

## Growth of JCVI-syn3B in SP4 medium with different glucose concentrations

### SP4 medium

SP4 growth medium, pH 7.0		
Component	Amount	Company/Details
Basal SP4, autoclaved	300 ml	-
20% yeastolate	10 ml	Gibco/Bacto TC yeastolate
15% yeast extract	35 ml	Carl Roth
7.5% NaHCO <sub>3</sub>	14.6 ml	Honeywell
20% glucose	25 ml	Carl Roth
400000 U/ml penicillin G, Na-salt	2.5 ml	Carl Roth/Cellpure > 1550 U/mg, Penicillin G Na-salt
25 mg/ml L-glutamine	5 ml	Carl Roth/> 99% purity, Cellpure
Fetal Bovine Serum	170 ml (17%)	Biowest/Heat inactivated, sterile filtered
CMRL (if liquid)	400 ml	PAN Biotech/ Liquid CMRL-1066, no glutamine, with 2.2 g/L NaHCO <sub>3</sub>
CMRL (if powder, no NaHCO <sub>3</sub> )	3.92 g + 0.88 g powder NaHCO <sub>3</sub>	Himedia AT226A-20L

To prepare basal SP4 (per 1L total SP4):

Difco PPLO Broth	3.5 g	Difco/Per 1L: Beef heart infusion from 50 g – 6 g Peptone – 10 g NaCl – 5 g
Tryptone (peptone from casein)	10 g	Sigma Aldrich
Peptone (peptone from gelatin)	5.3 g	Sigma Aldrich

→ typically, **5 g/L glucose** are added to the medium

→ Additionally, liquid CMRL 1066 contains 1 g/L glucose (170 mL → extra 0.17 g/L glucose)

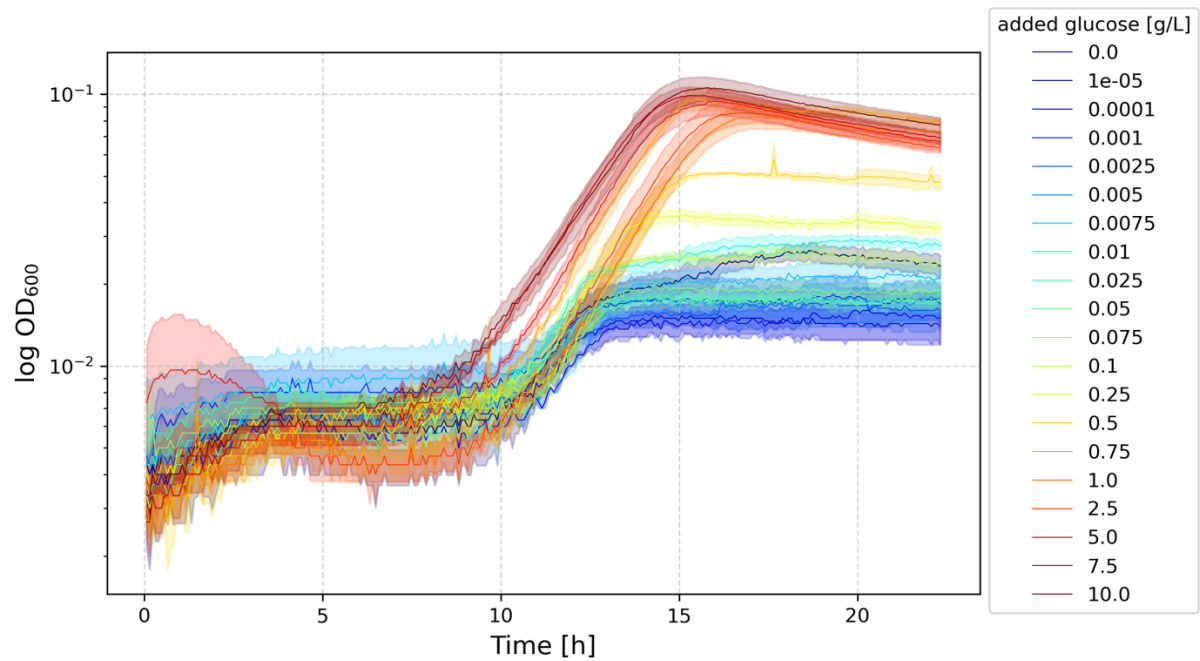
→ However, we use more and use powder: usually 9.8 g CMRL powder for 1 L CMRL (\*0.17 = 1,67g)

We add however 3.92 g powder in 1 L (this equals 9.8g/L CMRL \* 0.4 L = 3,92 g CMRL)

