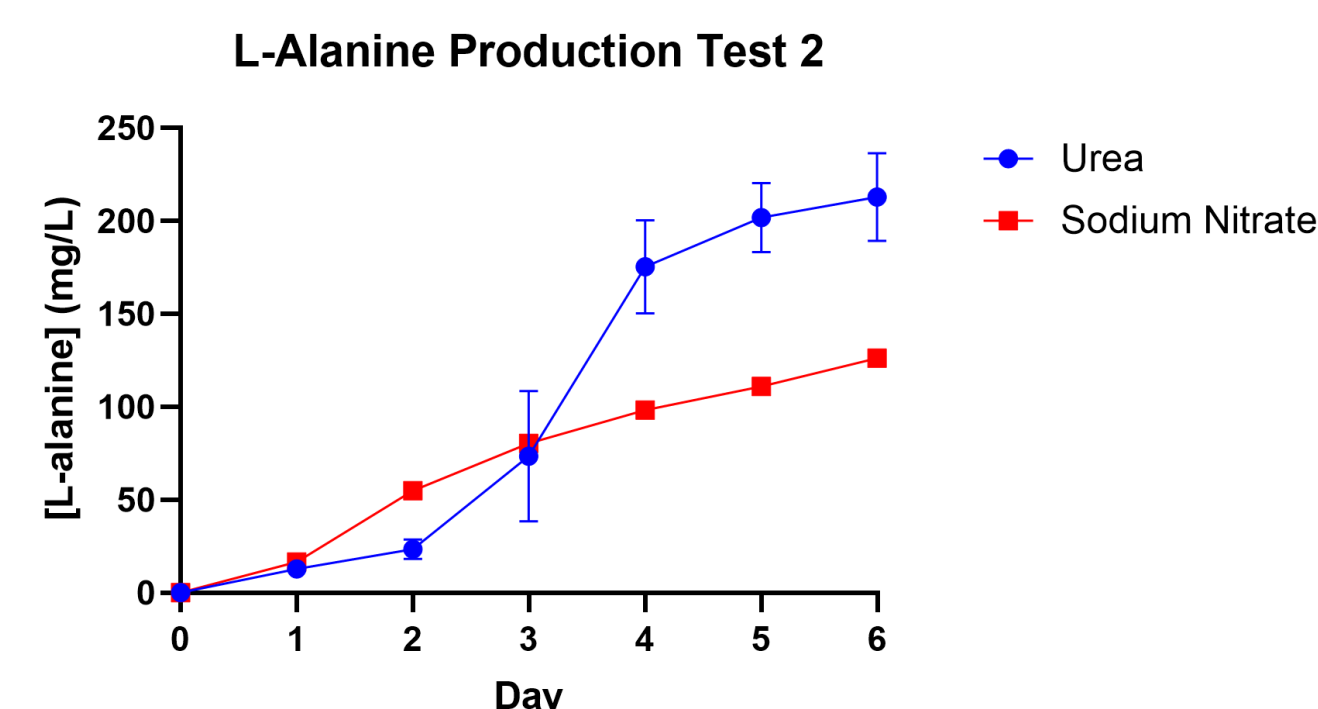
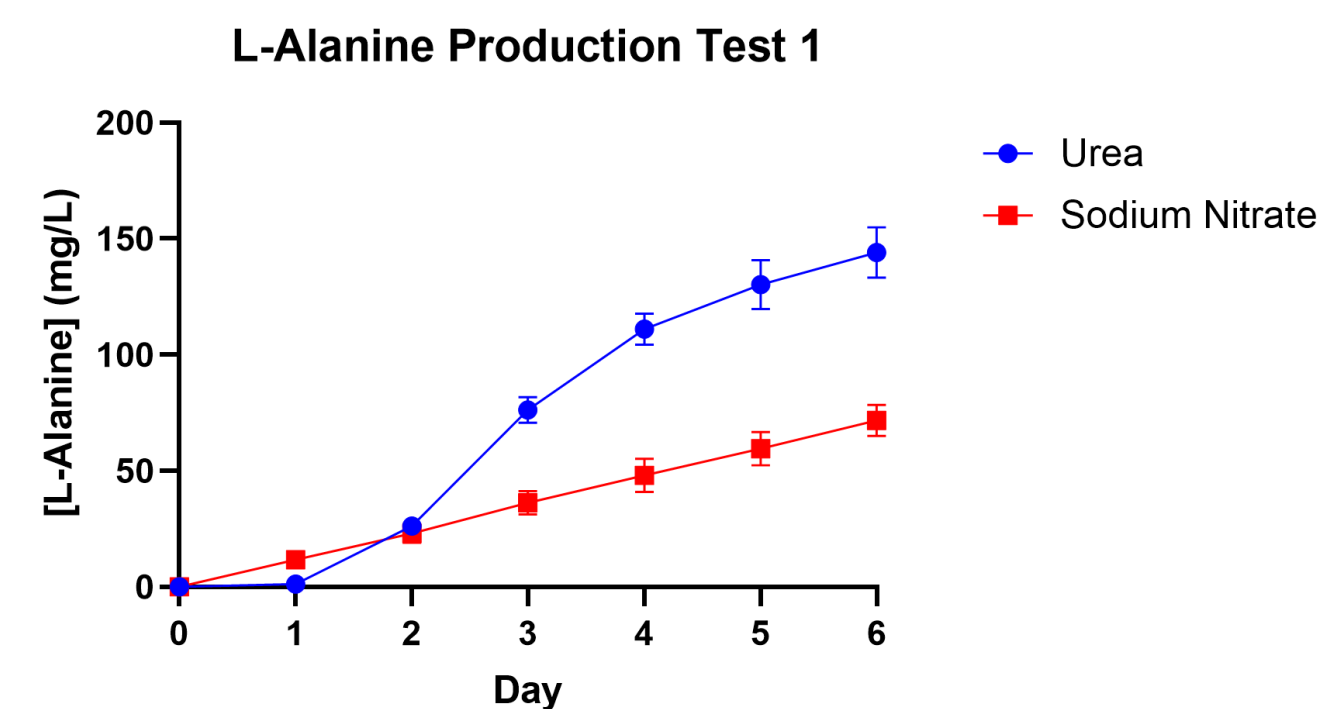
