Production of Pyruvic Acid by Metabolically Engineered *Escherichia coli*

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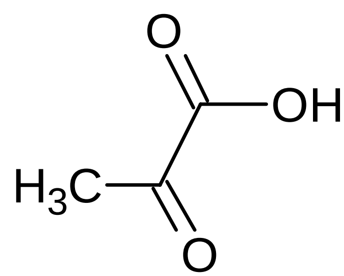
Introduction

Pyruvate is widely used in food, chemical, and pharmaceutical industries.

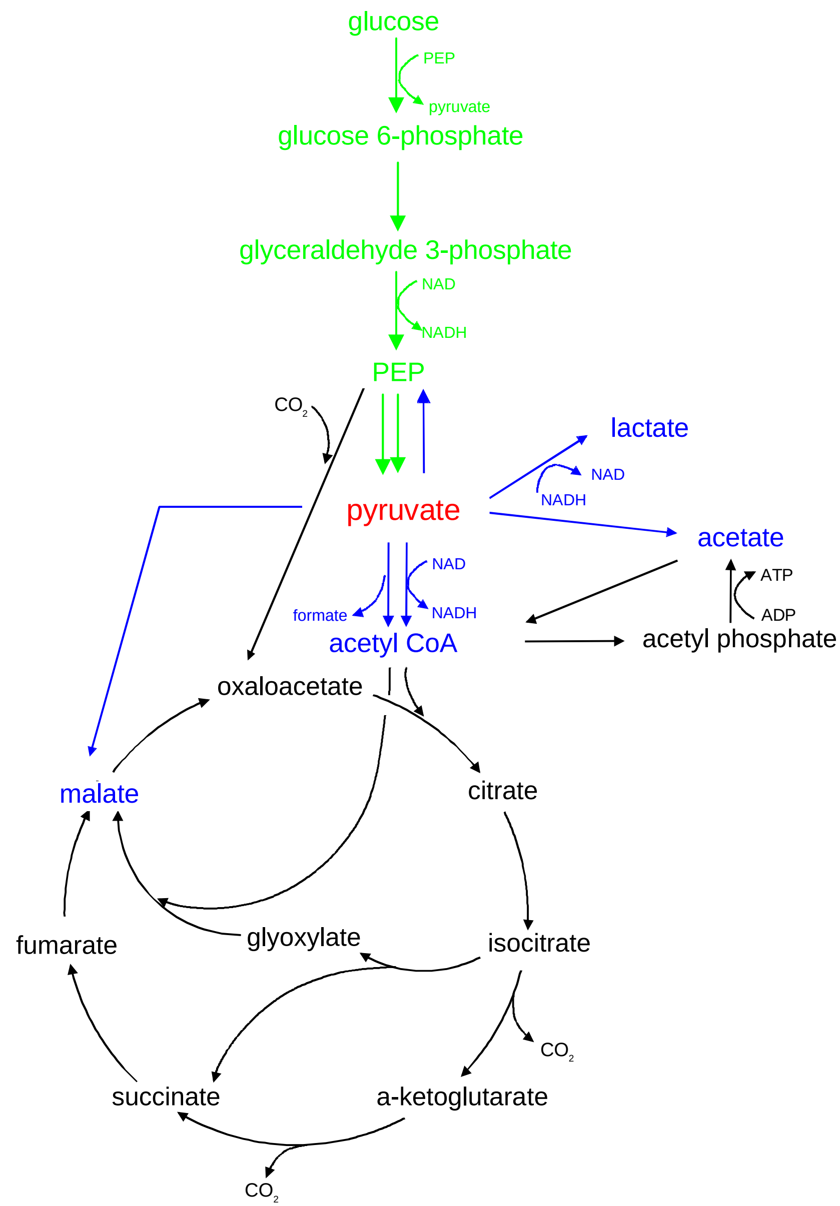


Pyruvic acid

Biochemical Pathways

Pathways which generate pyruvate

Pathways which consume pyruvate



