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SYSTEMS BIOLOGY
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Mathematical Modelling of the pH-induced Metabolic Shift in *C. acetobutylicum* Unravels a Heterogeneous Phase Transition

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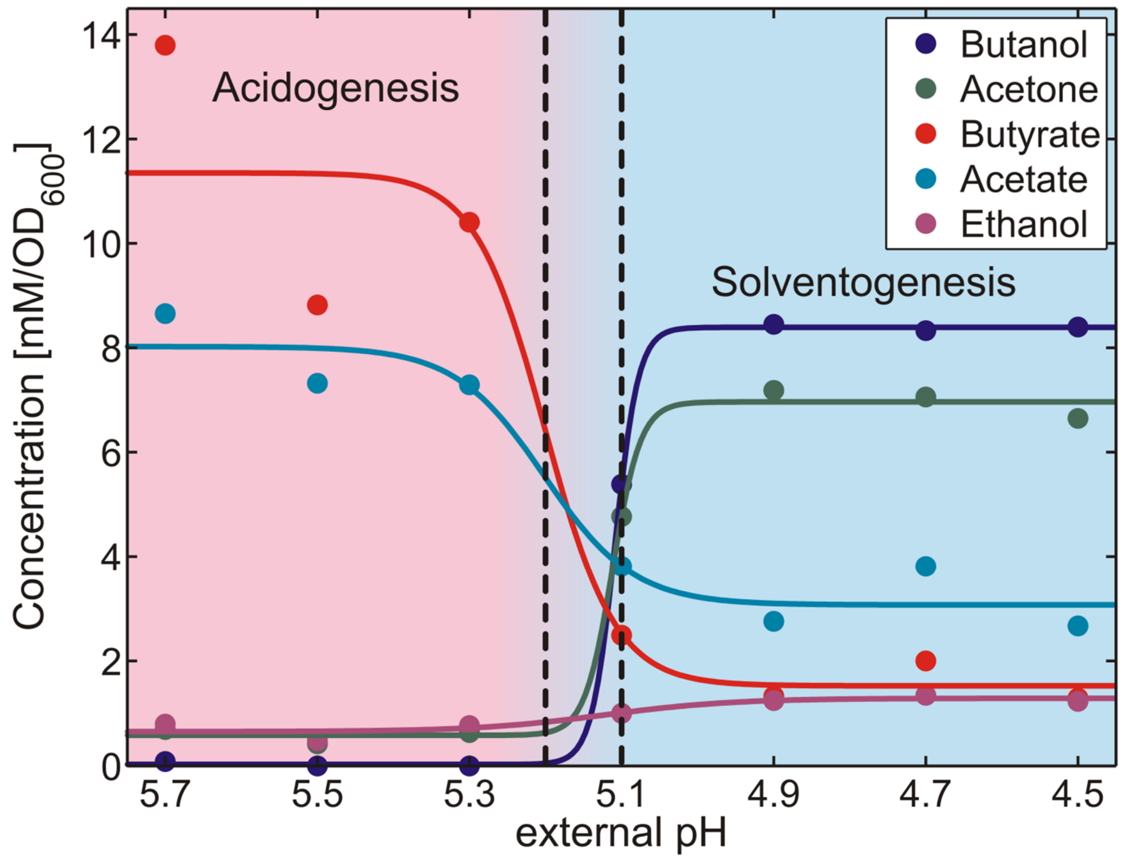
pH-induced metabolic shift in *C. acetobutylicum*



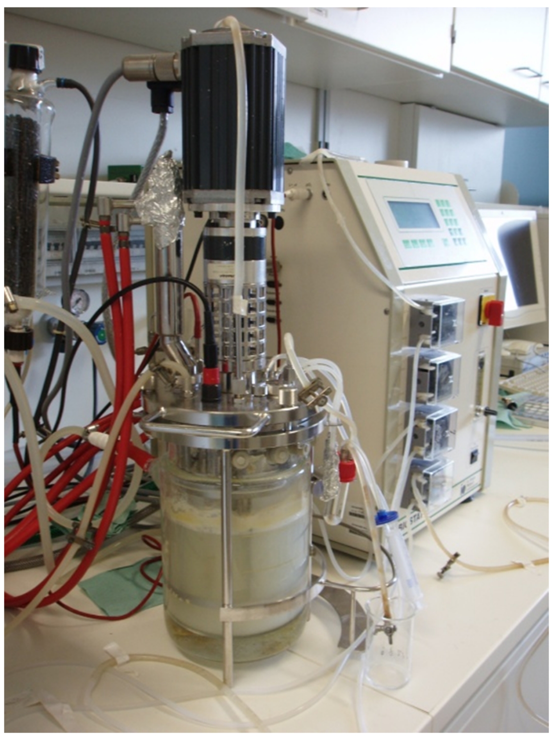
pH>5.2 acid formation

pH<5.1 solvent formation

Millat et al., Microbial Biotechnol. submitted

Standardized experimental setup

- *C. acetobutylicum* strain ATCC 824
- pH-controlled continuous culture using phosphate limitation
- 4% glucose in medium
- Constant diluation rate
- Temperature 37 °C

