Fundamentals of enzyme kinetics and thermodynamic analysis for microbial communities

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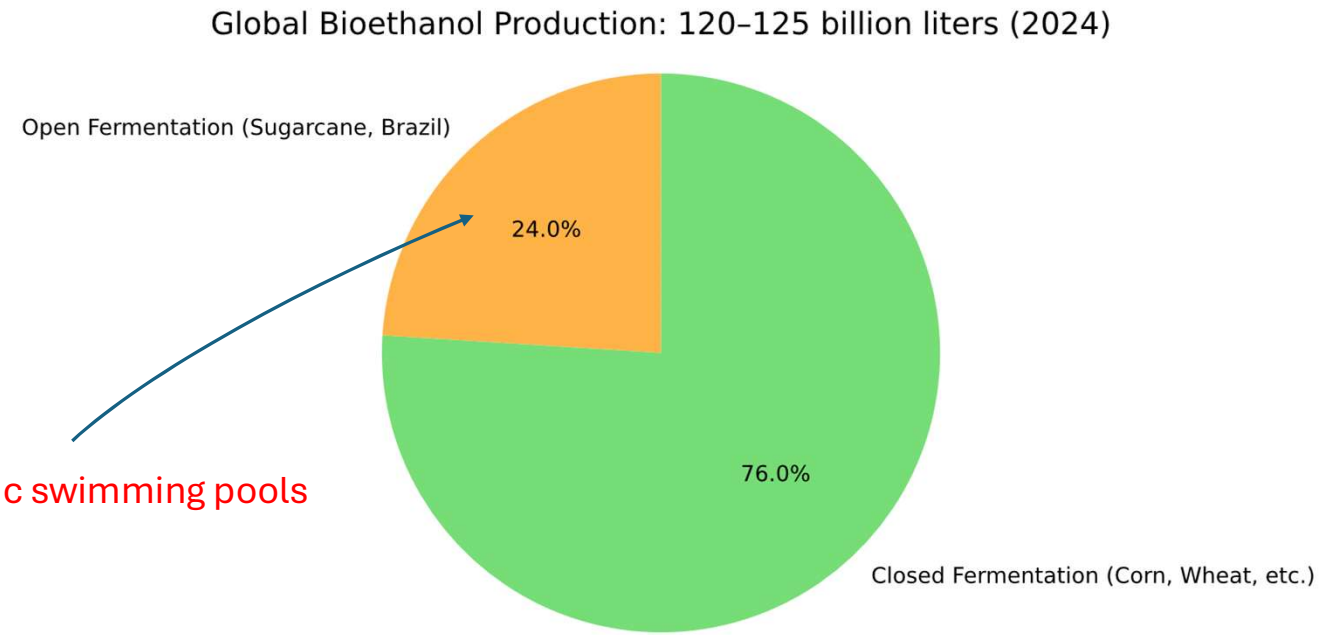
Today:

- 10:00 – 13:00 Djordje and Timmy – theoretical models in microbial ecology.
- **14:00 - 14:45 *Invited Lecture* –enzyme kinetics for microbial communities**
- **15:00 – 18:00 Flux Balance Analysis: Timmy**

Tomorrow:

- **9:00 – 11:30 Thermodynamics and computational practice**
- 12:00 – 13:00 *Invited lecture – Djordje.* Dynamic metabolic models
- 14:00 – 18:00 Djordje – Dynamic FBA y Resource allocation.

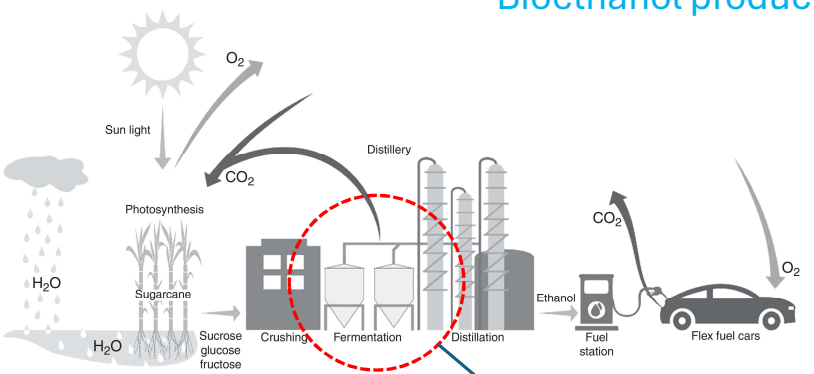
The size of the problem we are going to study today



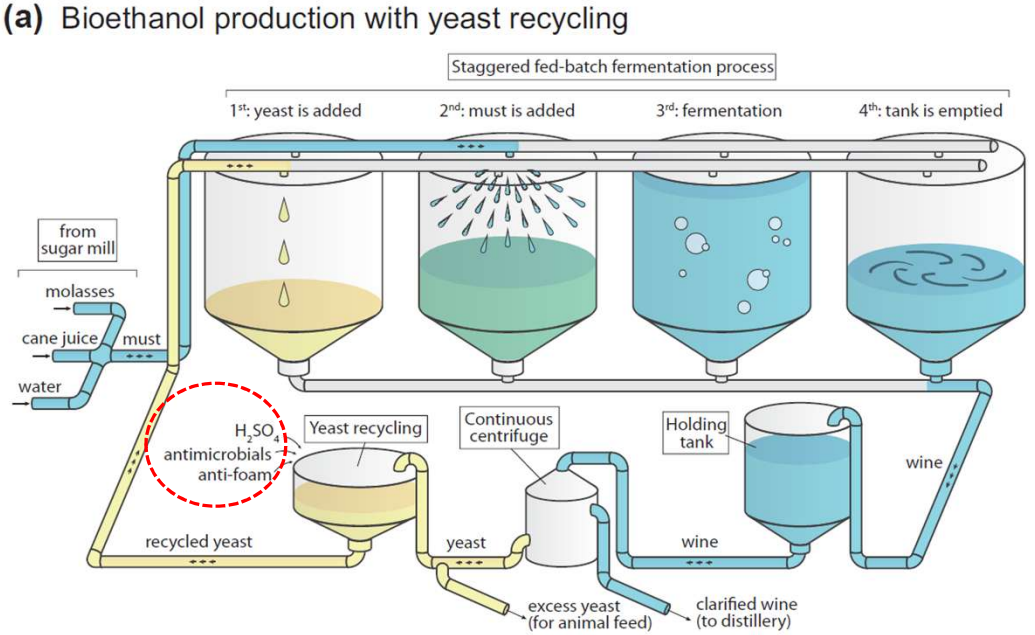
12000 Olympic swimming pools

Bioethanol in Brazil is mainly produced in open fermentation

Bioethanol production, using open fermentations, in a nutshell



Source: https://doi.org/10.1016/j.bjm.2016.10.003



Source: https://doi.org/10.1093/g3journal/jkad104

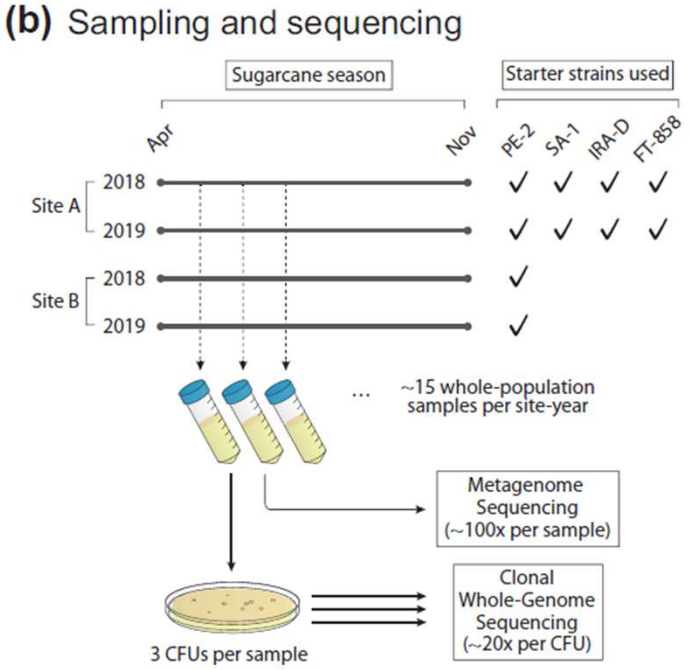
Challenges of the open fermentations

OXFORD G3 Genes | Genomes | Genetics

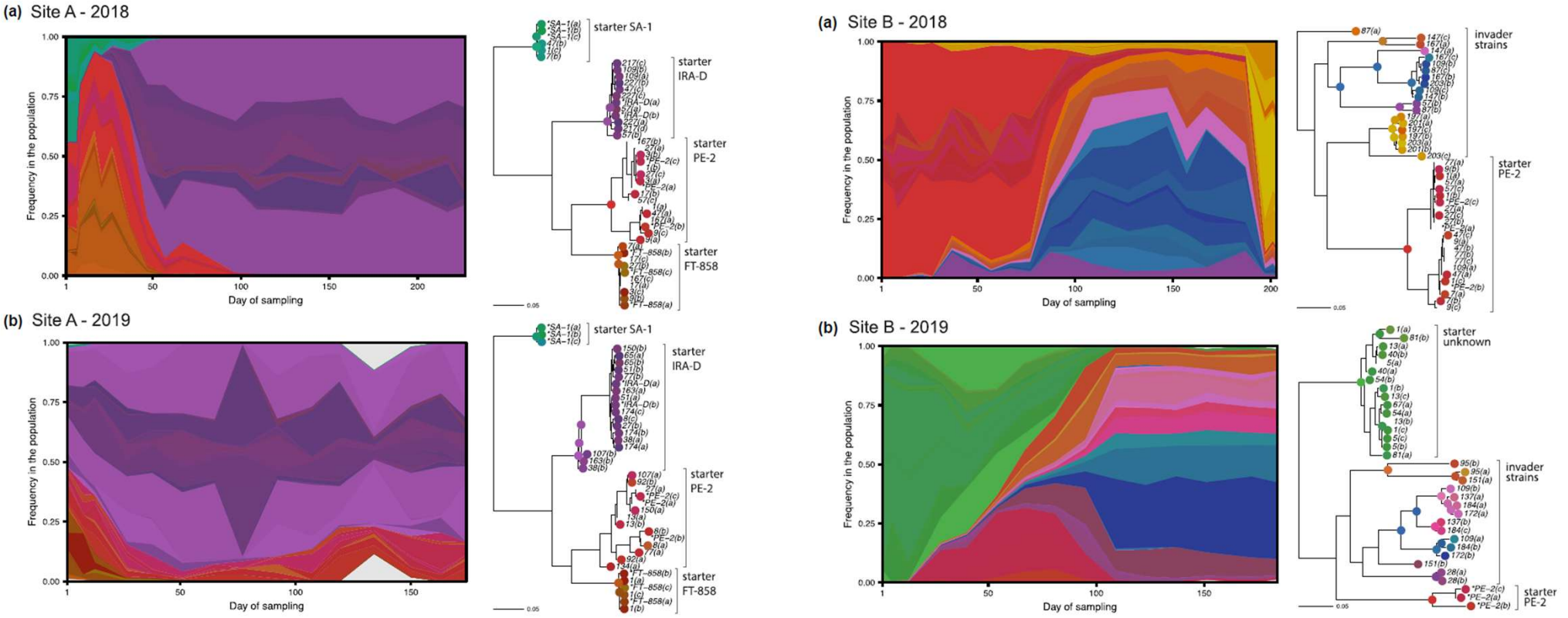
G3, 2023, 13(7), jkad104
https://doi.org/10.1093/g3journal/jkad104
Advance Access Publication Date: 2 June 2023
Investigation

