1. Dokumentinformation

Angaben zur Modellierung von basierend auf der Diplomarbeit von Lars Blank (Quelle: Frank Eiden).

2. Angaben zur Modellierung

2.1 Organismus

C(lostridia). acetobutylicum (EG925) Wildtyp = ATCC 824

anaerob

2.2 Komponenten

„Solvent“ = butanol (max titer 255mM at pH5)

Enzym „alcohol/aldehyde dehydrogenase“ (aad)

Substrate: Glukose, Stärke, Cellulose, Pektion

Produkte: Aceton, Acetat, Acetoin, Butanol, butyrate, Ethanol, CO2, Lactat

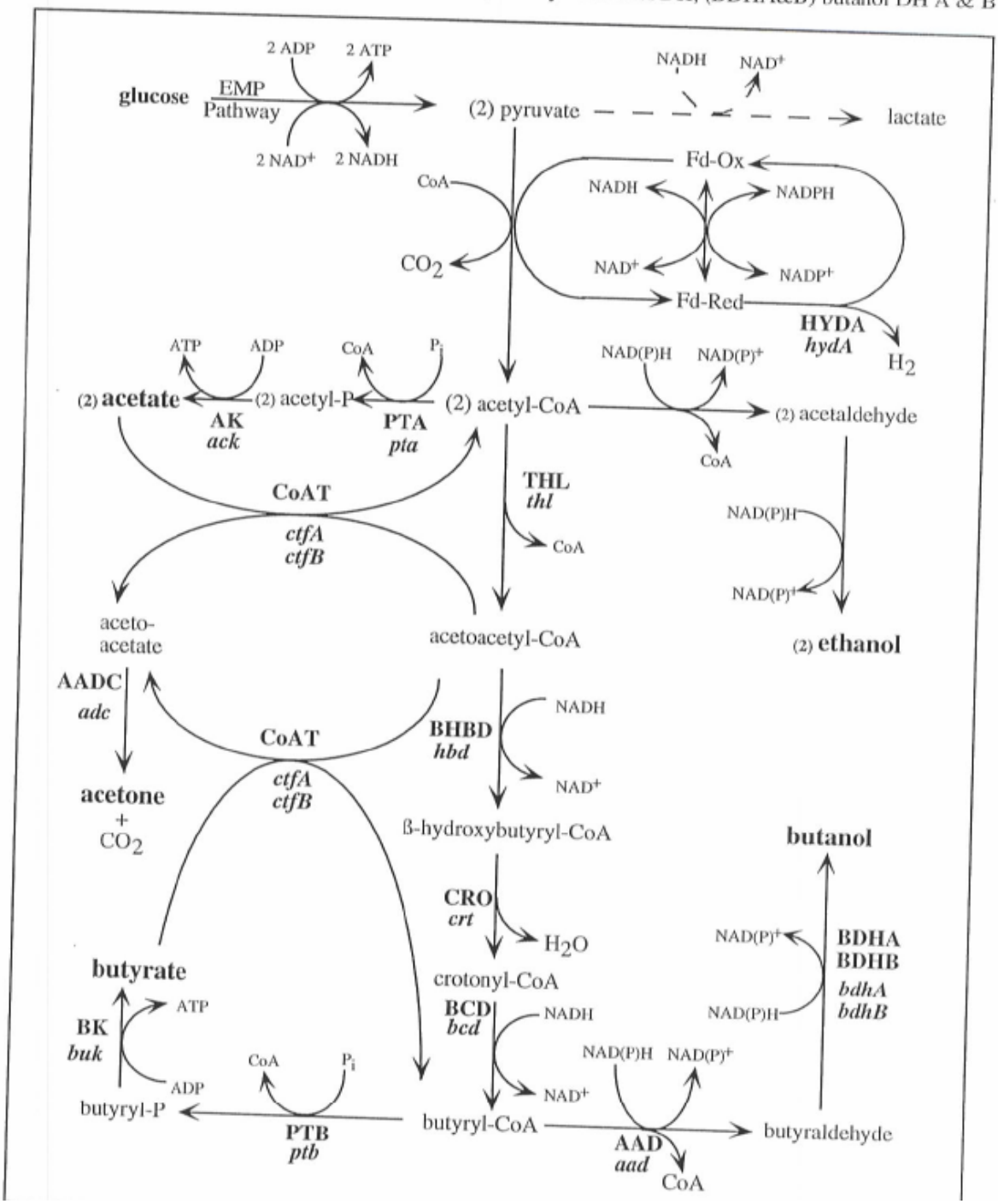
2.3 Reaktionen

2.3.1 Allgemein

at high AT (CoA transferase?) composition the shift from acidogenic to solventogenic phase