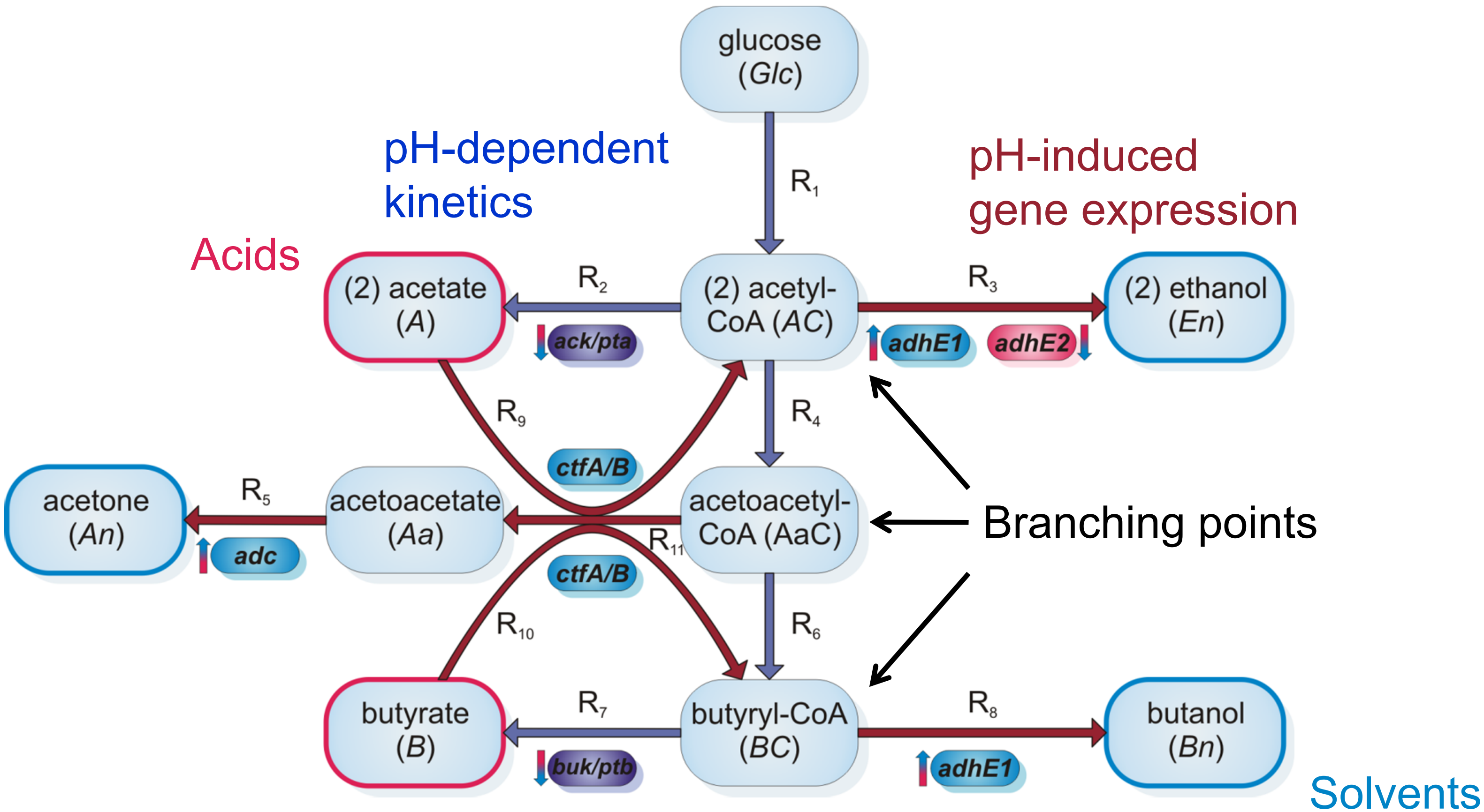


Dynamical change of the external pH
- Acidogenesis: pH 5.7
- Solventogenesis: pH 4.5

Experimental data:
- Environome
- Product spectrum
- Metabolome
- Proteome
- Transcriptome

Fischer et al., J. Bacteriol. 188, 5469 (2006)

AB fermentation pathway (reduced)