Yeast population dynamics in Brazilian bioethanol production

Artur Rego-Costa,[1,†] I-Ting Huang,[1,†] Michael M. Desai,[1,2,3,4] Andreas K. Gombert [5,*]



The yeast populations are dynamic



nature communications

Article

https://doi.org/10.1038/s41467-024-49683-2

Strain dynamics of contaminating bacteria modulate the yield of ethanol biorefineries

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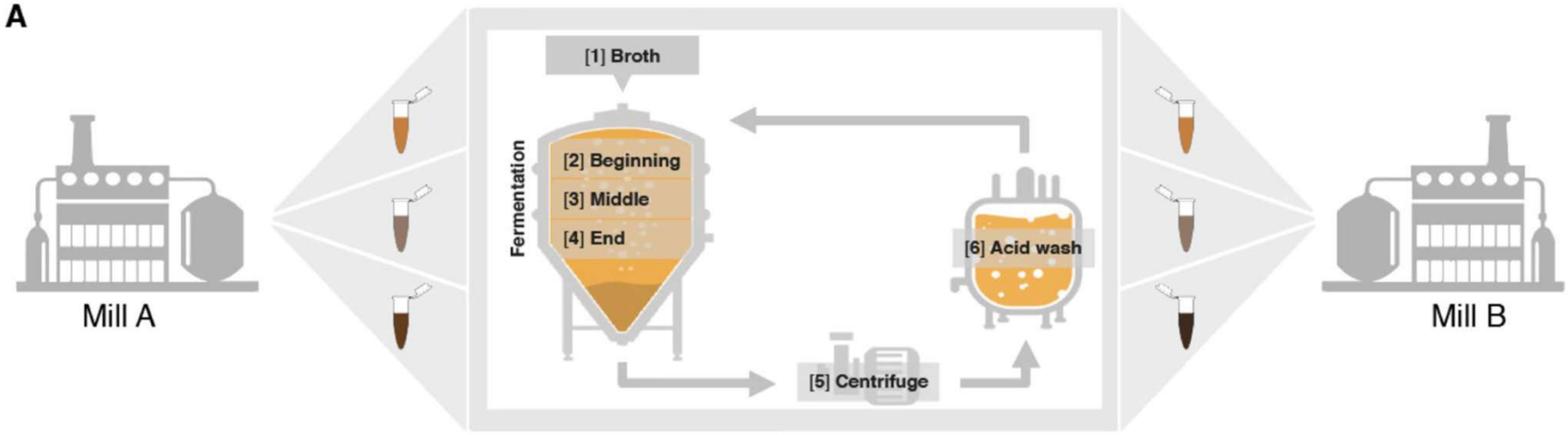
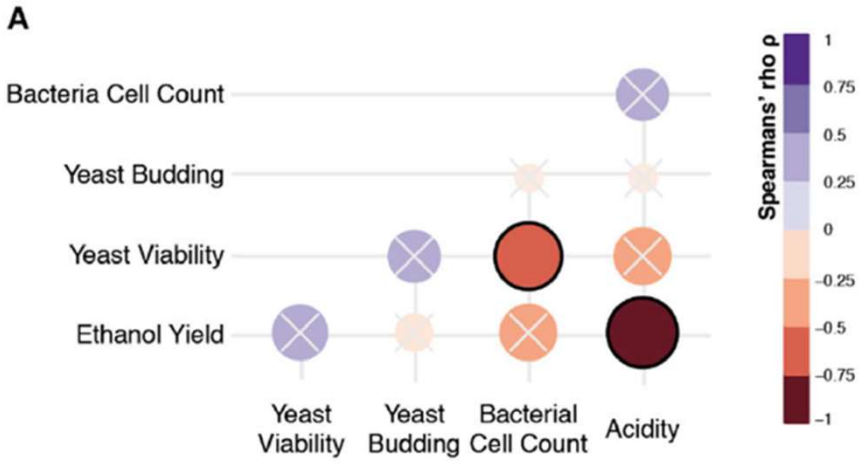
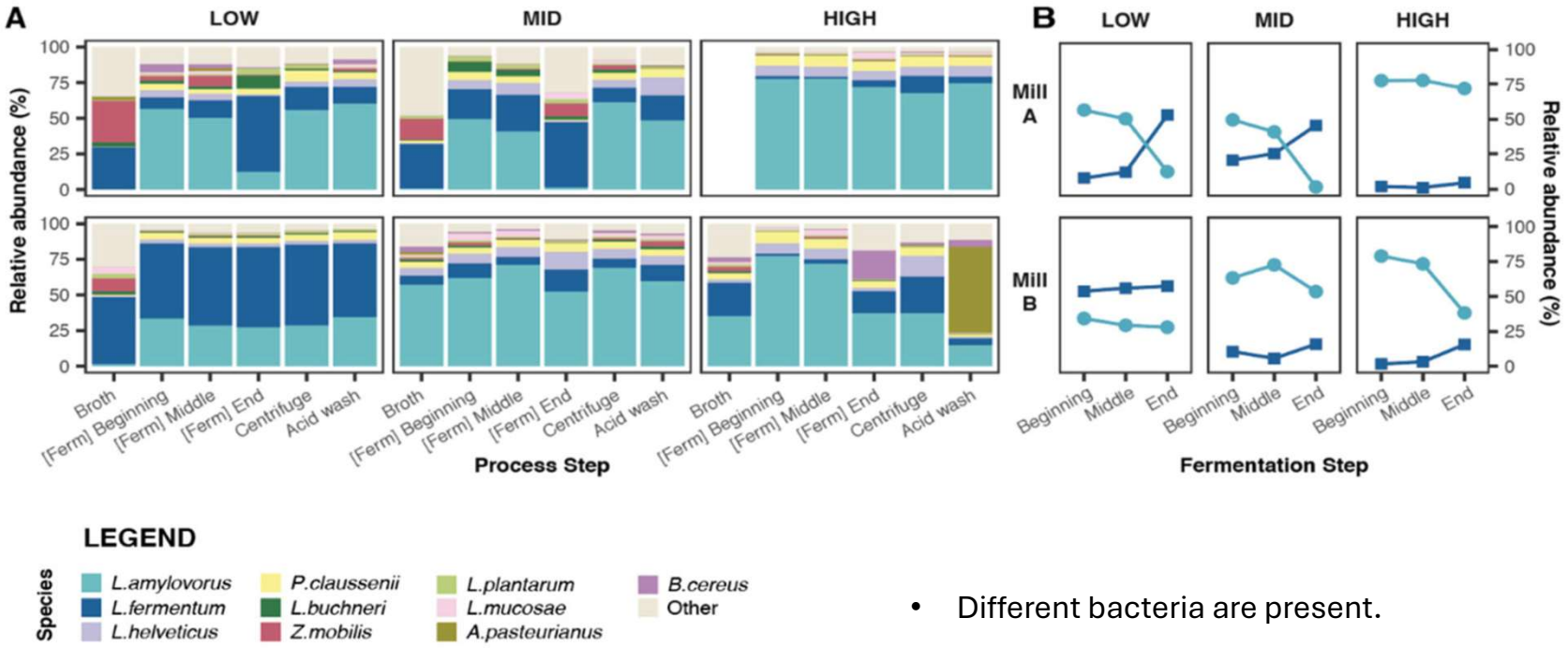
Felipe Senne de Oliveira Lino[1,6], Shilpa Garg[1,6], Simone S. Li[1,2], Maria-Anna Misiakou[1], Kang Kang[3], Bruno Labate Vale da Costa[4], Tobias Svend-Aage Beyer-Pedersen[1], Thamiris Guerra Giacon[5], Thiago Olitta Basso[5], Gianni Panagiotou[3] & Morten Otto Alexander Sommer[1]



Number of bacteria and the acids produced by them negatively affect ethanol production





- Different bacteria are present.
- *L. amylovorus* and *L. fermentum* are the dominant bacteria.
- High-performing batches contained more *L. amylovorus*.
- Across all batches and mills, *L. fermentum* increases and *L. amylovorus* decreases by the end of fermentation.

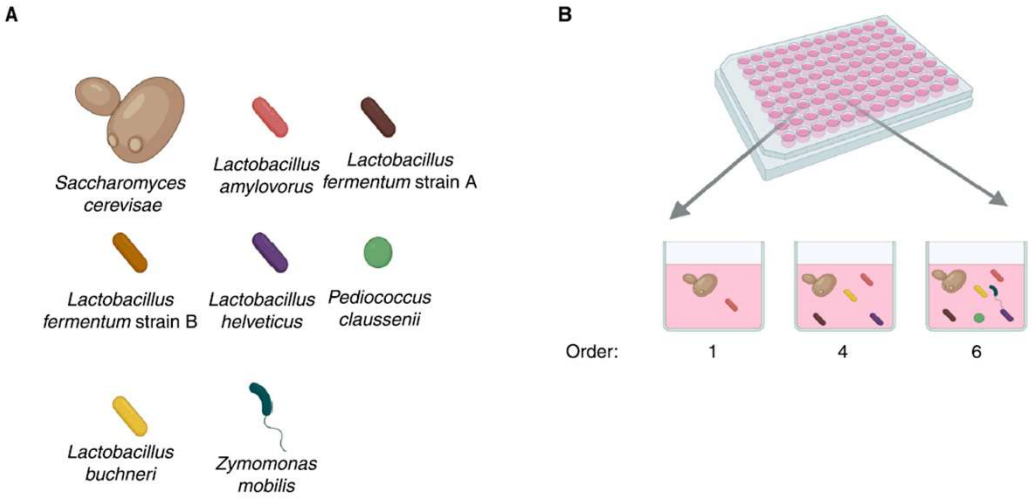
nature communications

Article | Open access | Published: 08 March 2021

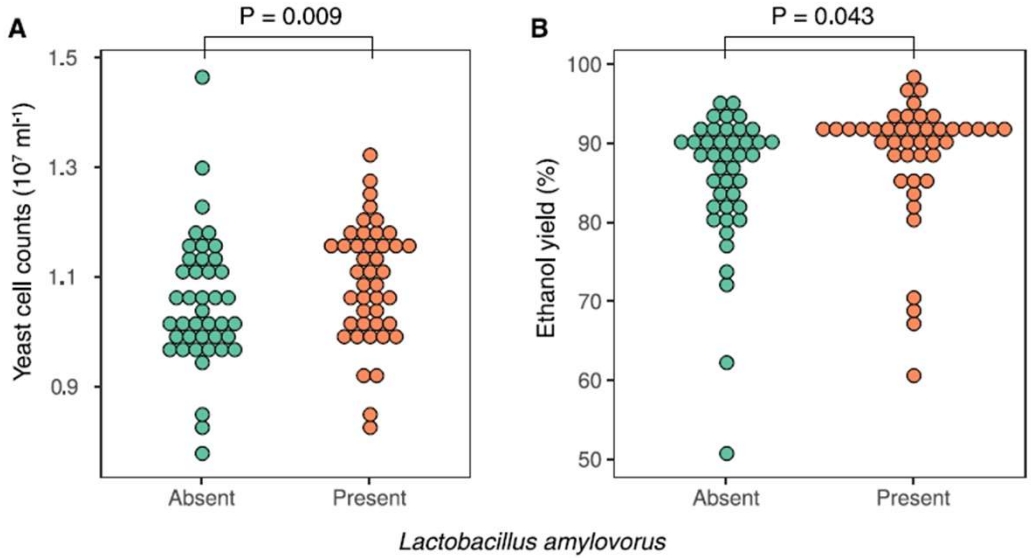
Complex yeast–bacteria interactions affect the yield of industrial ethanol fermentation