Figure 1.1

Product formation by *C. acetobutylicum* from glucose. Enzymes for cloned genes have been labelled in bold; corresponding genes are in italics: (HYDA) hydrogenase, (PTA) phosphotransacetylase, (AK) acetate kinase, (THL) thiolase, (CoAT) acetoacetyl-CoA:acetate-butyrate:CoA transferase, (AADC) acetoacetate decarboxylase, (BHBD) ß-hydroxybutyryl-CoA DH, (CRO) crotonase, (BCD) butyryl-CoA DH, (PTB) phosphotransbutyrylase, (BK) butyrate kinase, (AAD) aldehyde/alcohol DH, (BDHA&B) butanol DH A & B.



2.3.2 Acidogenic phase

cells are growing exponentially

Produkte: Wasserstoff, CO2, **Acetat**, **Butyrate**, und unter best. Bedingungen auch Lactat.

pH fällt in dieser Phase bis auf 3.8 (accumulation of carboxylic acids)

Wärmebildung

2.3.3 Solventogenic phase

stationäre Phase

Produkte: **Aceton, Butanol, Ethanol**, Acetoin

Substrate : Kohlenhydrate und carboxylic acids daher steigender pH

dauert solange, bis Substrat aufgebraucht oder toxische Butonal Konzentration von ca. 13 g/Ltr erreicht. Zellen bilden Sporen und Fermentation stoppt.

2.3.4 Offenen Fragen

- TODO Wodurch entsteht der Übergang von der „Acidogenic“ in die „Solventogenic“ Phase?

2.4 Kinetik

2.4.1 pH

Butanol Titer bei pH 5 besser als bei 4.7 und 5.5 über pH 5.8 keine Solvent Produktion

2.4.2 Sonstige

growing under low redox potential

3. Betriebsbedingungen

3.1 Temperatur

37°C

3.2 pO2

anaerob

3.3 Substrate

Glukose 80g /Ltr

3.4 Sonstige

Ethanol 10mM

3.5 Belüftung

N2 100%, 125ml/Min anfangs, später 25 ml/min bei höheren Biomassekonzentrationen

3.6 Rührer

200 rpm

3.7 Zufütterung

3.8M Glukose in der stationären Phase

4. Anlagen

2 (1.5) Liter und 5 (4) Liter

5. Ergebnisse

nach 32h Glukose aufgebraucht, dann 83mM Acetate und 86mM Butyrate, pH = 6.7
110 mM Aceton, 210 mM Butanol, 39mM Ethanol produziert
Verdopplungszeit = 1.4h

Table 3.1 Effect of pH on solvent production

C. acetobutylicum strain	pH	reactor	antibiotic	acetone (mM)	acetate (mM)	acetoin (mM)	ethanol (mM)	butanol (mM)	butyrate (mM)	lactate (mM)	acetone / butanol	additional glucose	glucose measured	glucose calculated	A_{600}	doubling time (hr)
EG925(pTAAD)	4.7	2.0 L	no	146	---	5	40	213	9	1.5	0.68	yes	440	378	10.5	1.82
EG925(pTAAD)	4.7	2.0 L	Clt	110	52	6	39	210	2	1	0.52	yes	480	369	7.5	1.9
EG925(pTAAD)	5.0	5.0 L	no	160	88	6	47	255	8	1	0.63	yes	590	468	10.5	1.44
EG925(pTAAD)	5.0	5.0 L	no	120	82	5.5	43	225	9	1	0.53	yes	450	406.5	10.5	1.7
EG925(pTAAD)	5.5	5.0 L	Clt	100	---	5	20	175	36	8	0.57	yes	401	323	13.0	1.64
EG925(pTAAD)	5.5	5.0 L	no	88	---	5	22	175	42	6	0.5	yes	360	317.5	10.0	1.83