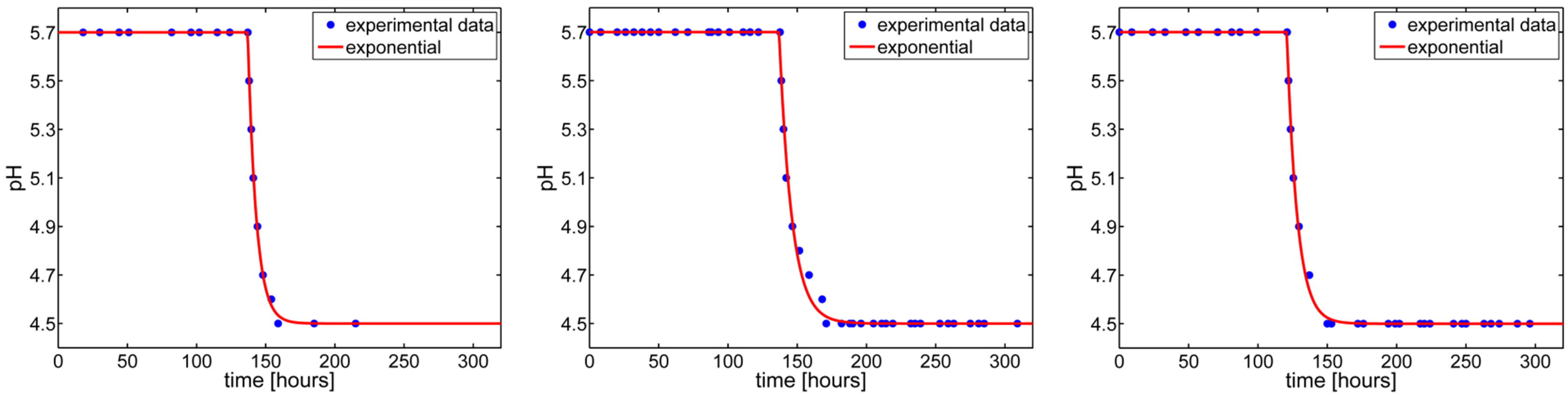


Janssen et al., Appl. Microbiol. Biotechnol. 87, 2209 (2010)
Grimmler et al., J. Mol. Microbiol. Biotechnol. 20, 1 (2011)

Jones & Woods, Microbiol. Rev. 50, 484 (1986)
Andersch et al., Appl. Microbiol. Biotechnol. 18,327 (1983)

Scaling of data

- Three independent ‘forward’-shift experiments



- Fit to measured pH data using an exponential function

$$pH(t) = 5.7 \cdot \Theta(\beta - t) + \left(4.5 + 1.2 \exp\{-\alpha (t - \beta)\}\right) \Theta(t - \beta)$$

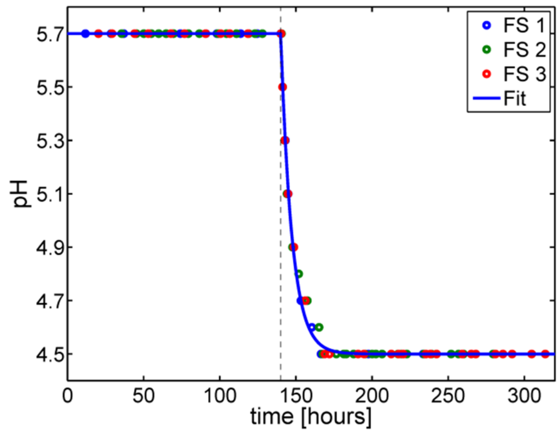
Parameters

α
- Time scale of the pH shift
- Scaling of time

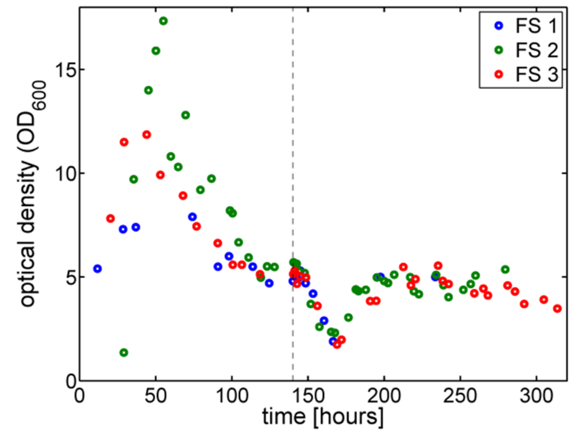
β
- Starting time of the pH shift
- Shifting of experiments