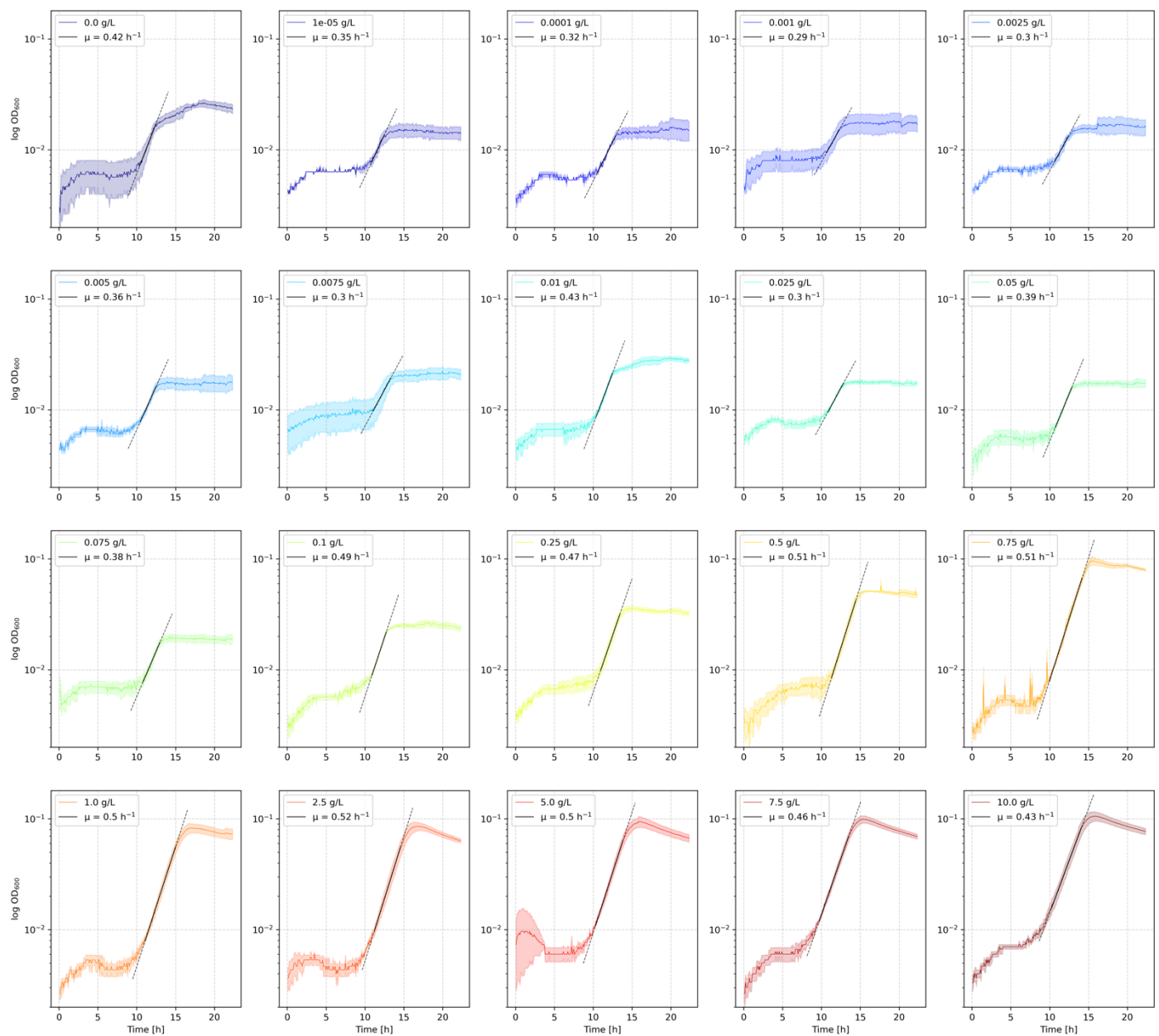
Thus we have an **additional of 0.4 g/L glucose** extra!