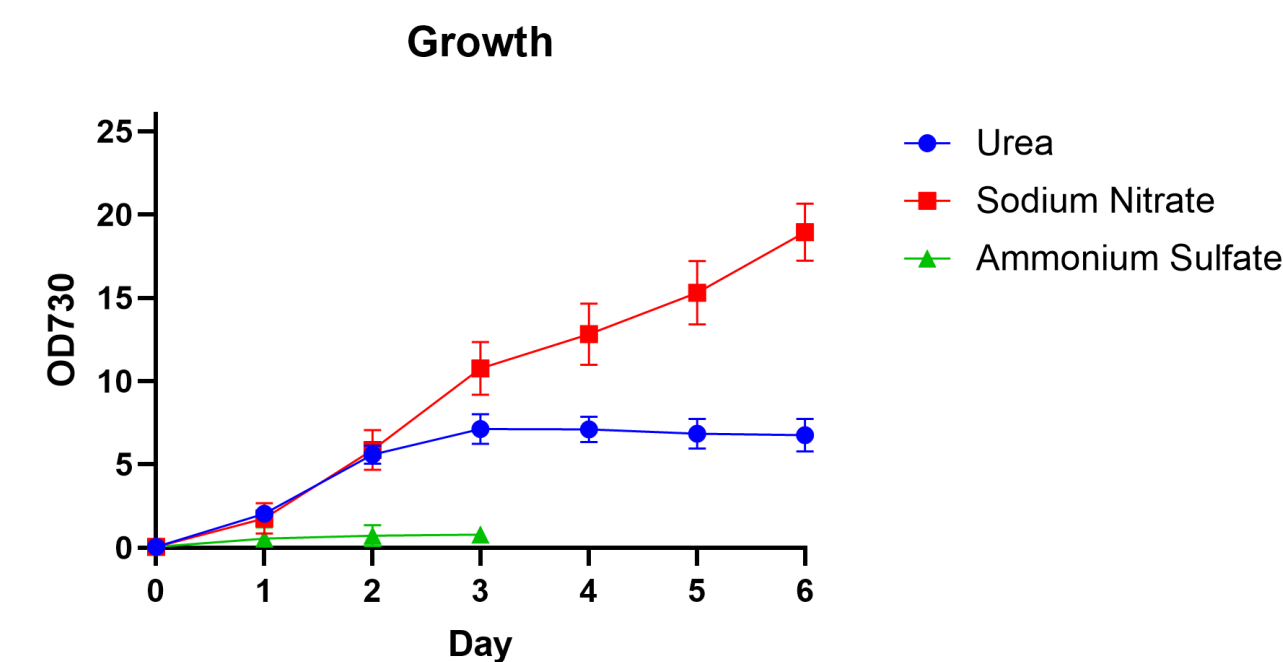
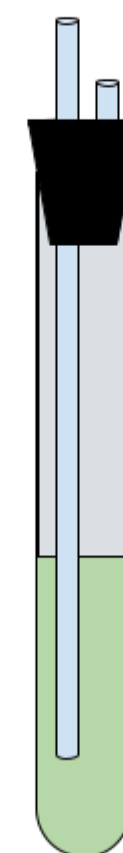