Scaling of data

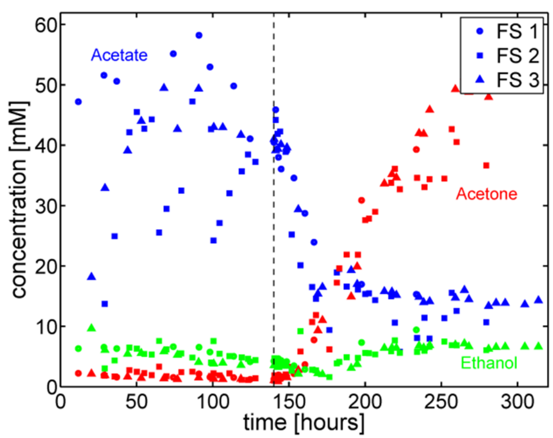
- pH



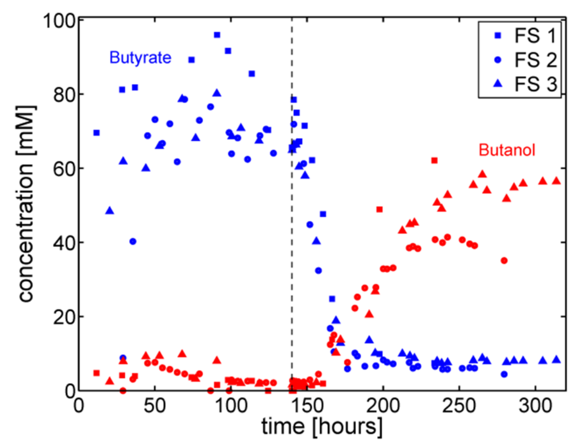
- Optical density (OD_{600})



- Acetate, acetone, and ethanol

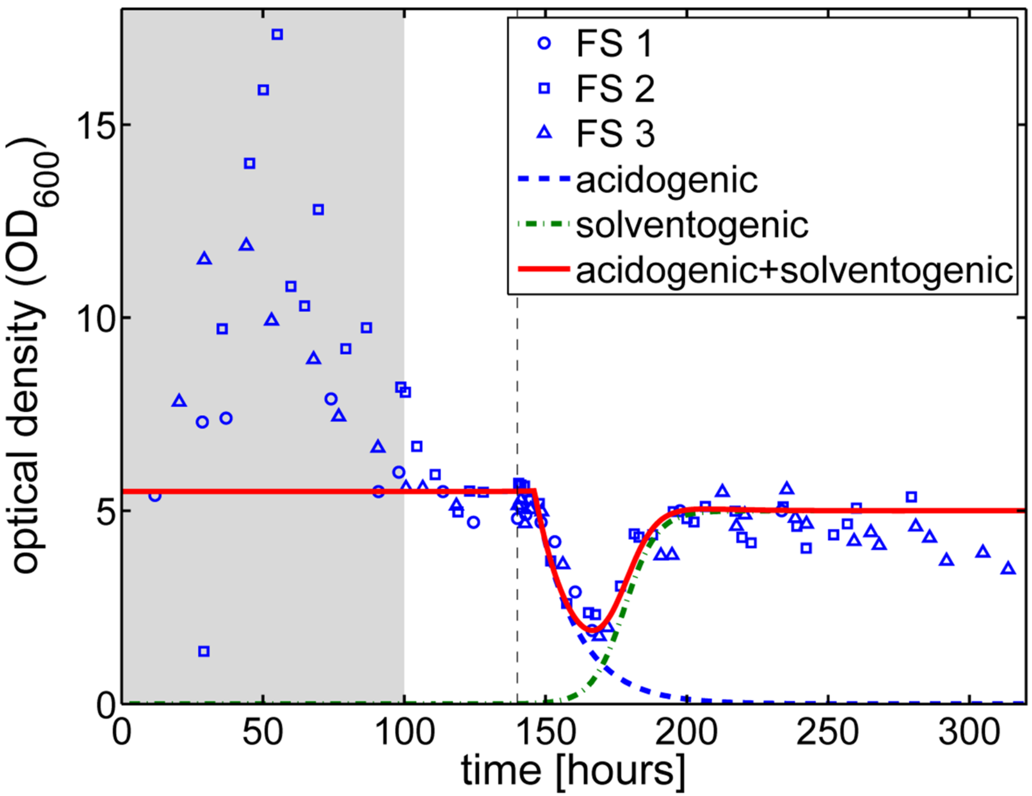


- Butyrate and butanol



Population size

- Population size changes during the transition and between both phases
- Assumption of an acid-forming and solvent-forming phenotype