--- Data not available
EG925 = ATCC 824 *solR*::pO1X

Table 3.5 Control fermentations with strains ATCC 824 and EG925

C. acetobutylicum strain	pH	reactor	antibiotic	acetone (mM)	acetate (mM)	acetoin (mM)	ethanol (mM)	butanol (mM)	butyrate (mM)	lactate (mM)	acetone / butanol	additional glucose	glucose measured	glucose calculated	A_{600}	doubling time (hr)
EG925	5.0	5.0 L	no	105	---	7	27	171	7	3	0.61	yes	460		7.1	1.63
EG925	5.0	2.0 L	Clt	85	---	5	28	146	10	5	0.58	no	430		8.0	1.8
ATCC 824	5.0	5.0 L	no	78	32	9	15	138	14	1	0.57	yes	375		7.5	1.25
ATCC 824	5.0	2.0 L	no	98	45	9	20	176	30	1	0.56	no	365		6.6	1.25

--- Data not available

EG925 = ATCC 824 *solR*::pO1X

Table 3.6 Specific formation and uptake rates for EG925 and ATCC 824 fermentations

C. acetobutylicum strain	start of solvent production after inoculation (hr)	specific glucose consumption rate (mM/(hr A_{600} unit))	specific acetone accumulation rate (mM/(hr A_{600} unit))	specific butanol accumulation rate (mM/(hr A_{600} unit))
EG925 #9	ca 14	3.62	1.09	1.83
ATCC 824 #7	ca 14	2.07	0.67	1.31

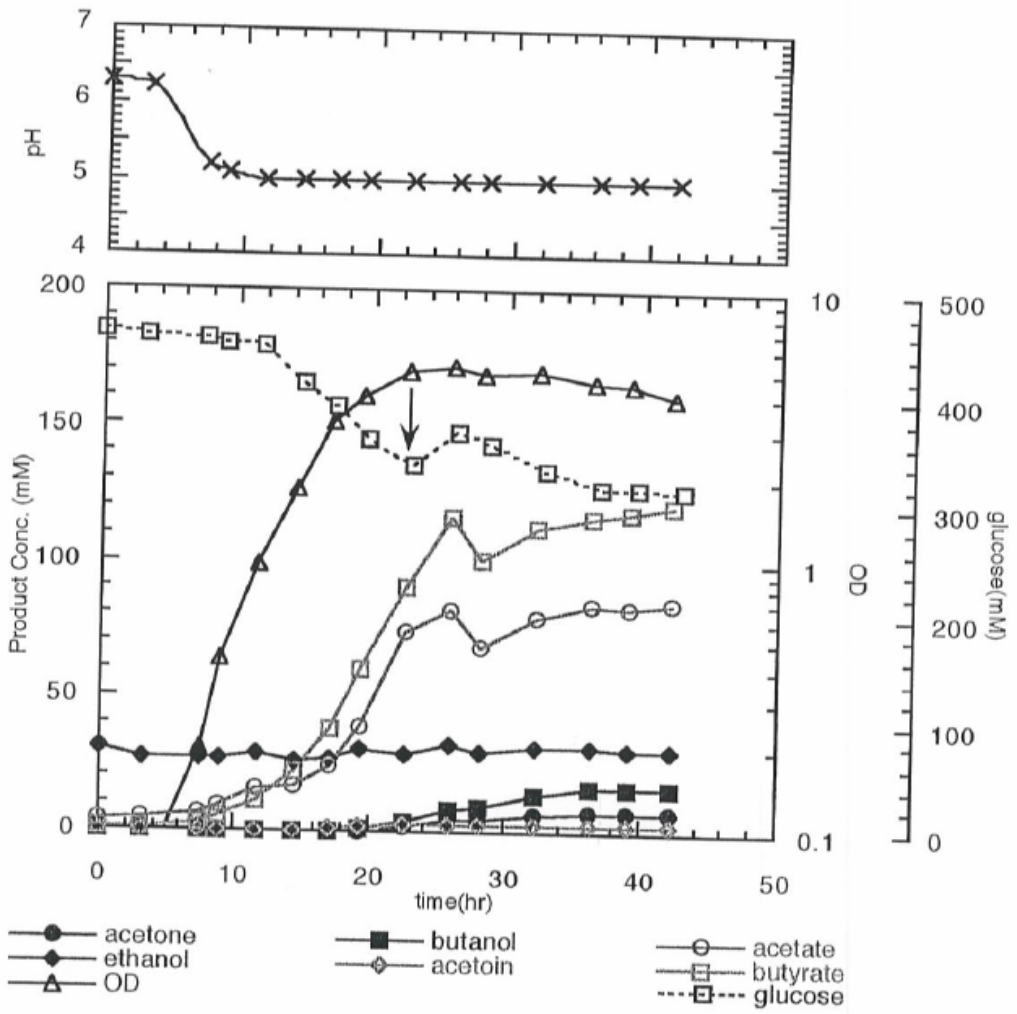


Figure 3.3 Fed-batch fermentation of EG925(pTAAD) controlled at pH 5.0. Tetracycline at a concentration of 10 µg/ml was used for the slection of the plasmid. The arrow indicates time point when glucose was added.

