Growth of *Bacillus subtilis* in Emulsion Droplets

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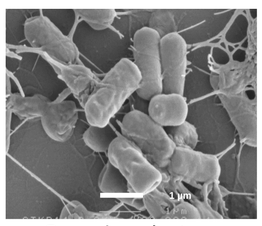
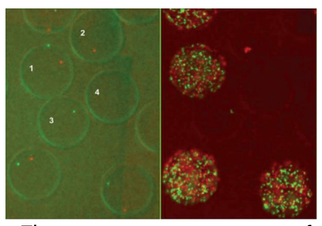
Motivation

B.subtilis produces **enzymes** that are important for biotechnology (food, antibiotics, household detergents).
- ➢ optimize yield of production, **understand metabolism**
- ➢ monitor **parameters** of individual cells



Scanning electron microscopic image of *B. subtilis* [1]

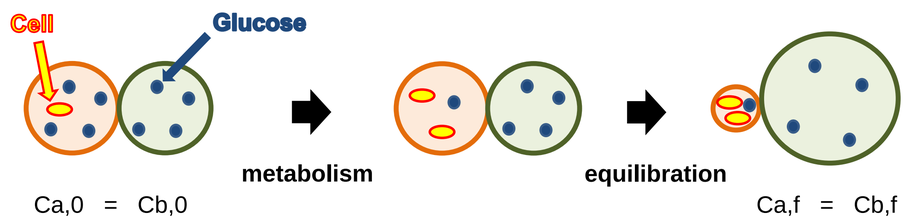

A colony of *B. subtilis* [2]



Fluorescence measurement of two *E.coli* strains (yellow and red), droplets of 1 nL [3]

Osmosis

Osmosis describes the spontaneous water flux to droplets with higher solute concentrations in order to **compensate osmostic pressure differences**.

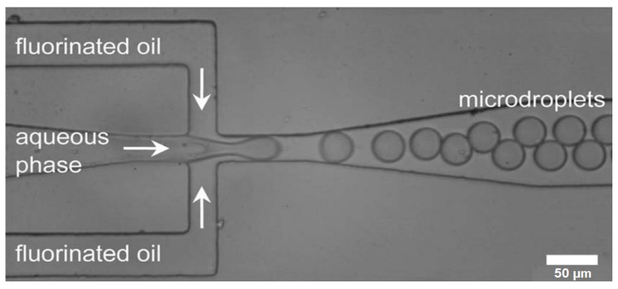


For *Bacillus subtilis*, 2 opposite effects can change osmotic pressure:

1) Glucose depletion
2) Extracellular Matrix (ECM) Production

Microfluidics technology

- ➢ **Encapsulation** of single cells in aqueous droplets dispersed in oil phase -> **inverse emulsions**
- ➢ Identically sized (pL - µL) droplets generated in chip geometries (T-junction [7] or **flow focusing geometry** [4])
- ➢ **Variety of applications**: detection (fluorescence [3], pH [9], volume evolution [5]), antimicrobial susceptibility analysis [10], microbial interactions [3,6])

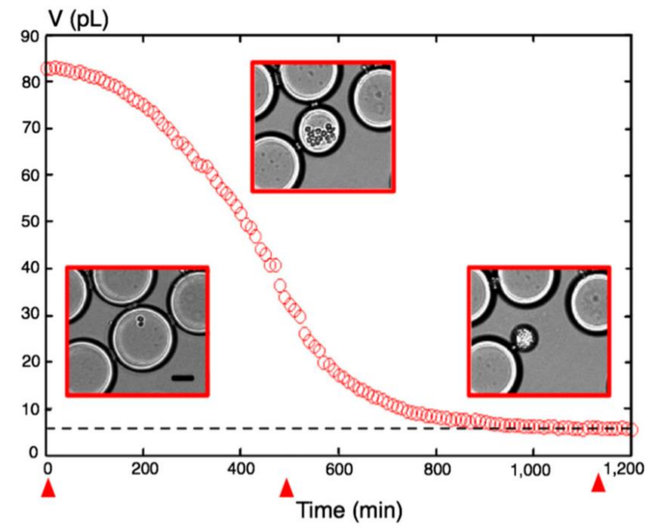


Droplet production using flow focussing geometry. Scale bar is 50 µm. [4]