Findings from the fit

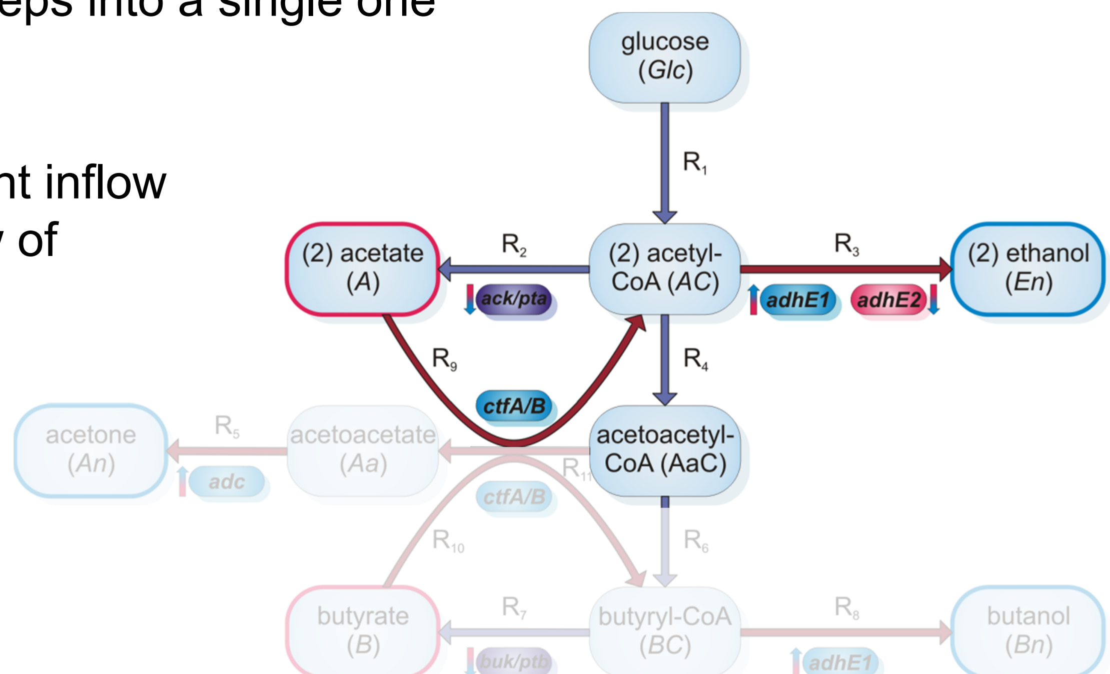
1. Delay between the initiation of the pH shift and the cellular response of around 5-6 hours
2. Acidogenic cells decline nearly with the speed of the dilution rate
3. Solventogenic population is established about 50 hours after the pH-shift

Metabolic level – The conversion of acetyl-CoA

$$\frac{dAC}{dt} = R_1 - R_2 - R_3 - 2R_4 + R_9 - D \cdot AC$$

- R_i biochemical conversions
- Combination of several steps into a single one
- Transport term
- Open system with constant inflow (e.g. glucose) and outflow of biochemicals and cells
- Constant dilution rate D
- **Sum over phenotypes**