Felipe Senne de Oliveira Lino, Djordje Bajic, Jean Celestin Charles Vila, Alvaro Sánchez & Morten Otto Alexander Sommer

Nature Communications **12**, Article number: 1498 (2021) | Cite this article



The bacterium *Lactobacillus amylovorus* has a positive effect on the ethanol production



In open fermentations:

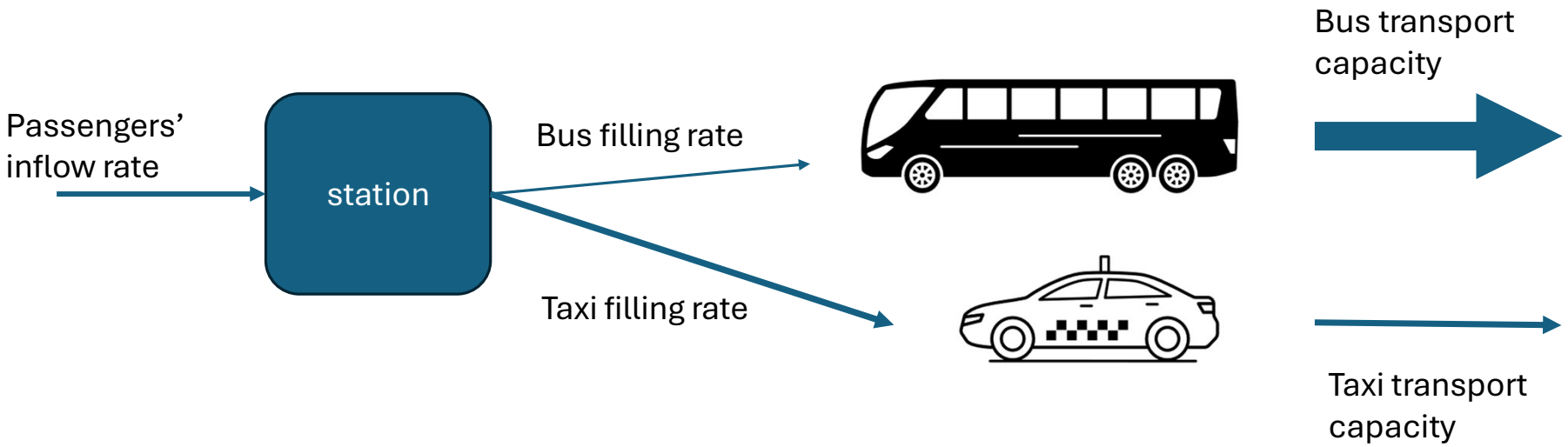
- Yeast populations are dynamic.
- Different bacteria are present and influence the ethanol production.
- Some bacteria have negative effects on the ethanol production, but some bacteria have a positive effect.

Our goals:

- Concepts from enzyme kinetics useful to study microbial communities
- Stoichiometric analysis of metabolic networks (Timmy's lecture)
- Integrating enzyme kinetics, stoichiometric analysis and thermodynamics

Let's start with a fundamental concept: **saturation**

- One bus station
- Passengers arriving to the station at different rates
- Passengers can be transported out of the station by bus or by taxi



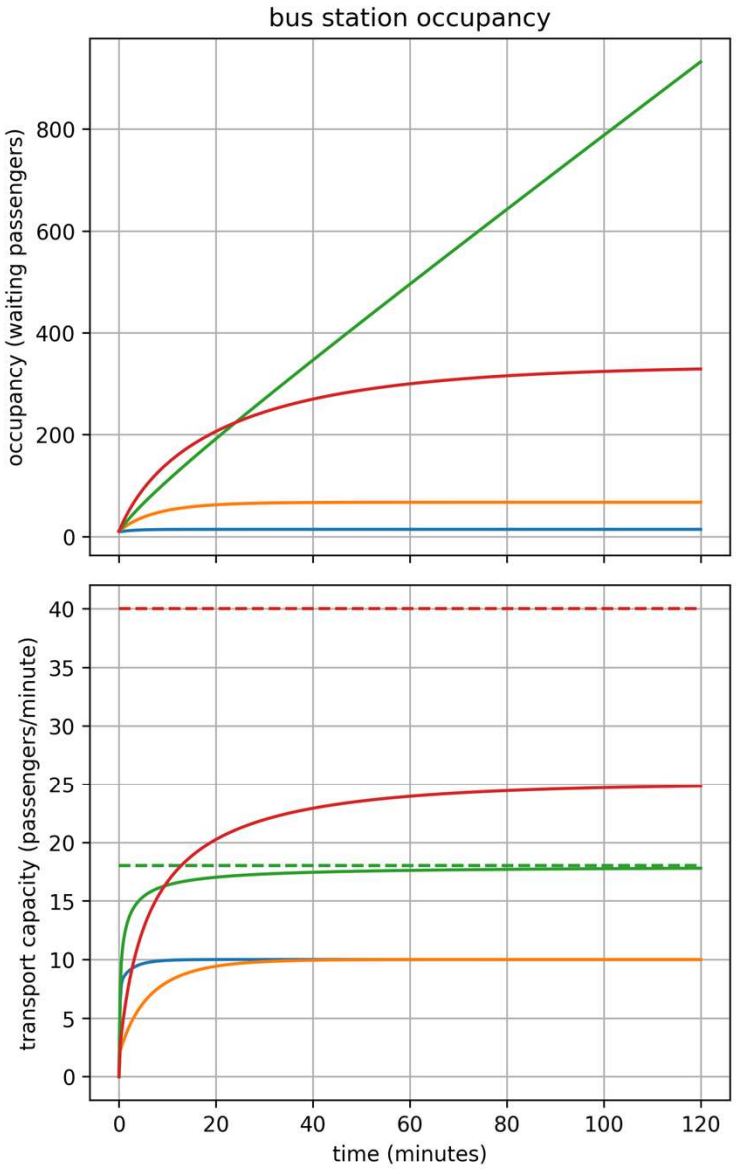
Which is the most effective way of transportation?

S : passengers waiting at the station
E : empty vehicles ready to be filled
ES : vehicles currently being filled with passengers
P : cumulative passengers already transported

k1 : rate at which passengers enter vehicles
k1r : rate at which passengers leave vehicles (before departure)
k2 : rate at which filled vehicles depart
f : external passenger influx (new arrivals per minute)

taxis are easy to fill but small capacity
buses are harder to fill but large capacity

```
dS  = f - k1*S*E + k1r*ES      # passengers at the station
dE  = -k1*S*E + (k1r + k2)*ES  # empty vehicles
dES = k1*S*E - (k1r + k2)*ES   # filled vehicles
dP  = k2*ES                    # passengers transported out of the station
```



Set:

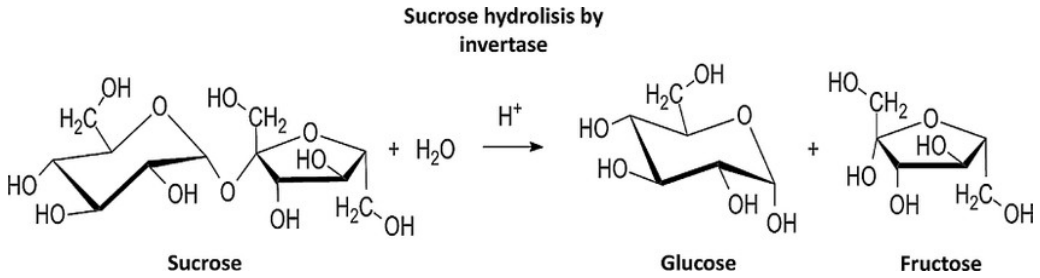
- Taxis carry 3 passengers
- Buses carry 20 passengers

taxis (n=6), passengers arriving rate=10
buses (n=2), passengers arriving rate=10
taxis (n=6), passengers arriving rate=25
buses (n=2), passengers arriving rate=25

- **What are representing the dashed lines?**
- What happens with the transportation by **taxi** when the passenger arriving rate is **25 passengers/minute**?