Previous Work

- ➢ Droplets to monitor cell metabolism: experiments on yeast cells [4,5,8], *B. subtilis* [8], *E.coli* [9]
- ➢ Determination of metabolism through pH change [9], metabolite profiling [8] or volume evolution of droplets [4,5]
- ➢ Investigating effect of culture conditions (oxygen, nutrition) [5,8]

Here: Analysis of osmosis-driven **volume evolution** of droplets over time -> label-free monitoring of cell metabolism



Volume evolution of droplets containing yeast cells [5]

Objectives

➔Understanding coupling between osmotic pressure and ECM production

➔Simulating growth of *B. subtilis* under variation of parameters and addition of ECM production

Simulation Parameters

Equations:

- $dV = -F \times vwat \times \left(\frac{(s0+s20)}{V0} - \frac{s1}{V} - \frac{s2}{V} - \frac{Xpol}{V}\right) \times dt$
- $\mu = \frac{\mu max}{\left(1+\frac{K}{c}\right)}$
- $dXcell = \mu \times Xcell \times dt$
- $ds = -\frac{dXcell}{Y}$
- $dXpol = Xcell \times f \times dt$

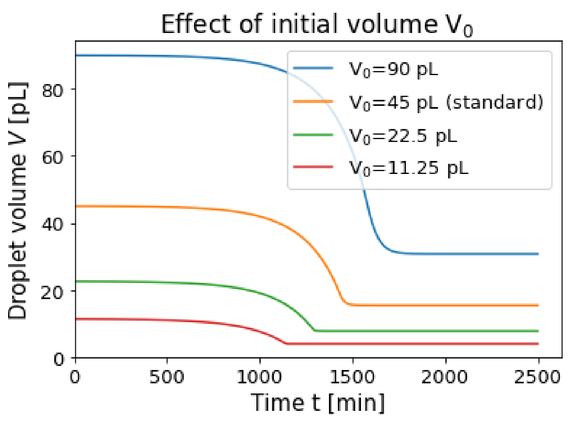
Parameters:

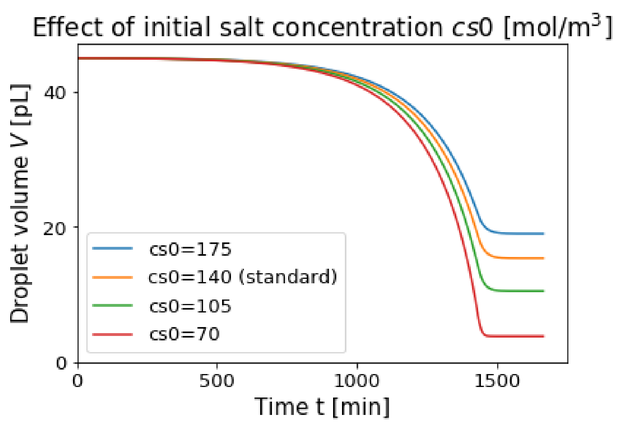
Literature:
µ: growth rate
Y: growth yield
K: substrate saturation
v_{wat}: molecular volume of water

System:
s0,s(t): initial,current substrate quantity
V0,V(t): initial,current droplet volume
c: glucose concentration (*s1/V*)
F: transport factor
Xcell: number of cells per droplet