$$R_3 = R_3^{A} + R_3^{S}$$



Proteomic level – The pH-induced cellular adaptation

1. Michaelis-Menten like expression

$$R_4 = \frac{V_4 \cdot AC}{2(K_4 + AC)} \cdot OD^{(\mathrm{A,S})}$$

Conversion of 2 acetyl-CoA to acetoacetyl-CoA

constant enzyme concentration

2. Product of metabolite and enzyme concentration

$$R_3^{\mathrm{A}} = \alpha_{32} \cdot AC \cdot AdhE2 \cdot OD^{\mathrm{A}}$$

$$R_3^{\mathrm{S}} = \alpha_{31} \cdot AC \cdot AdhE1 \cdot OD^{\mathrm{S}}$$

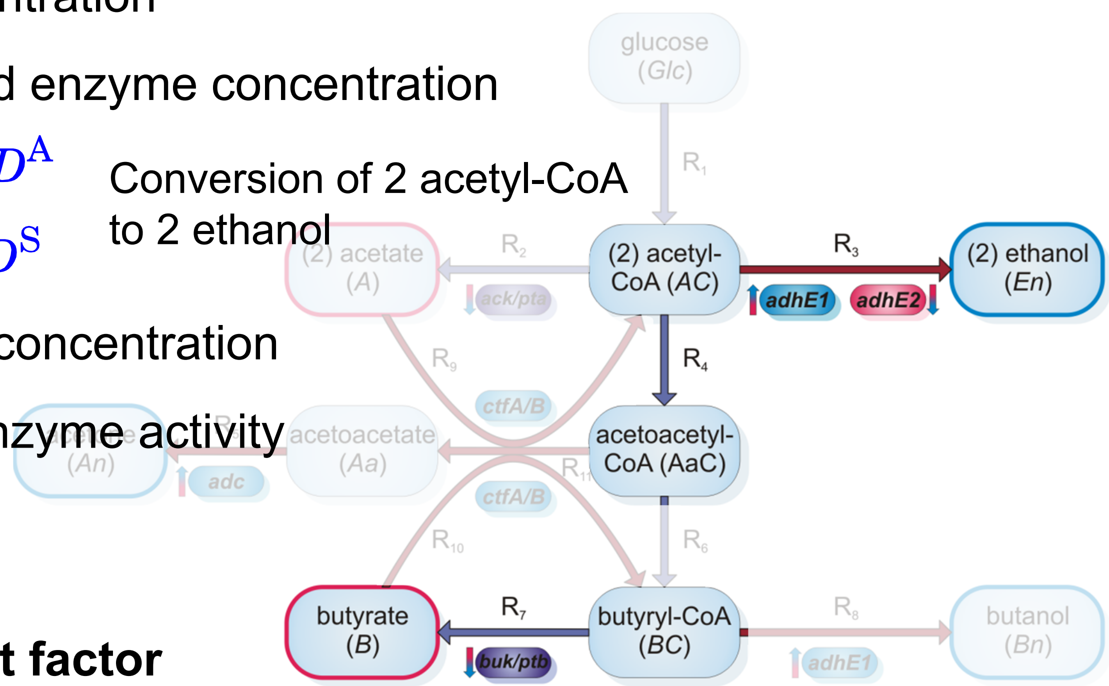
Conversion of 2 acetyl-CoA to 2 ethanol

pH-dependent enzyme concentration