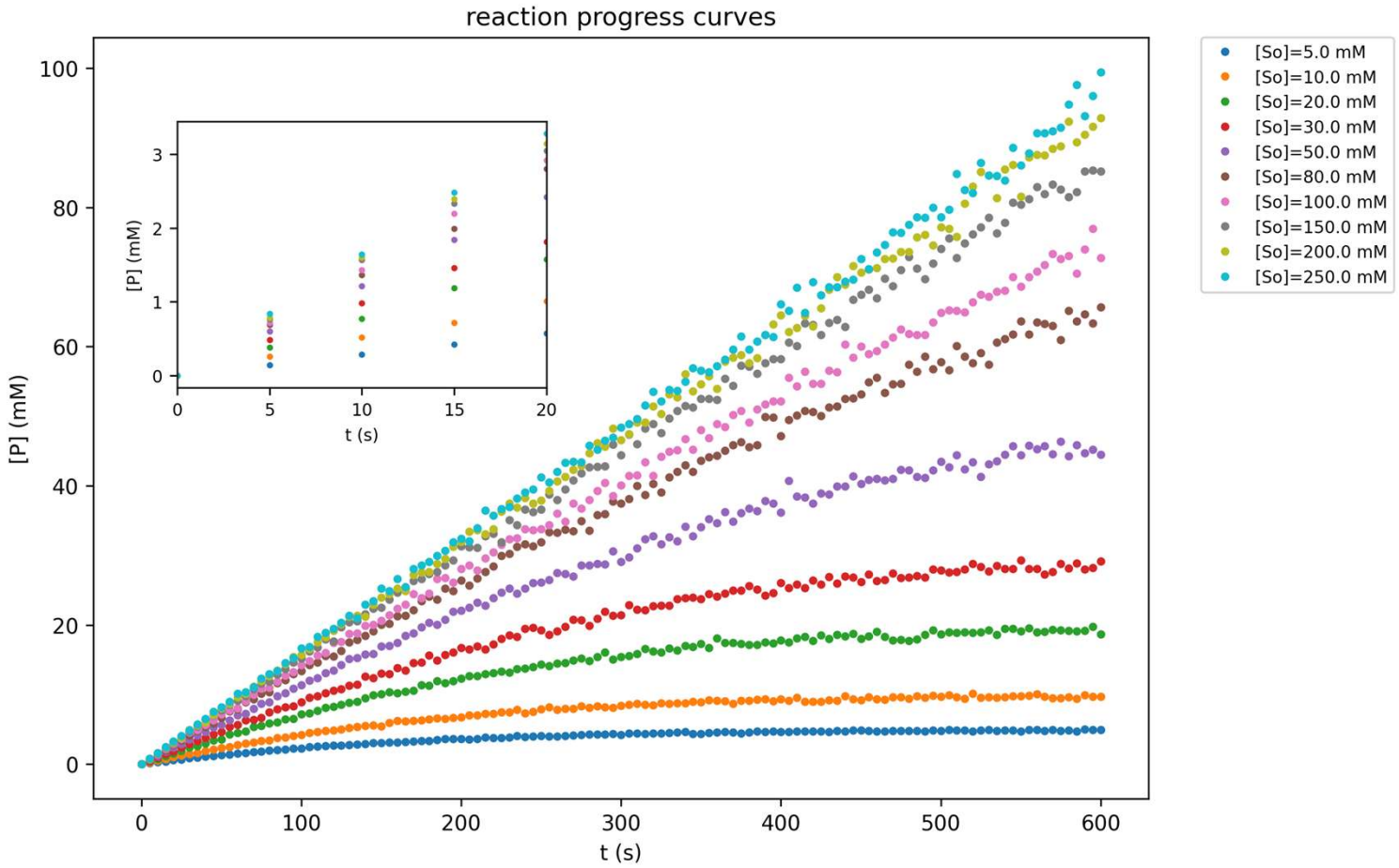
Maud Menten Leonor Michaelis

Michaelis, L., and Menten, M. (***1913***) Die kinetik der invertinwirkung, *Biochemistry Zeitung 49, 333-369*.



Happy coincidence! Sucrose is the most abundant sugar in the sugarcane juice

Michaelis and Menten also observed the phenomenon of saturation while studying the reaction catalyzed by the invertase



If there is one liter of a sucrose solution of 5 grams/liter, and we add 0.001 grams of invertase from yeast, how long it takes to hydrolyze half of the sucrose?

What do we need to know to answer this question?

Goal: predict the product formation rate in an enzyme-catalyzed reaction

E: concentration of free enzyme

S: concentration of sucrose

ES: concentration of the enzyme-substrate complex

They assumed that a ***rapid equilibrium*** between the species E, S and ES is established:

$k_1 >> k_2$ $\quad\quad$ $k_{1r} >> k_2$

They assumed that the rate-limiting step is the product formation:

$$product\ formation\ rate = k_2 * ES$$

In 1913, no experimental evidence of the existence of ES.

Rapid equilibrium model

$$E + S \underset{k_{1r}}{\overset{k_1}{\rightleftharpoons}} ES$$

$$rate^{\,ES\ complex\ formation} = rate^{\,ES\ complex\ dissociation}$$

$$k_1 * \boldsymbol{E}(t) * S(t) = k_{1r} * \boldsymbol{ES}(\boldsymbol{t})$$

Difficult to measure

$$K_S = \frac{k_{1r}}{k_1} \qquad K_S = \frac{E(t) * S(t)}{ES(t)} \qquad ES(t) = \frac{E(t) * S(t)}{K_S} = \frac{(E_{added} - ES(t)) * S(t)}{K_S} \qquad ES(t) = \frac{E_{added} * S(t)}{K_S + S(t)}$$

$$product\ formation\ rate = \frac{k_2 * E_{added} * S(t)}{K_S + S(t)} = \frac{V^{max} * S(t)}{K_M + S(t)}$$

If there is one liter of a sucrose solution of 5 grams/liter, and we add 0.001 grams of invertase from yeast, **how long** it takes to hydrolyze **half** of the sucrose?

$$\text{Product formation rate} = \frac{k_{cat} * E_{added} * S(t)}{K_M + S(t)}$$

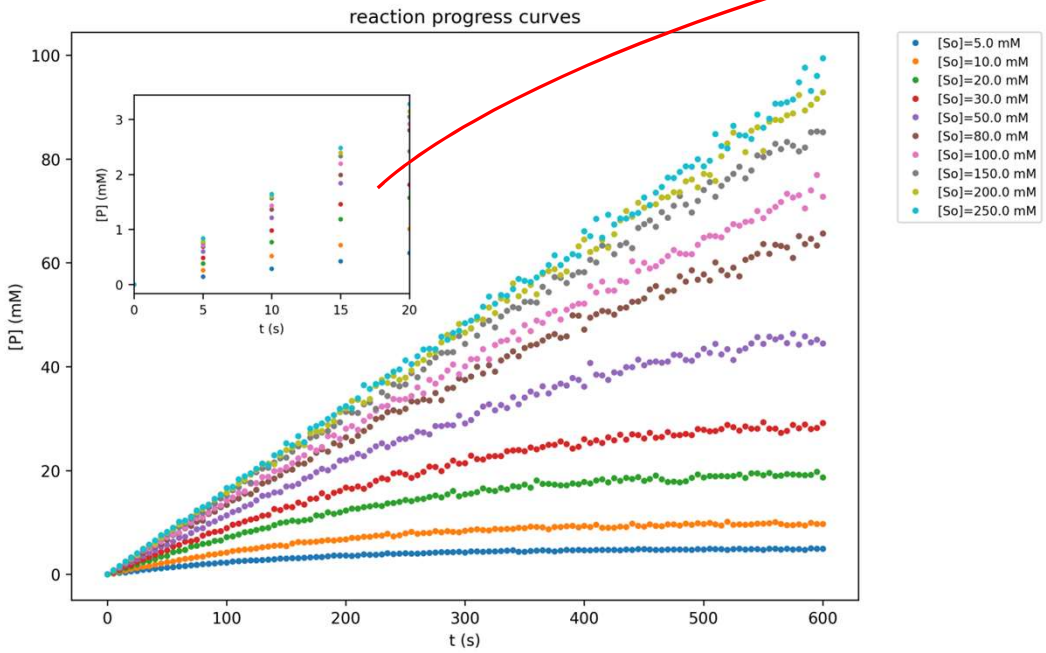
We know *So*=5 g/L, E_{added} = 0.001 g/L. However,

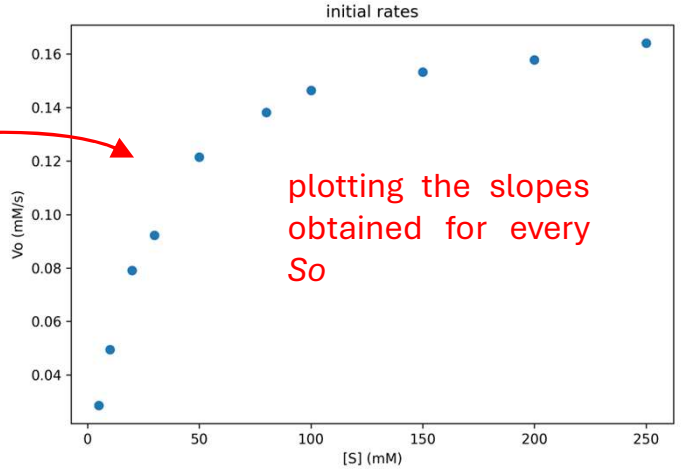
- How can we determine the kinetic parameters k_{cat} and K_M?
- S is changing in time.
- Where is the variable ***time***?

How can we determine the kinetic parameters k_{cat} and K_M?

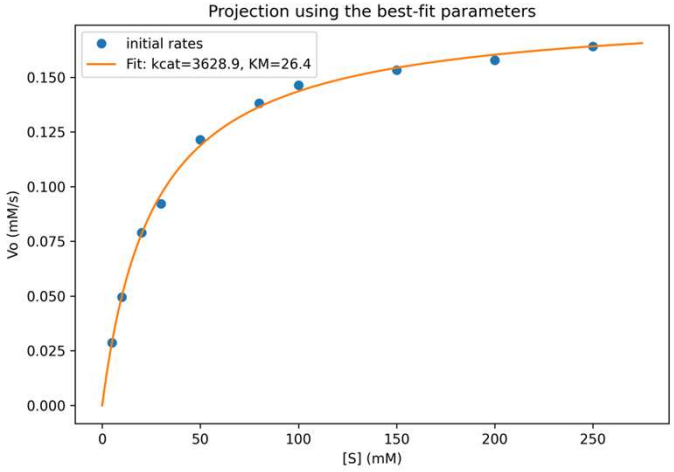
At the beginning of the reaction, *S* is *nearly* constant

$$initial\ rate\ (V_o) = \frac{k_{cat} * E_{added} * So}{K_M + So}$$





plotting the slopes obtained for every *So*



- S is changing in time. When half of the sucrose has been consumed, we are far from the initial rate conditions
- Where is the variable ***time***?

~~$initial\ rate\ (V_o) = \frac{k_{cat} * E_{added} * So}{K_M + So}$~~

$$product\ formation\ rate = \frac{dP}{dt} = -\frac{dS}{dt} = \frac{k_{cat} * E * S(t)}{K_M + S(t)}$$

$$-\frac{dS}{dt} = \frac{k_{cat} * E * S(t)}{K_M + S(t)}$$

$$S(t) + K_M * ln\frac{S(t)}{S_o} = -k_{cat} * E * t + S_o$$

Integrated form of the Michaelis-Menten equation

$$t = \frac{S(t) + K_M * ln\frac{S(t)}{So} - So}{-k_{cat} * E}$$

$$t = \frac{S(t) + K_M * ln\frac{S(t)}{So} - So}{-k_{cat} * E}$$

In our particular case, *S(t)* = *So*/2

$$t_{0.5} = \frac{So/2 + K_M * ln\frac{1}{2} - So}{-k_{cat} * E}$$

For *So*=5 g*/L, *E* = 0.001 g*/L, K_M = 25 mM and k_{cat} = 3600 s^{-1}, $t_{0.5} \approx$ 6 minutes

*Mw_sucrose = 342.3 g/mol
*Mw_invertase = 52000 g/mol

The integrated form of the Michaelis-Menten equation can also be employed to find the values of k_{cat} and K_M using reaction progress curves analysis

$$S(t) + K_M * ln\frac{S(t)}{S_o} = -k_{cat} * E * t + S_o$$

Between 1913 and 1997:

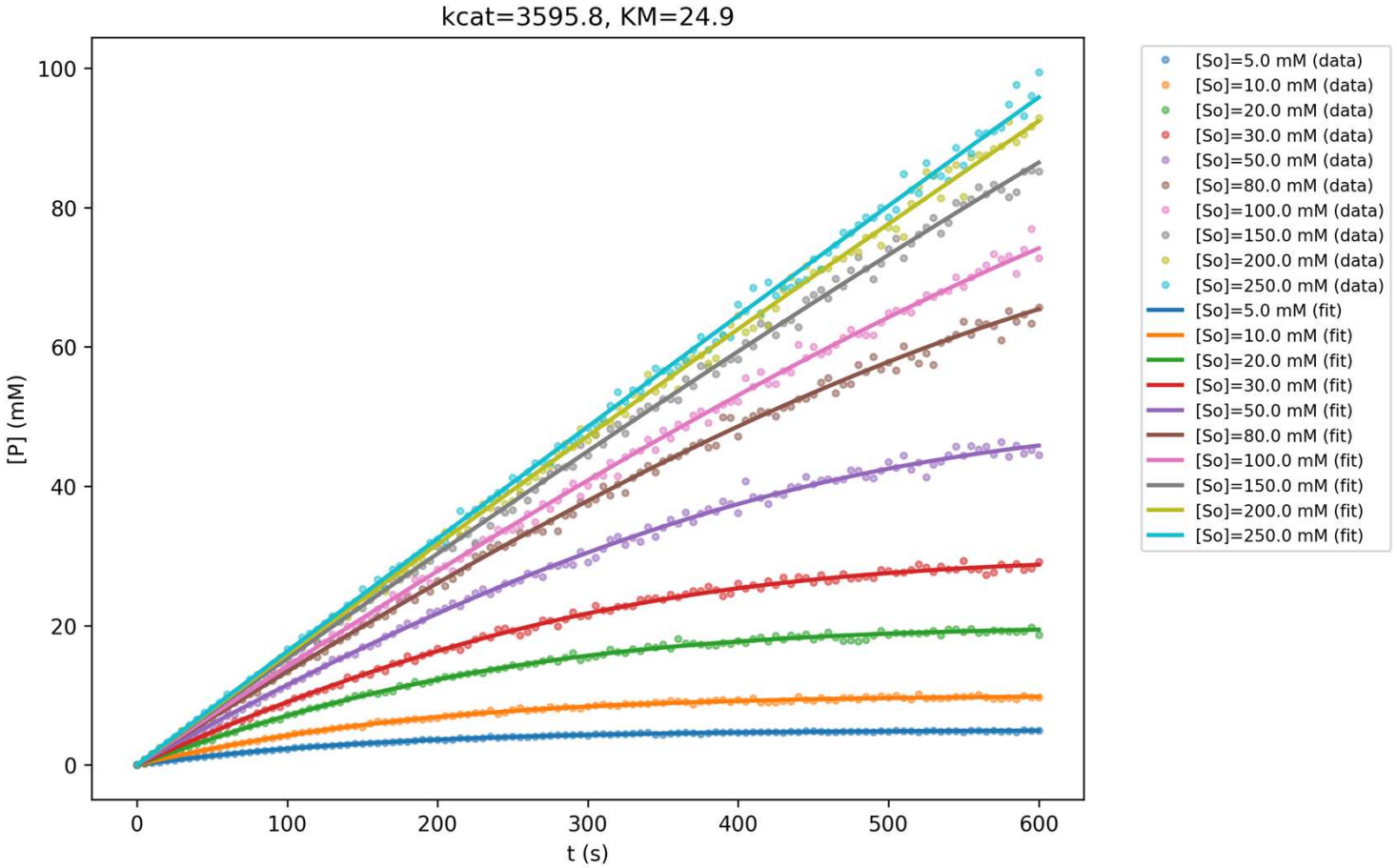
- Numerical integration
- (Complicated) algebraic methods

$$S(t) = K_M * \omega\left(\frac{S_o}{K_M} * e^{\frac{-k_{cat}*E*t+S_o}{K_M}}\right)$$

Santiago Schnell Claudio Mendoza

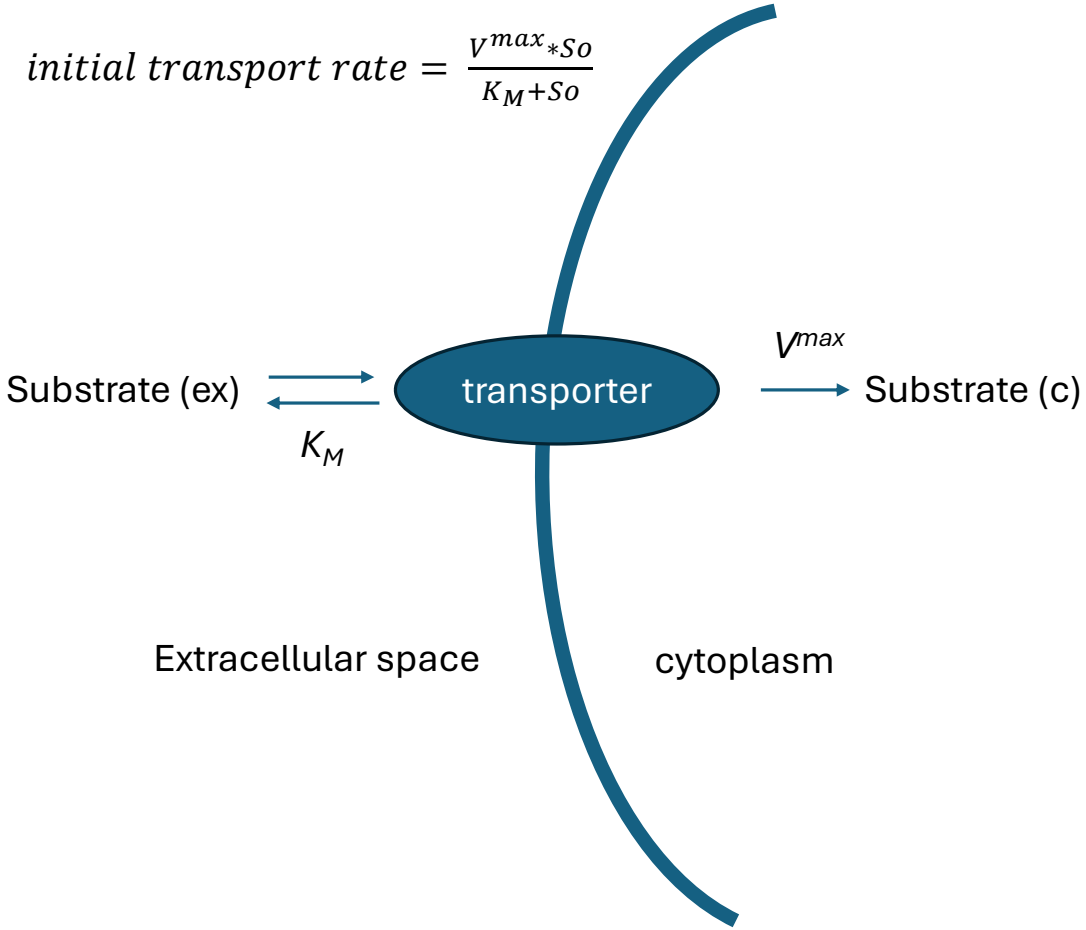
Latin got talent!

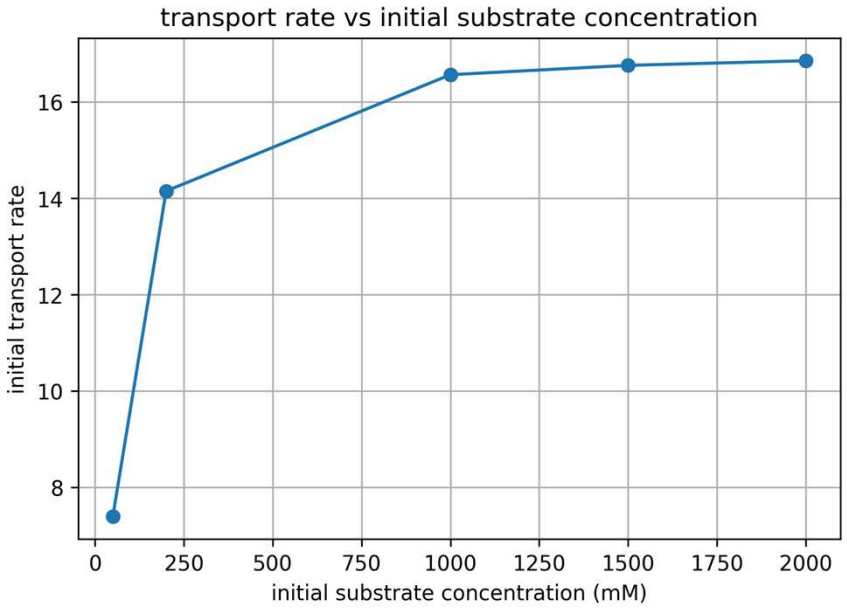
https://doi.org/10.1006/jtbi.1997.0425


kcat=3595.8, KM=24.9
[P] (mM)
t (s)
0
20
40
60
80
100
0
100
200
300
400
500
600
[So]=5.0 mM (data)
[So]=10.0 mM (data)
[So]=20.0 mM (data)
[So]=30.0 mM (data)
[So]=50.0 mM (data)
[So]=80.0 mM (data)
[So]=100.0 mM (data)
[So]=150.0 mM (data)
[So]=200.0 mM (data)
[So]=250.0 mM (data)
[So]=5.0 mM (fit)
[So]=10.0 mM (fit)
[So]=20.0 mM (fit)
[So]=30.0 mM (fit)
[So]=50.0 mM (fit)
[So]=80.0 mM (fit)
[So]=100.0 mM (fit)
[So]=150.0 mM (fit)
[So]=200.0 mM (fit)
[So]=250.0 mM (fit)

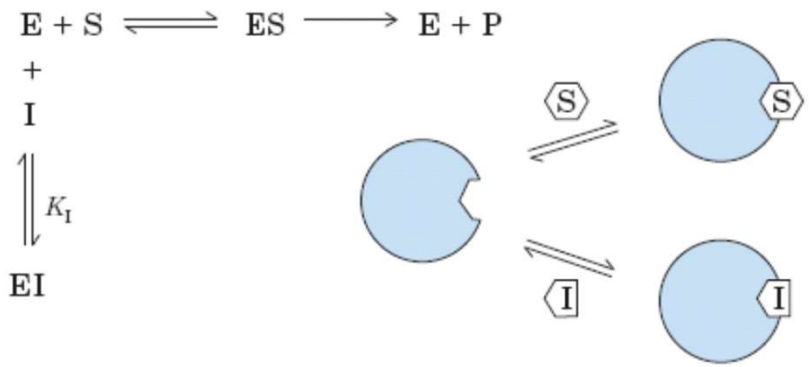
transporters can also be represented with the Michaelis-Menten equation