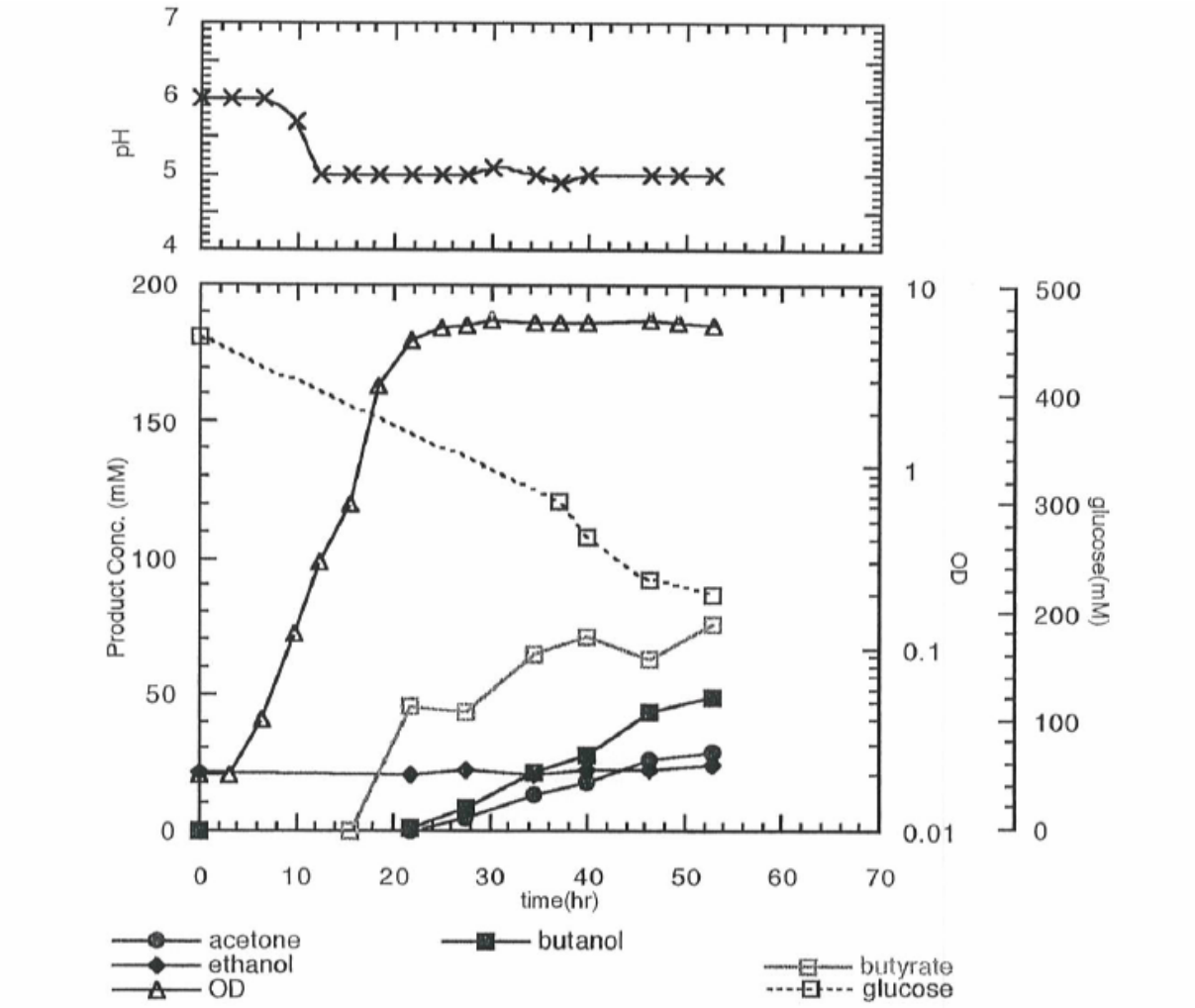


Figure 3.4 Batch fermentation of EG925(pTAAD) controlled at pH 5.0. Tetracycline at a concentration of 10 µg/ml was used for the slection of the plasmid.