3. pH-dependent specific enzyme activity

$$R_7 = \frac{V_7 \cdot BC}{f_7 (K_7 + BC)}$$

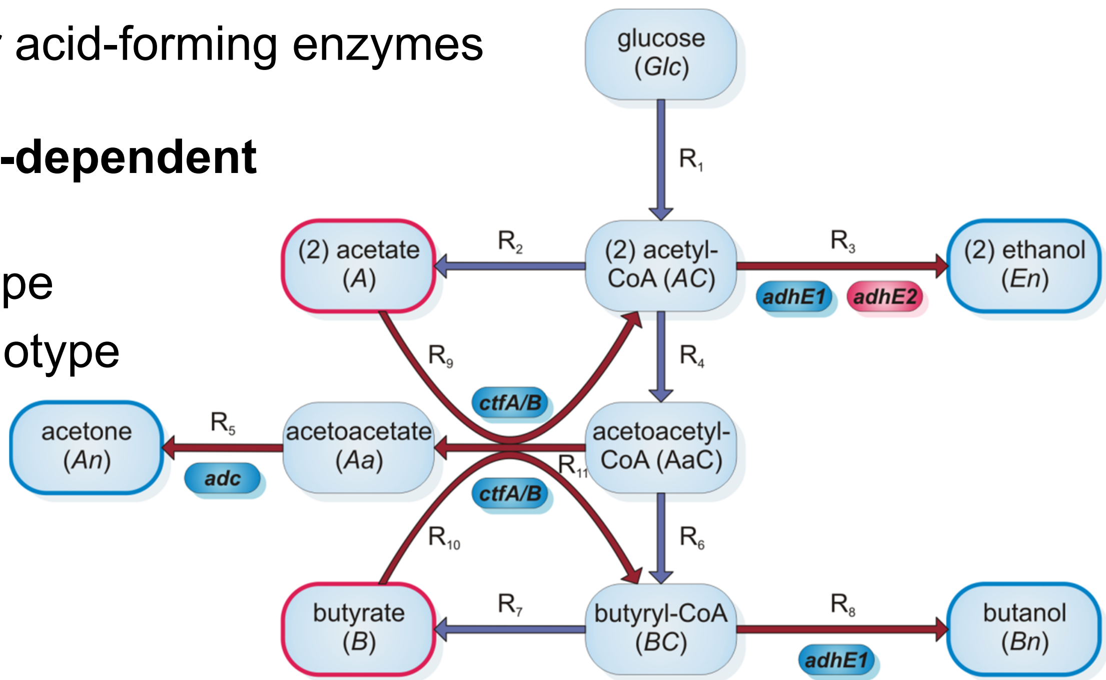
additional **pH-dependent factor**



Clostridial phenotypes in ABE fermentation

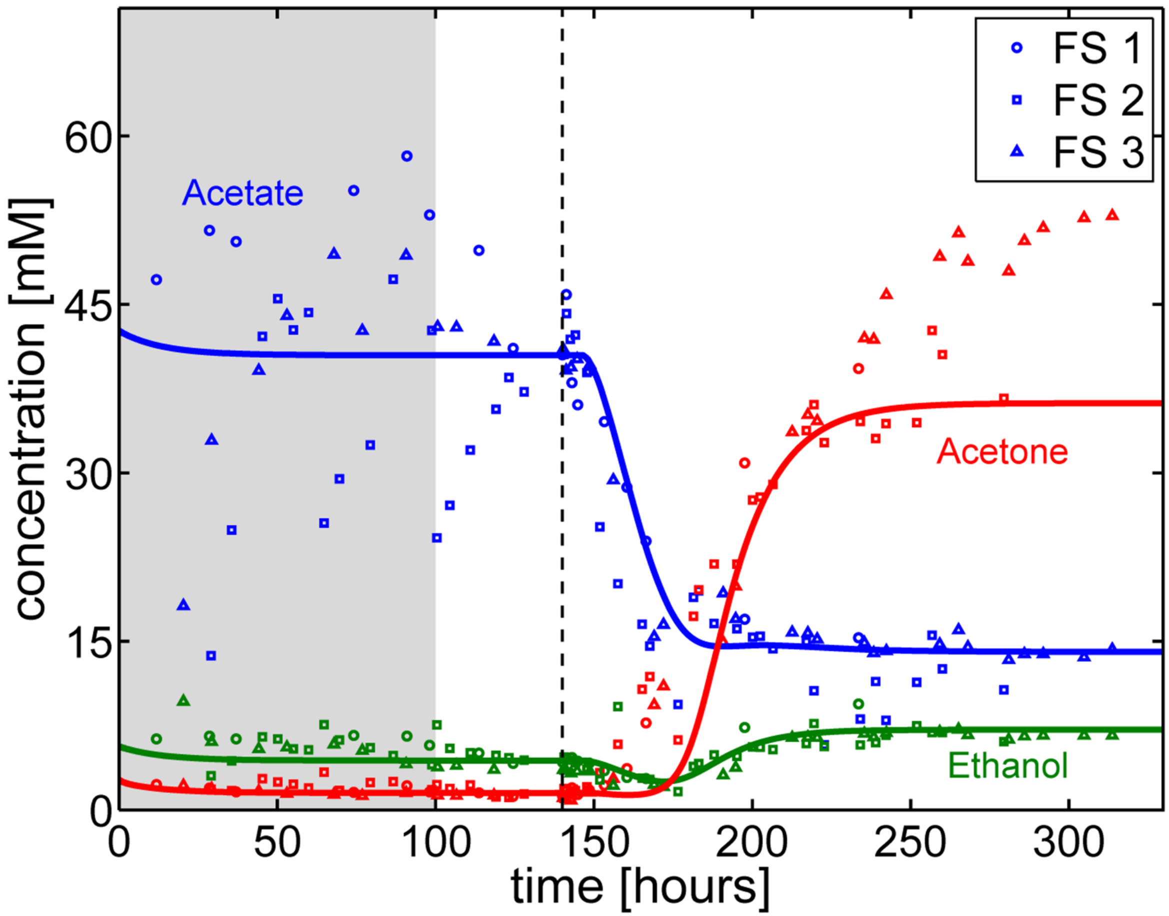
- Acid- and solvent-forming cells differ in their composition at steady state
- Induction of solvent-forming enzymes during solventogenesis
  - Antagonistic expression of AdhE1/2
  - Induction of Adc and CtfA/B
- No significant changes for acid-forming enzymes

- **constant but phenotype-dependent** proteome composition
  - Acid-forming phenotype
  - Solvent-forming phenotype