Pyruvate Biosynthesis

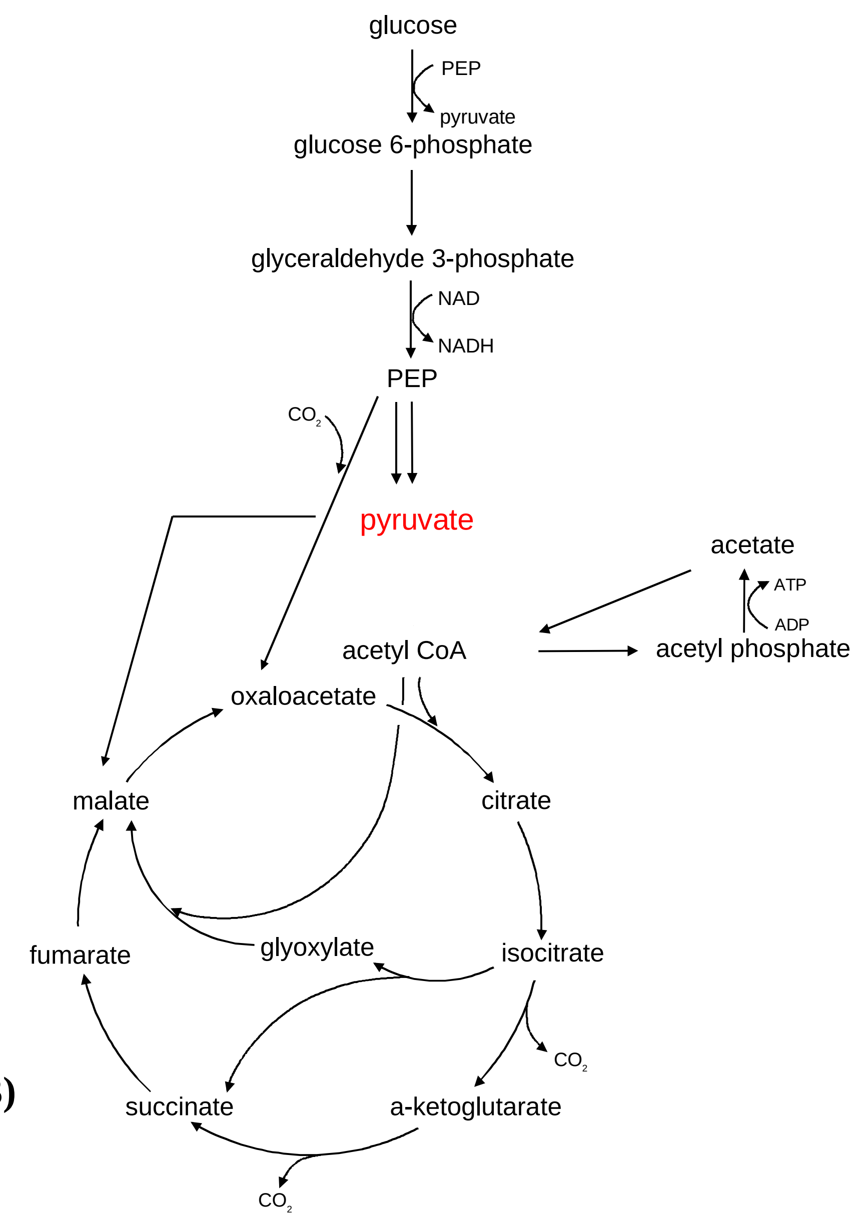
Must prevent metabolism at pyruvate!

Deletions

1. **pyruvate formate lyase (*pfl*)**
2. **pyruvate dehydrogenase (*aceEF*)**
3. **PEP synthase (*pps*)**
4. **pyruvate oxidase (*poxB*)**
5. **lactate dehydrogenase (*ldhA*)**

E. coli aceEF, pps, pfl, poxB, ldhA **(Zelic et al. 2003)**

Two carbon sources: Glucose and Acetate!