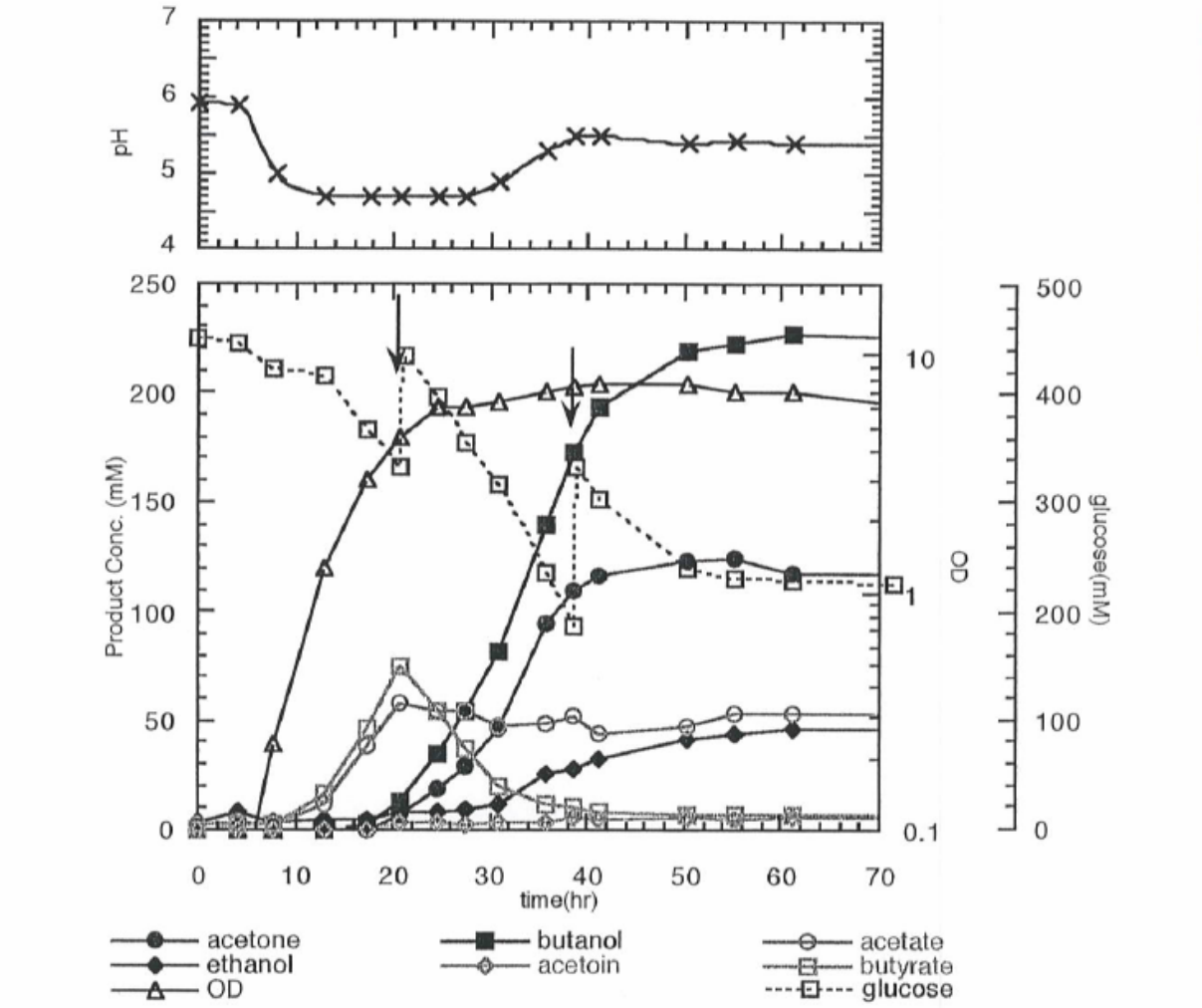


Figure 3.7 Fed-batch fermentation of EG925(pTAAD) controlled at pH 4.7. The arrows indicate time points when glucose was added.