$$initial\ transport\ rate = \frac{V^{max} * So}{K_M + So}$$

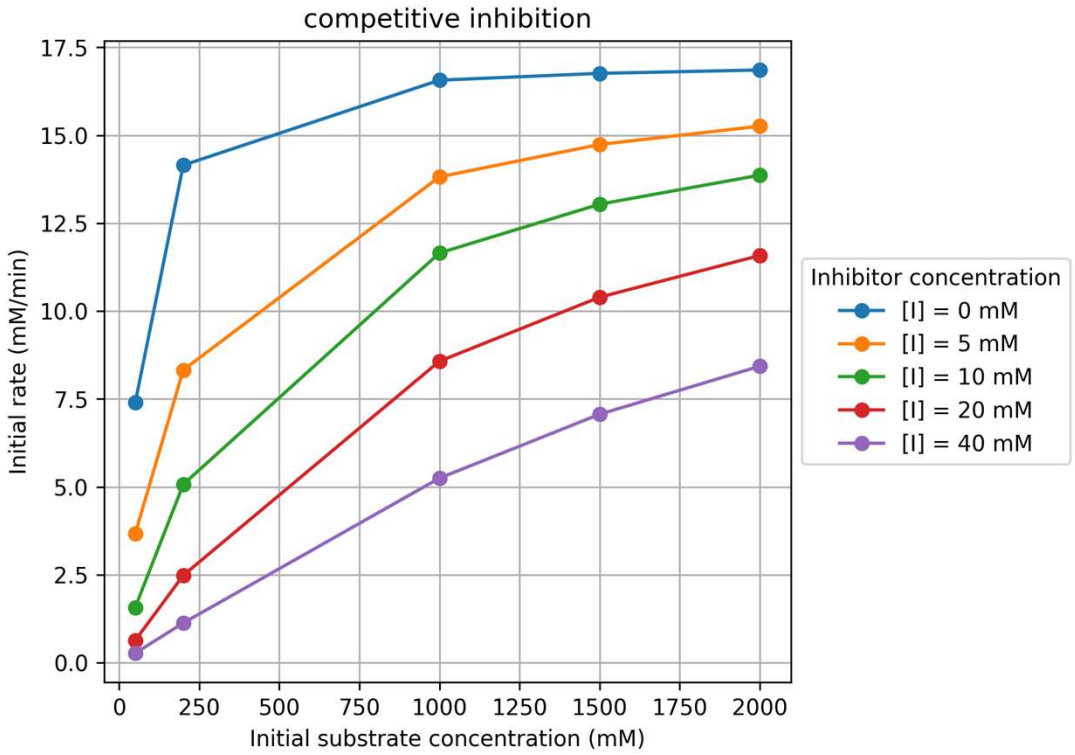




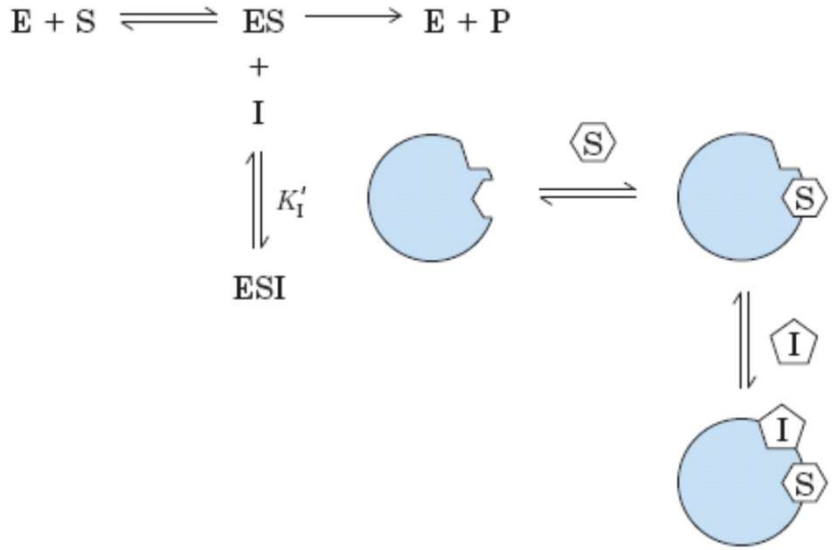
(a) Competitive inhibition



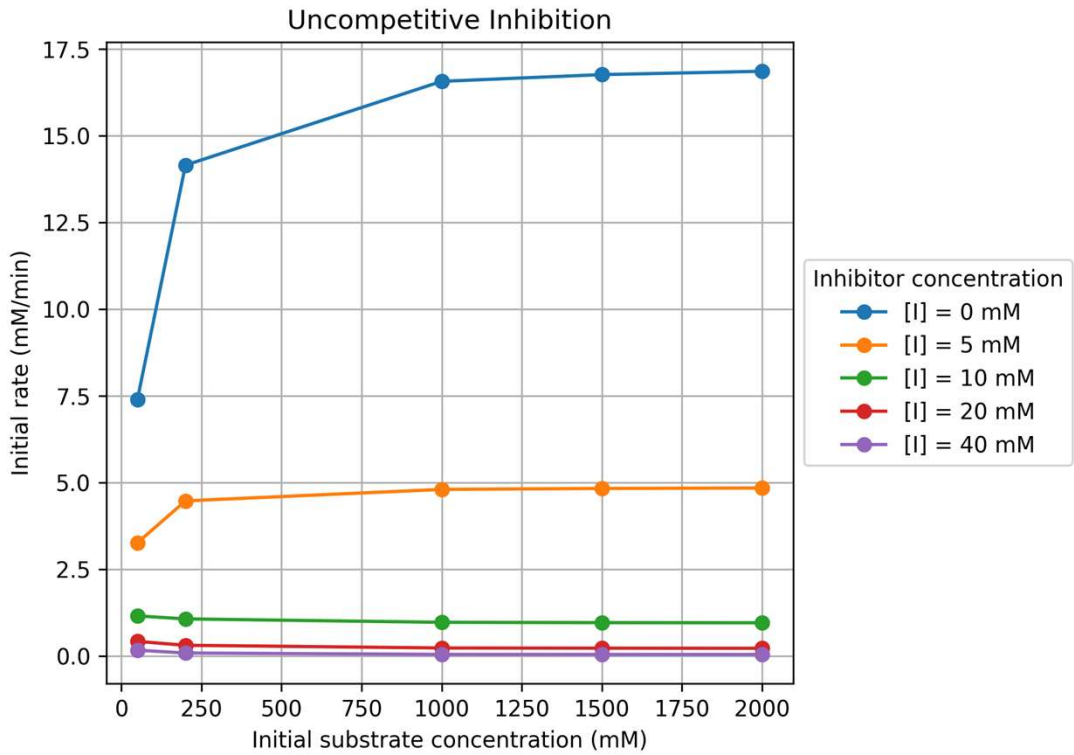
$$V_0 = \frac{V_{max}[S]}{\alpha K_m + [S]} \qquad \alpha = 1 + \frac{[I]}{K_I} \qquad K_I = \frac{[E][I]}{[EI]}$$



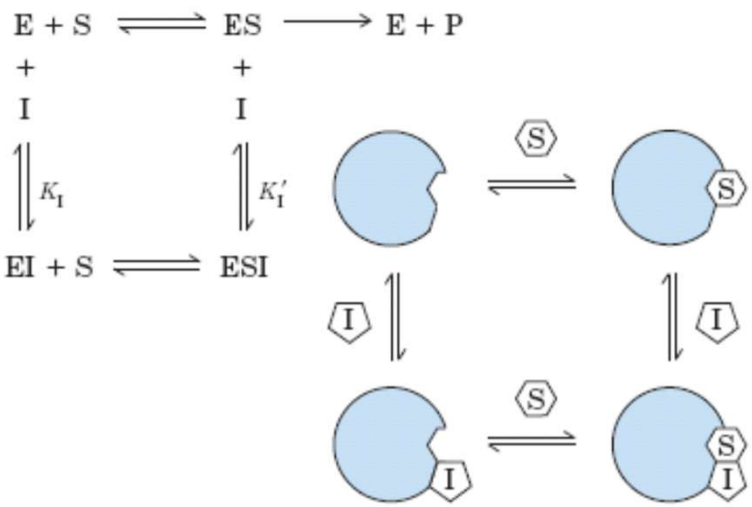
(b) Uncompetitive inhibition



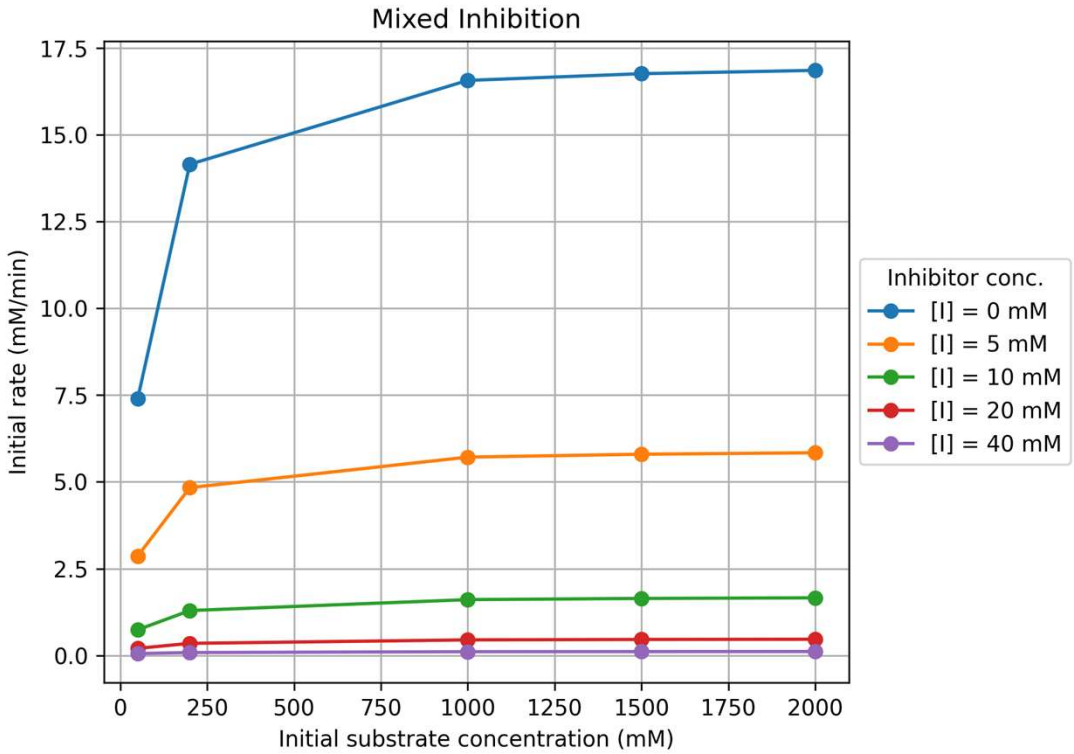
$$V_0 = \frac{V_{max}[S]}{K_m + \alpha'[S]} \qquad \alpha' = 1 + \frac{[I]}{K'_I} \qquad K'_I = \frac{[ES][I]}{[ESI]}$$



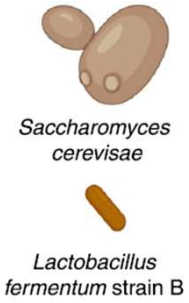
(c) Mixed inhibition



$$V_0 = \frac{V_{\max}\,[\mathrm{S}]}{\alpha K_\mathrm{m} + \alpha'[\mathrm{S}]}$$