Fermentation Balance

Competing Reactions:

Glucose + 2NAD$^+$ + 2ADP → 2Pyruvate + 2NADH + 2H$^+$ + 2ATP

Glucose → cells

Goals:

1) **Low biomass production – high pyruvate yield**

   Fed-batch process at growth rate of 0.15 h^{-1}

2) **High rate of glycolysis – high pyruvate productivity**

   Acetate-limited process

Selection of Limiting Nutrients

Chemostat Type		G - limited	A - limited	N - limited	P - limited
Dilution Rate	(h^{-1})	0.15	0.15	0.15	0.15
DCW	(g/L)	2.15	2.45	1.76	1.44
q_A	(g/g·h)	0.28	0.07	0.20	0.36
q_G	(g/g·h)	0.36	1.60	0.85	0.77
q_{Pyr}	(g/g·h)	0.08	1.11	0.57	0.20
$Y_{X/G}$	(g/g)	0.43	0.10	0.18	0.20
$Y_{X/A}$	(g/g)	0.54	2.39	0.76	0.42
$Y_{Pyr/G}$	(g/g)	0.24	0.70	0.67	0.26
$Y_{Pyr/X}$	(g/g)	0.56	7.15	3.72	1.32

Fermentation Balance

Competing Reactions:

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   Also:
   - Knockout of ATP synthase (Δ*atpFH*)
   - Knockout of *arcA*
   - Introduction of NADH oxidase

Comparison of Strains

Strains:	ALS929:	*aceEF pps pfl poxB ldhA*
	ALS1058:	ALS929 *atpFH*
	ALS1059:	ALS929 *atpFH arcA*
	ALS1062:	ALS929 *atpFH arcA* NOX^{+}

Medium: Defined (Glucose + Acetate + Isoleucine)

Conditions: Acetate-limited Chemostat

Dilution rate = 0.15 h^{-1}

Comparison of strains

Strains		ALS929	ALS1058	ALS1059	ALS1062
Dilution Rate	(h^{-1})	0.15	0.15	0.15	0.15
DCW	(g/L)	2.45	1.71	1.62	1.26
q_A	(g/g·h)	0.07	0.09	0.09	0.12
q_G	(g/g·h)	1.60	2.18	2.38	2.67
q_{Pyr}	(g/g·h)	1.11	1.65	1.86	2.01
$Y_{X/G}$	(g/g)	0.10	0.07	0.07	0.06
$Y_{X/A}$	(g/g)	2.39	1.82	1.70	1.33
$Y_{Pyr/G}$	(g/g)	0.70	0.76	0.78	0.75
$Y_{Pyr/X}$	(g/g)	7.15	10.67	11.97	12.94

Fed-Batch at Constant Specific Growth Rate

Strains: ALS929, ALS1059

Medium: Defined (Glucose + Acetate + Isoleucine)

Conditions: Aerobic (Air + Oxygen)