ECM production:
Xpol: number of polymers
f: polymer production rate

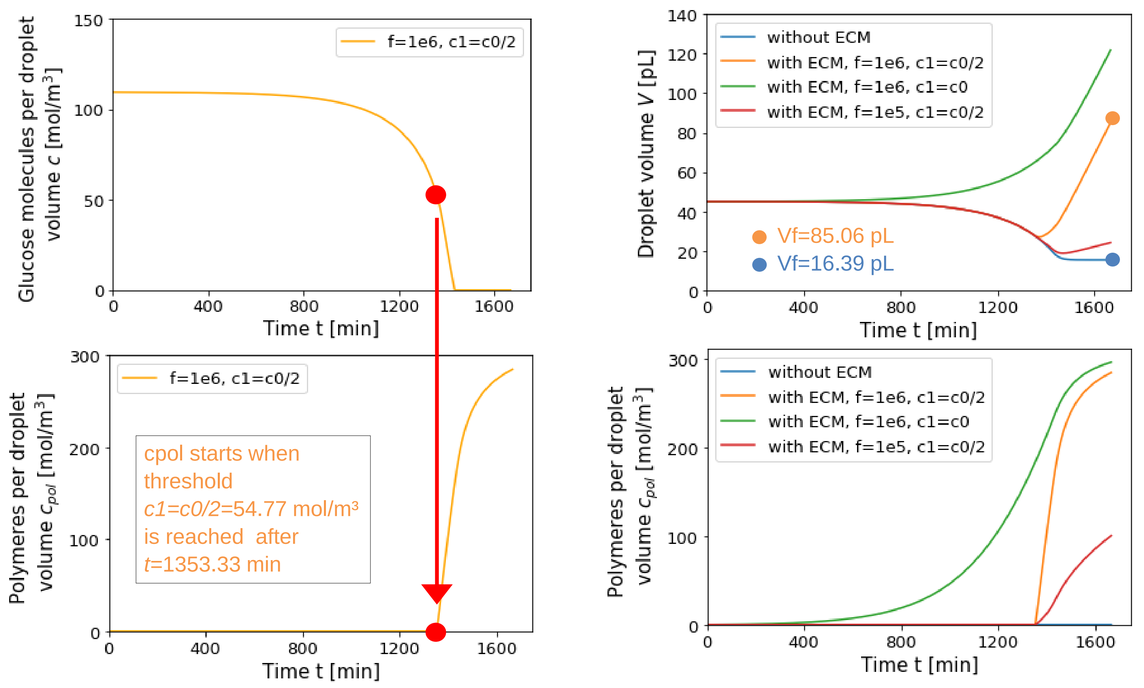
Effect of initial volume and salt concentration





- ➢ **„Salt"** defines **other, not limited solutes** in the growth medium like amino acids and ions that contribute to osmotic pressure and production of biomass [5].
- ➢ **High initial volume** (90 pL) and **low initial salt concentration** (70 mol/m³) show significant volume variation and are thus favorable for experiments.

Effect of ECM Production



Orange curve: **ECM production** (rate *f*=1e6 polymers per second per cell) triggered when **glucose conc. decreases** to *c1=c0/2* (*t*=1353 min). **Steep volume increase** after *t*=1372 min. When *c*=0 (*t*=1460 min) **bacteria stops proliferating**, but volume increases further to 85.06 pL (**without ECM** volume stabilizes at 16.39 pL).

Conclusions

- ➢ Simulations show which conditions are most favorable for experiments.
- ➢ Depending on polymer production rate *f* and threshold *c1* osmotic pressure increase caused by ECM production competes with osmotic pressure decrease from glucose depletion to different extent (for *f* =1e6 polymers per second per cell and for *c1=c0/2* both effects are measurable).
- ➢ Next steps: experiments, investigate other parameters like oxygen concentration in droplets

References

[1] Zweers et al., Microbial Cell Factories, 2008, 7(10), 1-20.
[2]https://www.faz.net/aktuell/wissen/natur/bakterien-biofilme-hort-gefaehrlicher-keime-1540036.html
[3] Park et al., PloS ONE, 2011, 6(2), e17019.
[4] Hofmann et al., Lab Chip, 2012, 12, 916–922.
[5] Boitard et al., PNAS, 2012,109(19), 7181–7186.
[6] Kaminski et al., Lab Chip, 2016, 16, 2168-2187.
[7] Martin et al., Lab Chip, 2003, 3, 202–207.
[8] Bjork et al., Biomicrofluidics, 2015, 9, 044128,1-10.
[9] Funfak et al., Microchim Acta, 2009, 164, 279–286.
[10] Kaushik et al., Biosens Bioelectron, 2017, 97, 260–266.