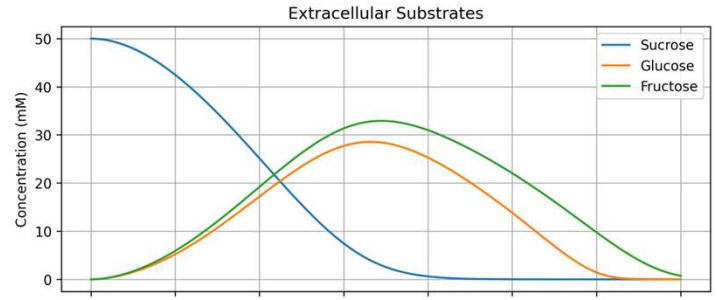


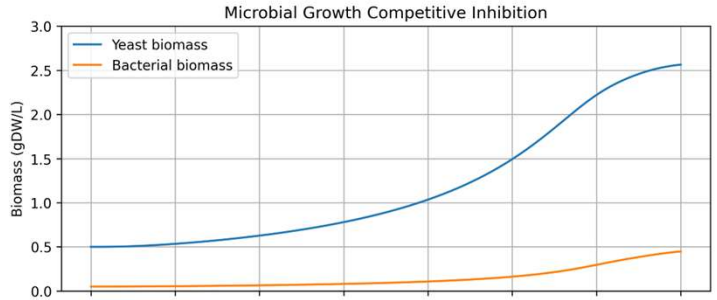
Coming back to our original problem:

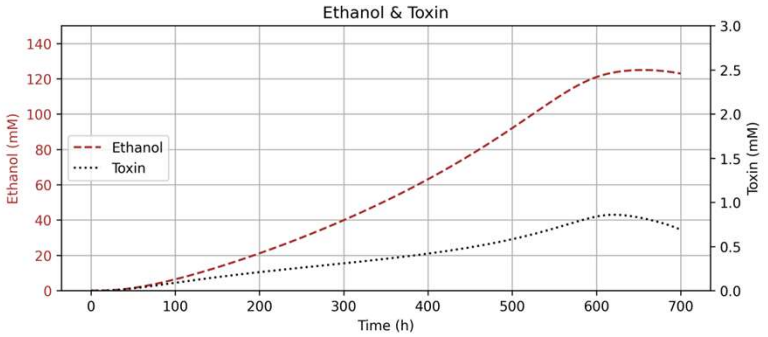


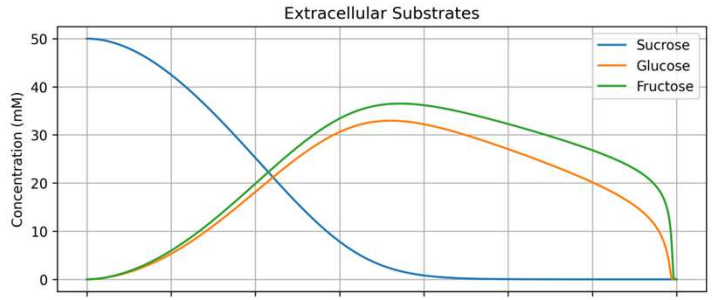
- Sucrose is hydrolyzed by invertase (produced by yeast) into glucose and fructose via Michaelis–Menten kinetics.
- Glucose and fructose are taken up by both yeast and bacteria.
- **Assuming: Yeast transporters for glucose and fructose are inhibited by a bacterial toxin. The inhibition mechanism can be competitive or non-competitive**.

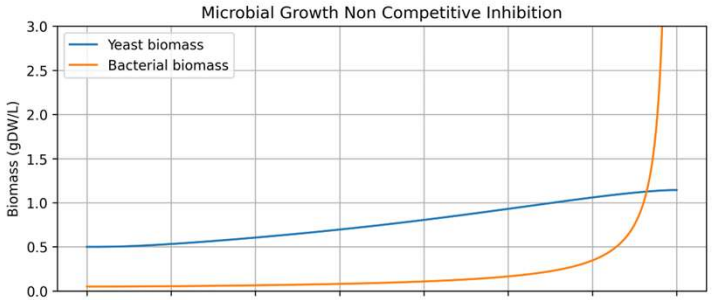
Which inhibitory mechanism is more effective for the bacteria?

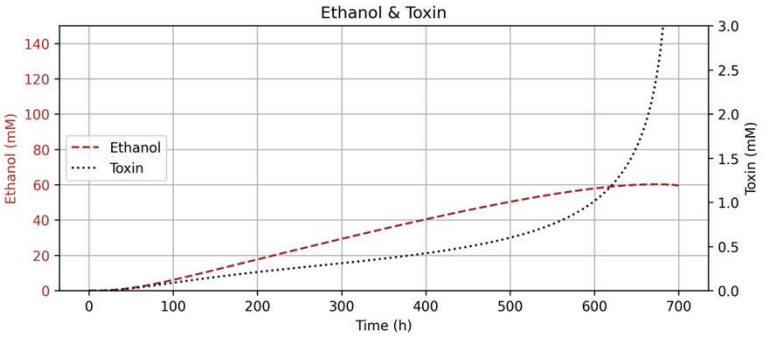

Extracellular Substrates
Sucrose
Glucose
Fructose
Concentration (mM)
0
10
20
30
40
50


Microbial Growth Competitive Inhibition
Yeast biomass
Bacterial biomass
Biomass (gDW/L)
0.0
0.5
1.0
1.5
2.0
2.5
3.0


Ethanol & Toxin
Ethanol
Toxin
Ethanol (mM)
Toxin (mM)
Time (h)
0
100
200
300
400
500
600
700

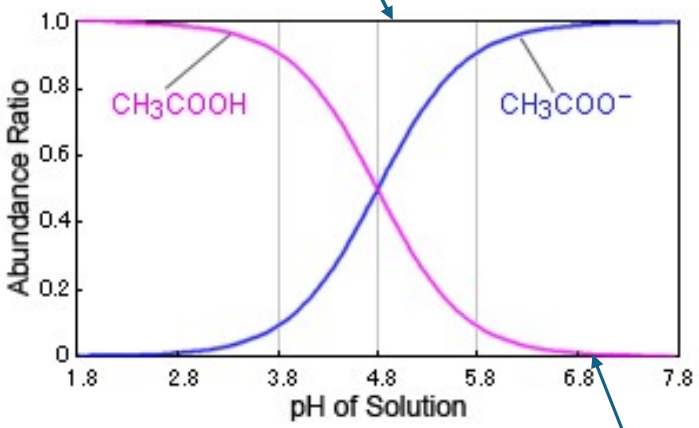

Extracellular Substrates
Sucrose
Glucose
Fructose
Concentration (mM)
0
10
20
30
40
50


Microbial Growth Non Competitive Inhibition
Yeast biomass
Bacterial biomass
Biomass (gDW/L)
0.0
0.5
1.0
1.5
2.0
2.5
3.0

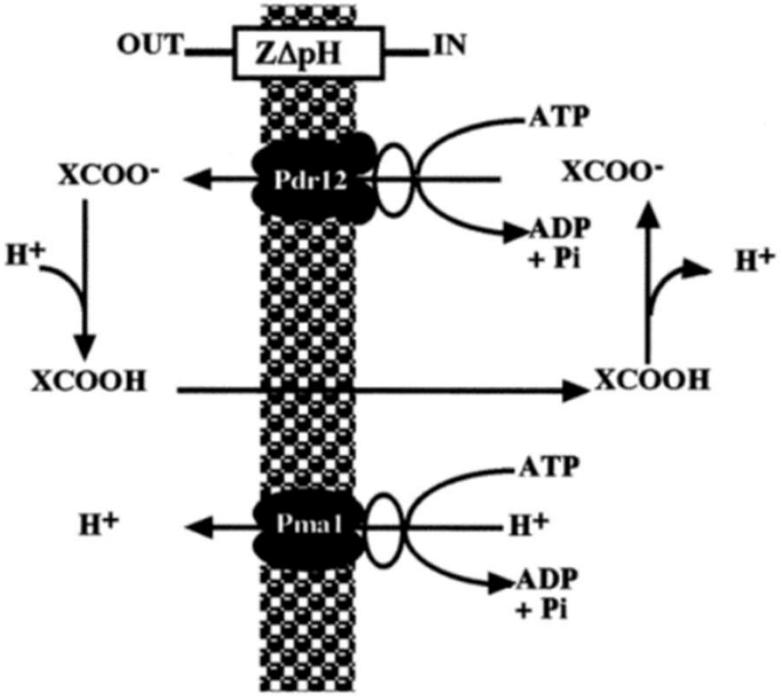

Ethanol & Toxin
Ethanol
Toxin
Ethanol (mM)
Toxin (mM)
Time (h)
0
100
200
300
400
500
600
700

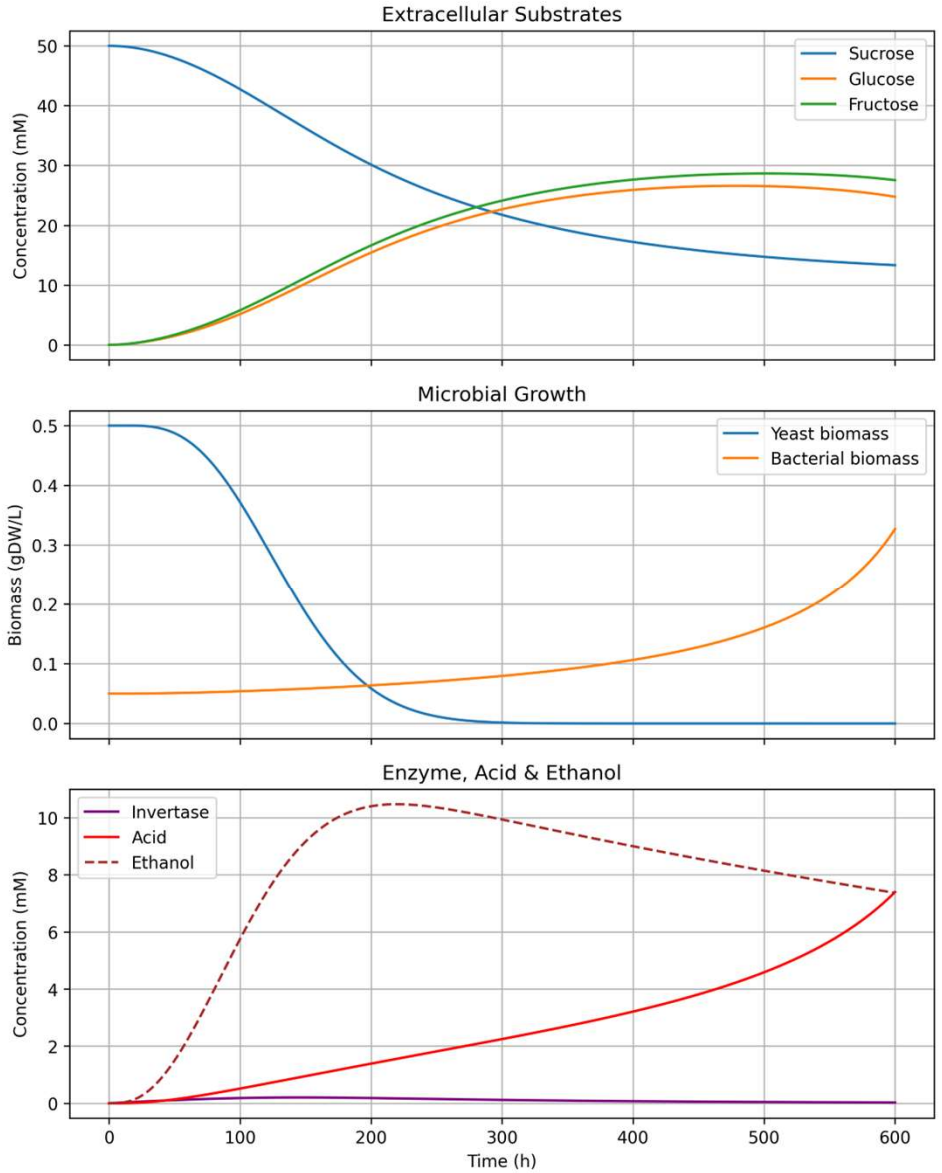
Energetic cost of the acids produced by bacteria