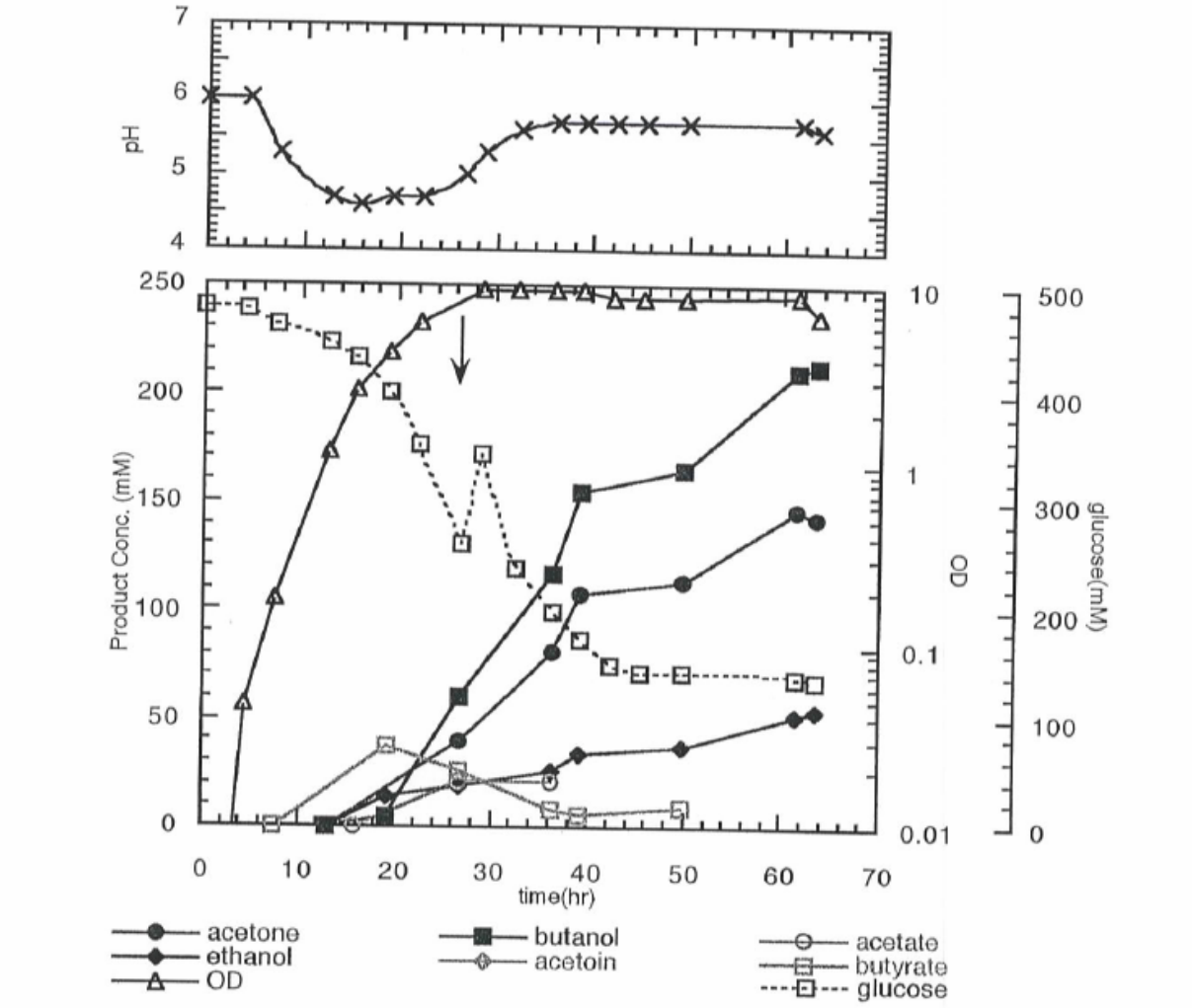