→ The other components should not have additional larger amounts of glucose

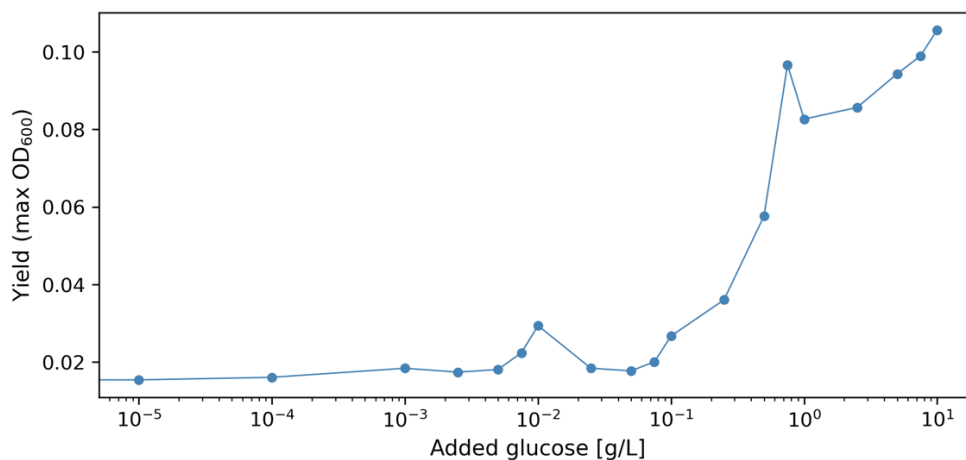
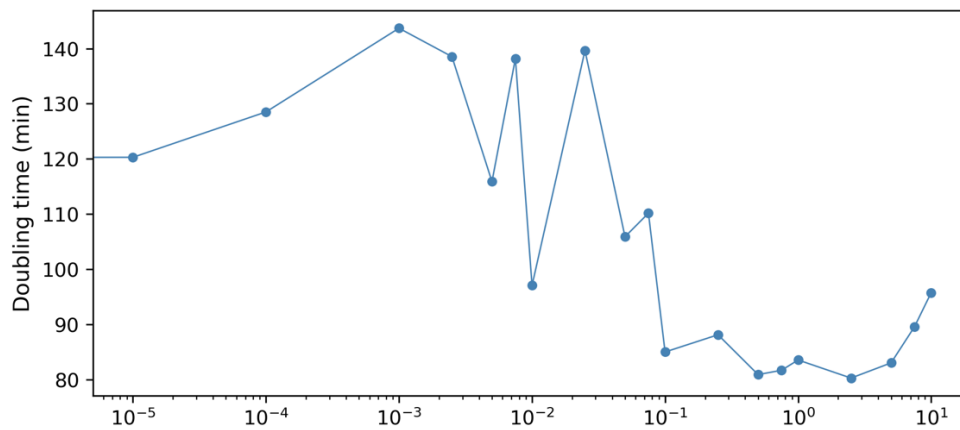
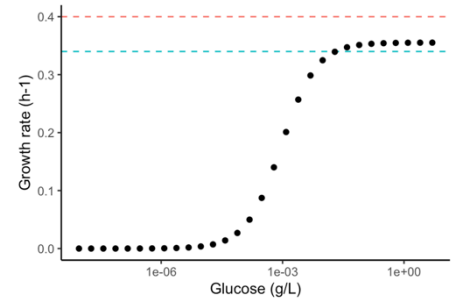
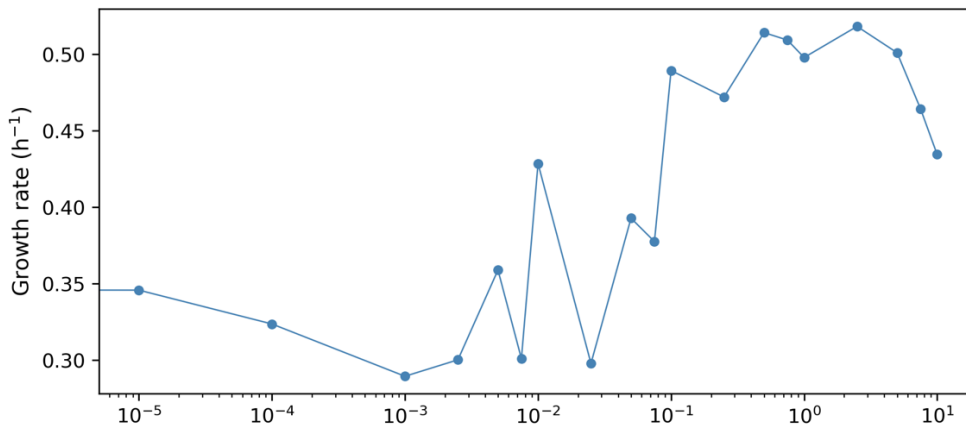
## Overview



## Determination of growth rate



## Growth rate and yield in dependence of glucose concentration



**Note: additional 0.4 g/L not included in data!**

→ Additional 0.4 g/L explains growth at “zero” added glucose

→ Growth rate in dependence of added glucose unfortunately looks quite wobbly, some trend of increase visible

→ Wobbly curve may be explained by

- difficulties of adding small amounts of glucose
- statistical issues
- at high glucose concentrations the amount of metabolized glucose should be the same – we know that in normal SP4, not all of the glucose is consumed (measured by HPLC in Jülich). This is because glucose is metabolized to lactate which acidifies the medium more and more until the cells die. There is probably also saturation of glucose transporter
- high amounts of glucose increase medium osmolarity, which can cause stress, may explain the slight decrease of growth rate

→ Big increase in yield for higher glucose, whereas the growth rate varies only within 0.3 to 0.5  $\text{h}^{-1}$

→ This variation is less than compared to the model, where it was as low as e.g. 0.1  $\text{h}^{-1}$

**Future experiments:**

- comparing experimental and predicted values in a single plot with corrected glucose values (maybe Charles, you can do it?)
- Make buffer system better, so glucose uptake is not limited by medium pH, then repeat experiment
- Will talk with the people in cologne that did glucose/ chemostat/ growth law experiments to ask what else we may do or how to better the experiment