Optimal medium pH for yeast

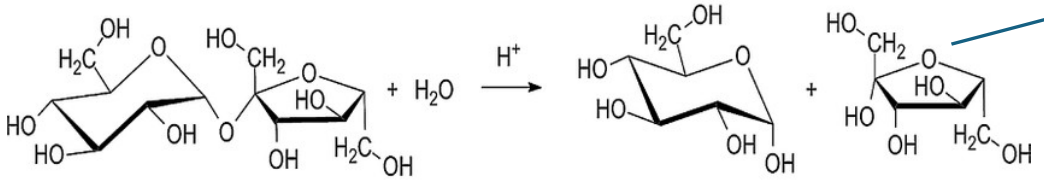


Cytoplasmic pH




Extracellular Substrates
Concentration (mM)
Sucrose
Glucose
Fructose
Microbial Growth
Biomass (gDW/L)
Yeast biomass
Bacterial biomass
Enzyme, Acid & Ethanol
Concentration (mM)
Invertase
Acid
Ethanol
Time (h)

Sucrose hydrolisis by invertase



Michaelis-Menten kinetics

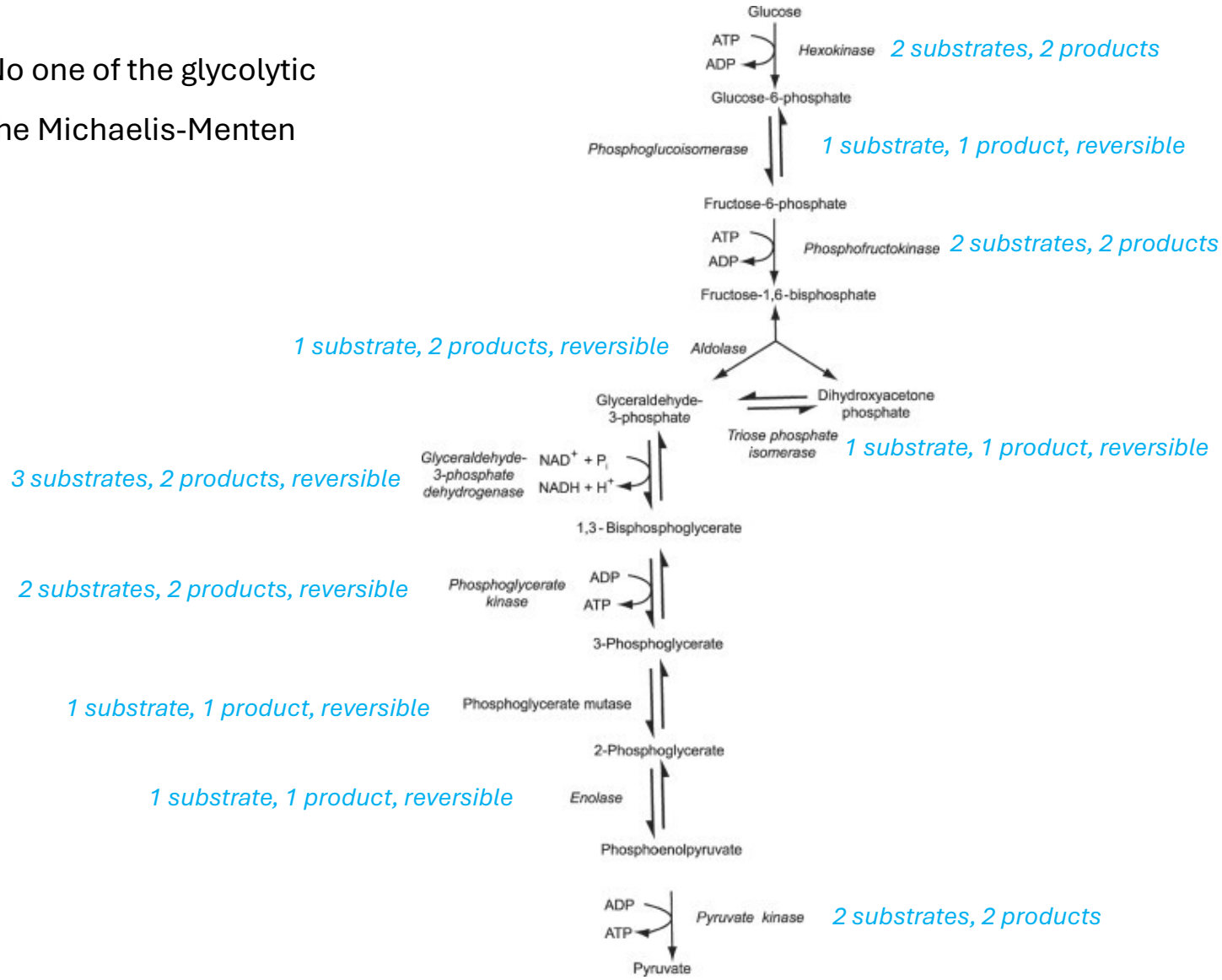
Michaelis-Menten kinetics

cytoplasm

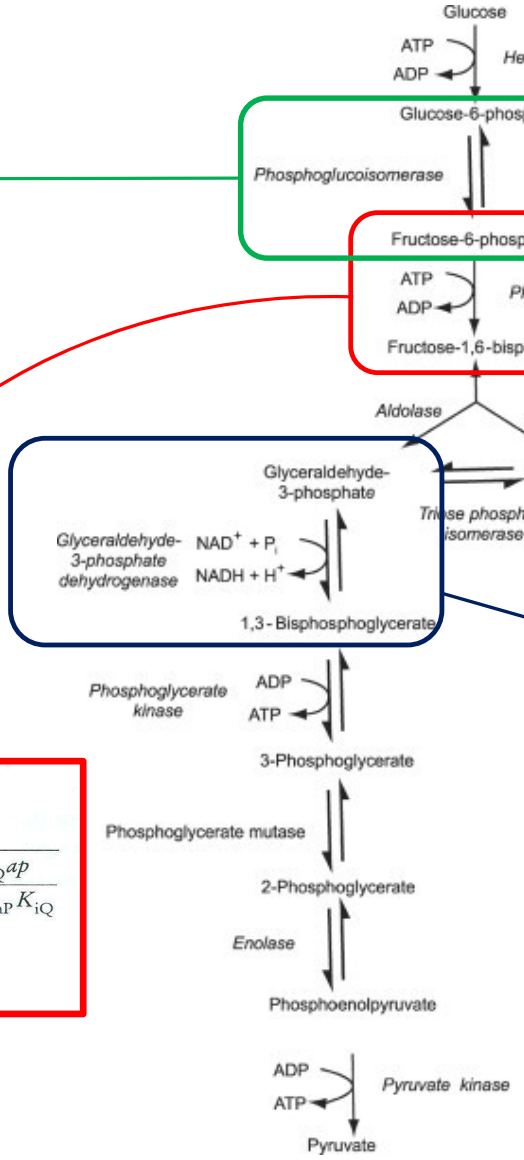
Michaelis-Menten kinetics

?

Houston, we have a problem: No one of the glycolytic reaction can be described with the Michaelis-Menten equation!



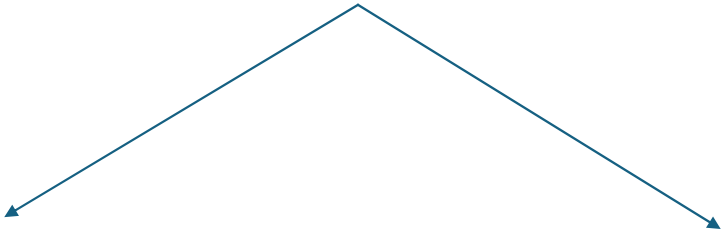
$$v = \frac{\frac{k^f_{cat}}{K_s} * E * s - \frac{k^r_{cat}}{K_p} * E * p}{1 + \frac{s}{K_s} + \frac{p}{K_p}}$$



$$v = \frac{\frac{V_+ ab}{K_{iA}K_{mB}} - \frac{V_- pq}{K_{mP}K_{iQ}}}{1 + \frac{a}{K_{iA}} + \frac{K_{mA}b}{K_{iA}K_{mB}} + \frac{K_{mQ}p}{K_{mP}K_{iQ}} + \frac{q}{K_{iQ}} + \frac{ab}{K_{iA}K_{mB}} + \frac{K_{mQ}ap}{K_{iA}K_{mP}K_{iQ}} + \frac{K_{mA}bq}{K_{iA}K_{mB}K_{iQ}} + \frac{pq}{K_{mP}K_{iQ}} + \frac{abp}{K_{iA}K_{mB}K_{iP}} + \frac{bpq}{K_{iB}K_{mP}K_{iQ}}}$$

$$v = \frac{V_f V_r \left([A][B][C] - \frac{[P][Q]}{K_{eq}} \right)}{V_r K_{ia} K_{ib} K_{m_C} + V_r K_{ib} K_{m_C}[A] + V_r K_{ia} K_{m_B}[C] + V_r K_{m_C}[A][B] + K_{m_B}[A][C] + V_r K_{m_A}[B][C] + V_r[A][B][C] + \frac{V_f K_{m_Q}[P]}{K_{eq}} + \frac{V_f K_{m_P}[Q]}{K_{eq}} + \frac{V_f[P][Q]}{K_{eq}} + \frac{V_f K_{m_Q}[A][P]}{K_{ia}K_{eq}} + \frac{V_f K_{m_Q}[A][B][P]}{K_{ia}K_{ib}K_{eq}} + \frac{V_f K_{m_Q}[A][B][C][P]}{K_{ia}K_{ib}K_{ic}K_{eq}} + \frac{V_r K_{ia} K_{m_B}[C][Q]}{K_{iq}} + \frac{V_r K_{m_A}[B][C][Q]}{K_{iq}} + \frac{V_r K_{ia} K_{m_B}[C][P][Q]}{K_{ip}K_{iq}} + \frac{V_r K_{m_A} K_{ic}[B][P][Q]}{K_{ip}K_{iq}} + \frac{V_r K_{m_A}[B][C][P][Q]}{K_{ip}K_{iq}}}$$

Fortunately, there are methods to deal with this problem



Analysis of metabolic network under metabolic steady-state (Timmy's lecture)

Integrating enzyme kinetics and thermodynamics (second lecture)

- We reviewed some fundamental concepts of enzyme kinetics
- We learnt how some enzyme kinetics equations can help us to represent simple microbial interactions
- We saw that the actual representation of the metabolic processes can be very complex

To be continued....