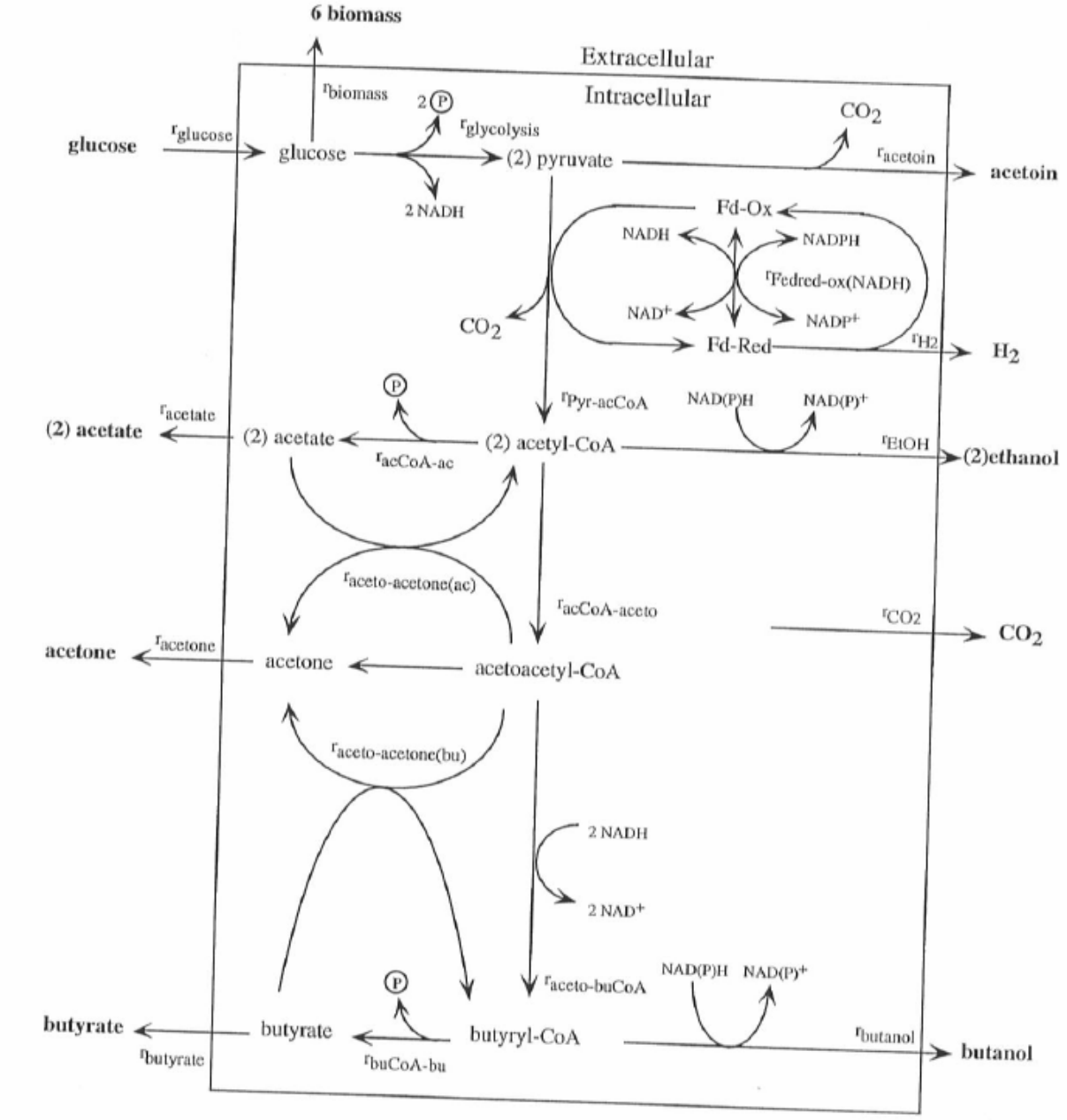