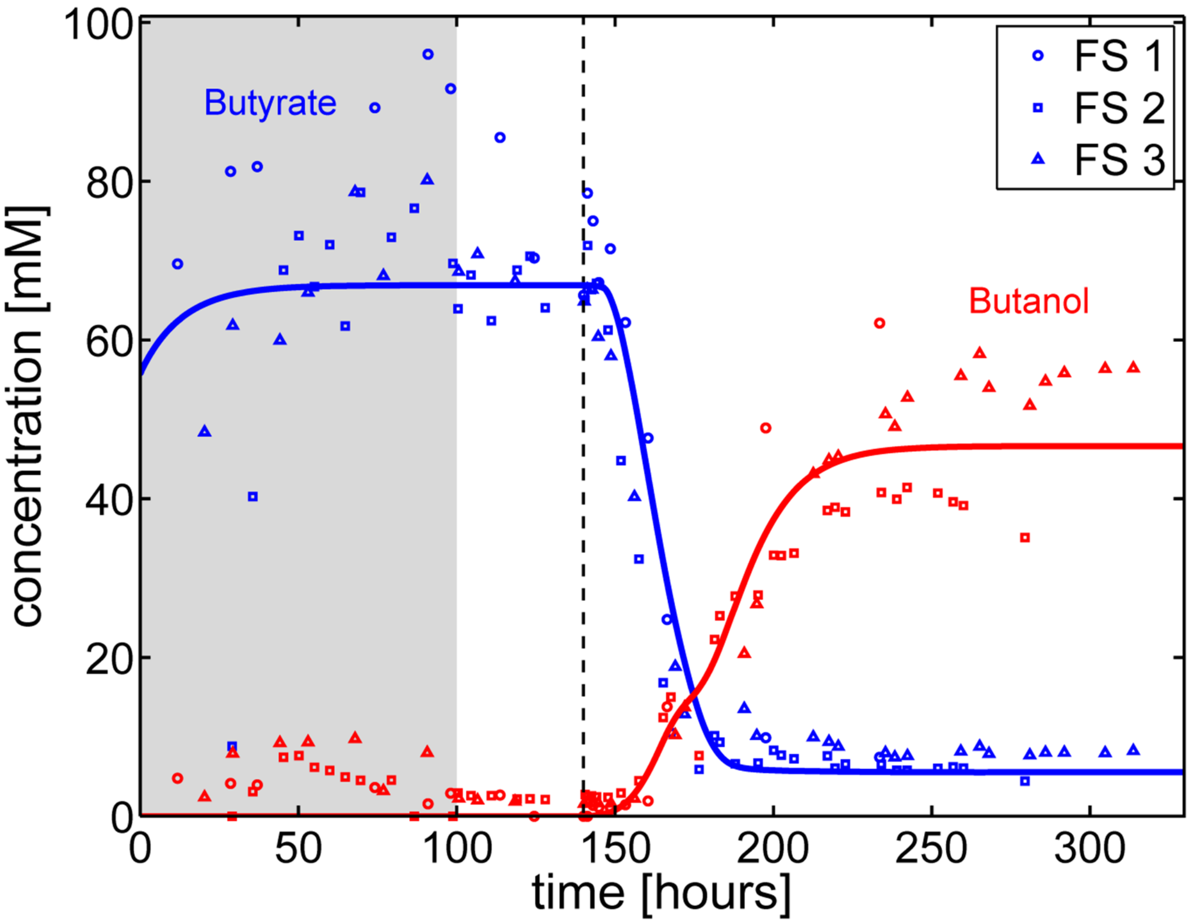


Janssen et al., Appl. Microbiol. Biotechnol. 87, 2209 (2010); Grimmler et al., J. Mol. Microbiol. Biotechnol. 20, 1 (2011)

Simulation and experimental data



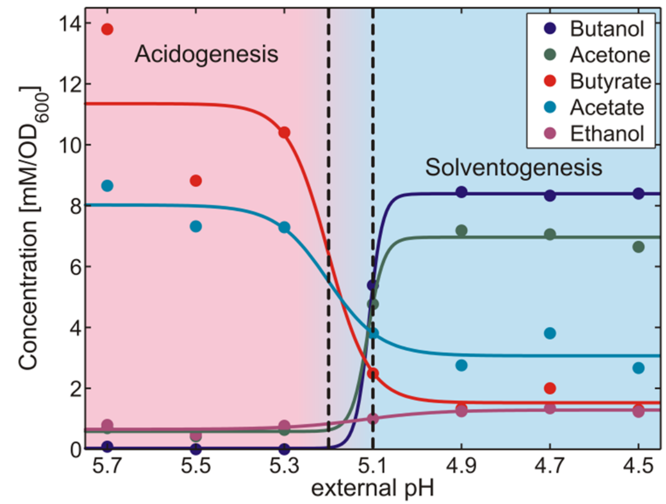
Simulation and experimental data



Conclusions

- The pH-induced metabolic switch involves changes on transcriptomic, proteomic, and metabolomic as well as on kinetic levels.
- The hypothesis of a heterogeneous mixture of acid- and solvent-forming subpopulations is supported by the model and simulation results for continuous cultures.
- Following the initiation of the pH-shift, the cells are adapting to the changing but also acidogenic conditions.
- After the pH level falls below a pH=5.2, acidogenic metabolism fails to ensure stable growth conditions, resulting in a decline of acid-forming cells.
- During the decline, a solvent-forming population rises from a few cells.
- The metabolic state in continuous cultures is determined by the pH level:
  - pH > 5.2 acidogenesis
  - pH < 5.1 solventogenesis

Outlook / Open Questions



- What is the origin of the subpopulations?
    1. Are acid- and solvent-forming cells always present in the culture?
    2. Do only few cells, selected by an unidentified criteria, start to produce solvents?
- The antagonistic expression patterns of the alcohol/aldehyde dehydrogenases AdhE1 and AdhE2 seem to be crucial for the pH-induced shift.
    - Does AdhE2 loose its functionality at low pH levels?
- Which kind of clostridial behaviour is established for the intermediate pH range 5.2 > pH > 5.1 in continuous cultures?
    - Our analysis suggests that cells are neither involved in acidogenesis or solventogenesis.

Team

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