Urea Improves L-Alanine Production

Culture Conditions:

- 270-280 μE light
- 5% CO₂
- 48 mM total N

Each experiment performed in triplicate. Tyson tubes allow for more consistent light.

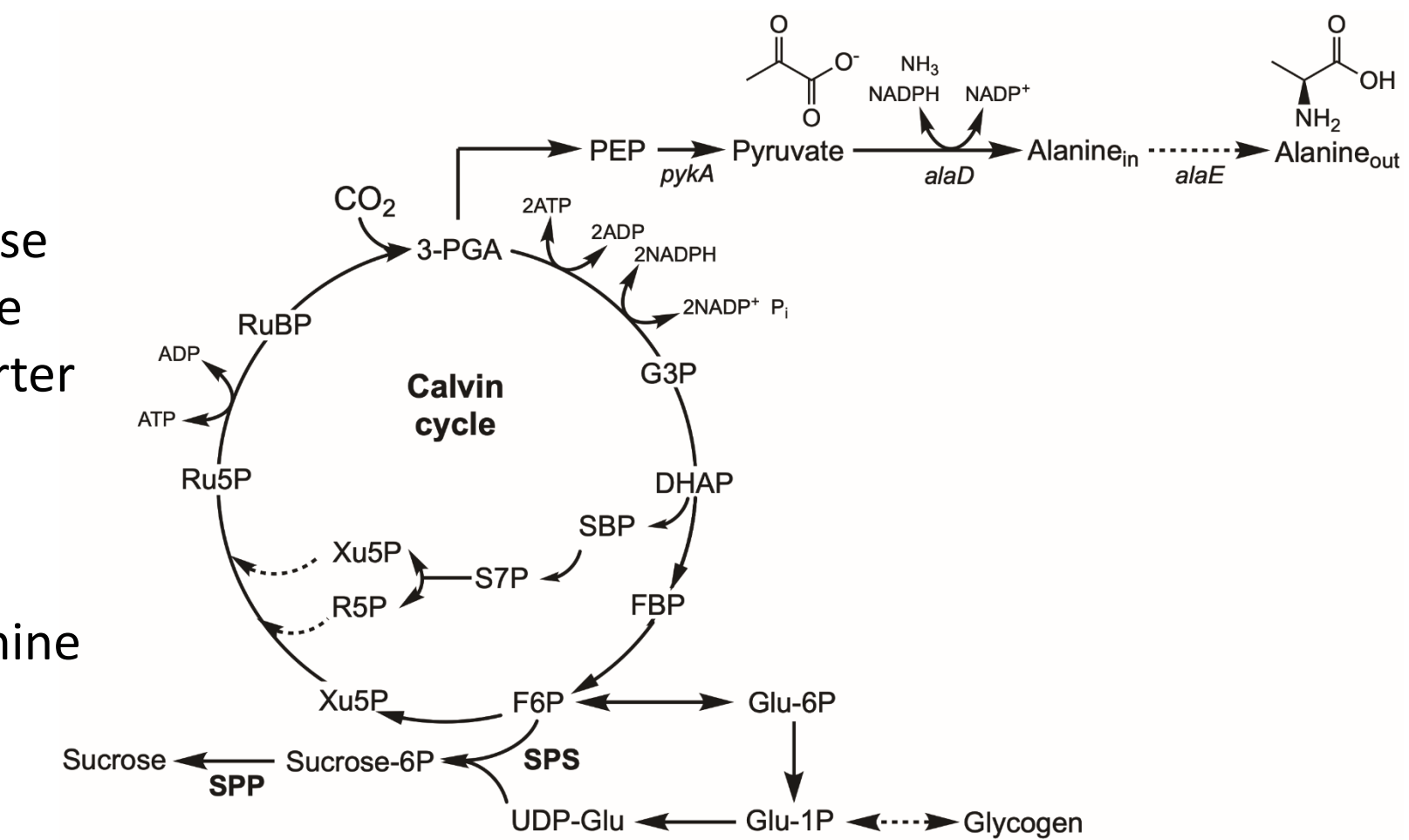


Results:

- Ammonium sulfate did not support growth or production
- Urea supported production of >150 mg/L L-alanine
- Sodium Nitrate supported production of ~100mg/L L-alanine

Pathway

Alanine Dehydrogenase (AlaD) and the Alanine exporter (AlaE) work together to produce and excrete L-alanine



Future Work

- Urea titration at 24, 32, and 40mM
- Overexpress FBPAse gene from PCC 7002 at the *glpK* integration site
- Overexpress *pykA* from *E. coli* at the *NS2* integration site to further catalyze the conversion of phosphoenolpyruvate (PEP) into pyruvate

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