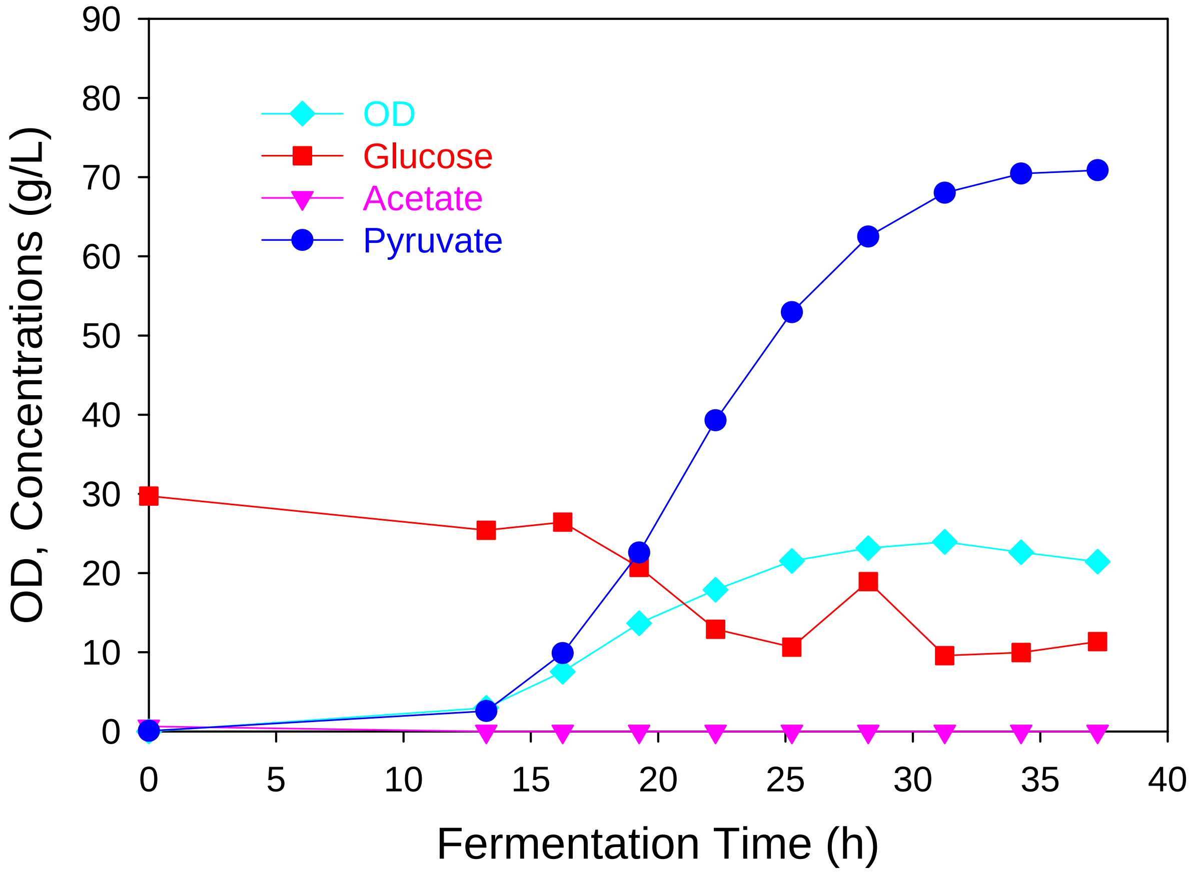
Specific growth rate = 0.15 h^{-1}

Feed glucose:acetate (30:1) exponentially

Limiting substrate: Acetate

With or Without 5 mM Betaine

Fed-Batch with ALS929



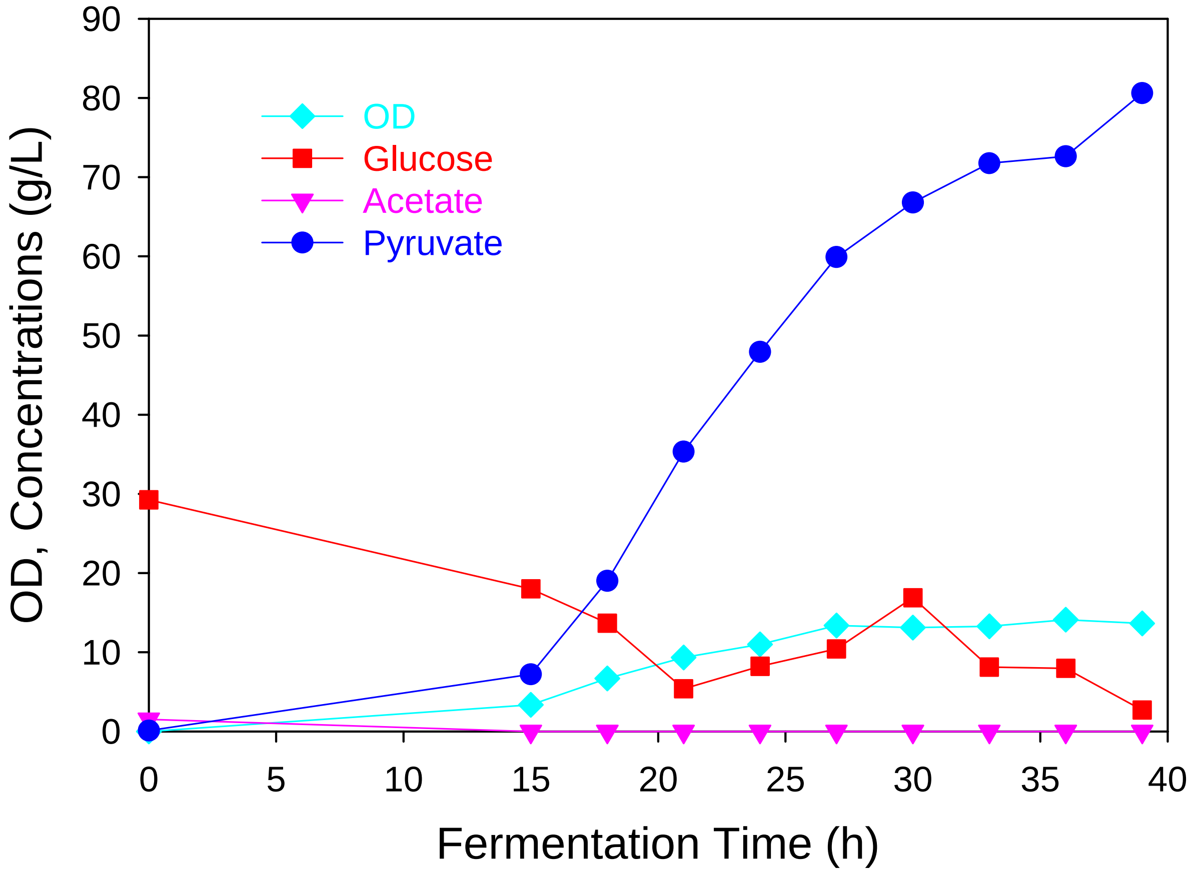
Pyruvate: **70.4 g/L**

Productivity: **2.06 g/L·h**

Yield: **0.62 g/g**

OD: **24.0**

Fed-Batch with ALS1059



Pyruvate: **80.6 g/L**

Productivity: **2.07 g/L·h**

Yield: **0.73 g/g**

OD: **14.1**

Fed-Batch Results

Strains		ALS929	ALS929
Betaine	(mM)	0	5.0
Max. OD		16.8	24.0
Pyruvate conc.	(g/L)	56.3	70.4
Q_{pyr}	(g/L·h)	2.05	2.06
Max. Q_{pyr}	(g/L·h)	5.78	5.92
Max. q_{pyr}	(g/g·h)	1.25	1.16
$Y_{Pyr/G}$	(g/g)	0.71	0.62
$Y_{Pyr/X}$	(g/g)	8.6	8.26

Fed-Batch Results

Strains		ALS929	ALS1059
Betaine	(mM)	5.0	5.0
Max. OD		24.0	14.1
Pyruvate conc.	(g/L)	70.4	80.6
Q_{pyr}	(g/L·h)	2.06	2.07
Max. Q_{pyr}	(g/L·h)	5.92	5.58
Max. q_{pyr}	(g/g·h)	1.16	1.87
$Y_{Pyr/G}$	(g/g)	0.62	0.73
$Y_{Pyr/X}$	(g/g)	8.26	14.78

Summary

1) **Acetate is the best limiting nutrient**
2) **Glycolysis enhanced by introducing *atpFH* knockout, *arcA* knockout and NADH Oxidase**
3) **Betaine protects cells from osmotic stress and increases cell growth and pyruvate production**
4) **80 g/L pyruvate**

   Overall productivity of 2.07 g/L·h

   Yield of 0.73 g/g

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