Figure 3.8 Fed-batch fermentation of EG925(pTAAD) controlled at pH 4.7. Clarithromycin at a concentration of 100 µg/ml was used for the slection of the gene deletion mutant. The arrow indicates the time point when glucose was added.

6. Modell von Papoutsakis (1984)

Dies findet sich in der DA im Teil 3 auf den Seiten 14 bis 26 und im Teil 4 (Matlab-Code des Modells).

Figure 3.38 Metabolic pathway of *C. acetobutylicum*



The model consists of 20 reactions:

$r_{glucose}$:	$glucose_{extracellular} = glucose_{intracellular}$	(1)
$r_{biomass}$:	glucose + 0.873 NADH + x ATP = 6 biomass^{+}	(2)
$r_{glycolysis}$:	glucose = 2 pyruvate + 2 NADH + 2 ATP	(3)
$r_{Pyr\text{-}acCoA}$:	pyruvate = acetyl-CoA + CO_2 + Fd-red*	(4)
$r_{acetoin}$:	pyruvate = acetoin + 2 CO_2	(5)
r_{EtOH}:	acetyl-CoA + 2 NADH = ethanol	(6)
$r_{acCo\text{-}ac}$:	acetyl-CoA = acetate + ATP	(7)
$r_{acetate}$:	$acetate_{intracellular} = acetate_{extracellular}$	(8)
$r_{acCoA\text{-}aceto}$:	acetyl-CoA = acetoacetyl-CoA	(9)
$r_{aceto\text{-}acetone(ac)}$:	acetoacetyl-CoA + acetate = acetone + CO_2 + acetyl-CoA	(10)
$r_{aceto\text{-}acetone(bu)}$:	acetoacetyl-CoA + butyrate = acetone + CO_2 + butyryl-CoA	(11)
$r_{acetone}$:	$acetone_{intracellular} = acetone_{extracellular}$	(12)
$r_{aceto\text{-}buCoa}$:	acetoacetyl-CoA + 2 NADH = butyryl-CoA	(13)
$r_{buCoA\text{-}bu}$:	butyryl-CoA = butyrate + ATP	(14)
$r_{butyrate}$:	$butyrate_{intracellular} = butyrate_{extracellular}$	(15)
$r_{butanol}$:	butyryl-CoA + 2 NADH = butanol	(16)
r_{CO2}:	$CO_{2intracellular} = CO_{2extracellular}$	(17)
r_{ATP}:	ADP + P = ATP	(18)
r_{H2}:	Fd-red* = H_2	(19)
$r_{Fdred\text{-}ox(NADH)}$:	Fd-red* = NADH	(20)

$^{+}$ Carbon utilized in biomass.

* Fd-red refers to the reduced form of ferredoxin.

The pseudo-steady-state approximation has been validated for the stoichiometric model of *C. acetobutylicum* (Papoutsakis, 1984) as well as numerous other stoichiometric models (Stephanopoulus, 1993). For *C. acetobutylicum*, twelve mass balances can be formulated.

Glucose	$r_{glucose} - 1/6\, r_{biomass} - r_{glycolysis}$	$= 0$	(22)
Acetyl-CoA	$r_{Pyr\text{-}acCoA} + r_{aceto\text{-}acetone(ac)} - r_{acCoA\text{-}aceto} - r_{acCoA\text{-}ac}$	$= 0$	(23)
Acetate	$r_{acCoA\text{-}ac} - r_{ac} - r_{aceto\text{-}acetone(ac)}$	$= 0$	(24)
Acetoacetyl-CoA	$r_{acCoA\text{-}aceto} - r_{aceto\text{-}buCoA} - r_{aceto\text{-}acetone(ac)} - r_{aceto\text{-}acetone(bu)}$	$= 0$	(25)
Acetone	$r_{aceto\text{-}acetone(ac)} + r_{aceto\text{-}acetone(bu)} - r_{acetone}$	$= 0$	(26)
Butyryl-CoA	$r_{aceto\text{-}buCoA} + r_{aceto\text{-}acetone(bu)} - r_{butanol} - r_{buCoA\text{-}bu}$	$= 0$	(27)
Butyrate	$r_{buCoA\text{-}bu} - r_{aceto\text{-}acetone(bu)} - r_{butyrate}$	$= 0$	(28)
Feredoxin-red	$r_{Pyr\text{-}acCoA} - r_{Fedred\text{-}ox(NADH)} - r_{H2}$	$= 0$	(29)
CO_2	$2\, r_{acetoin} + r_{aceto\text{-}acetone(ac)} + r_{aceto\text{-}acetone(bu)} + r_{Pyr\text{-}acCoA} - r_{CO2}$	$= 0$	(30)
NADH	$2\, r_{glycolysis} + r_{Fedred\text{-}ox(NADH)} - 2\, r_{aceto\text{-}buCoA} - 2\, r_{EtOH} - 2\, r_{butanol}$	$= 0$	(31)
Pyruvate	$2\, r_{glycolysis} - 2\, r_{acetoin} - r_{Pyr\text{-}acCoA}$	$= 0$	(32)
ATP	$2\, r_{glycolysis} + r_{acCoA\text{-}ac} + r_{buCoA\text{-}bu} - r_{ATP}$	$= 0$	(33)

It was shown by de Kok and Roels (1980) that there is no loss in information if you split the matrix into measured and non measured elements. The new equation is:

$$E_m \cdot r_m + E_c \cdot r_c = 0 \qquad (35)$$

where r_m is the m-dimensional measured part of r; r_c is the c-dimensional unmeasured part of r (m is the number of measured conversions and c the number of unmeasured rates: $c + m = n$). In our system, we have as mentioned above eight measured rates and twelve unmeasured rates.

$$Em = \begin{pmatrix} 1 & -\frac{1}{6} & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 \\ 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 2 & 0 \\ 0 & 0 & 0 & 0 & 0 & -2 & 0 & -2 \\ 0 & 0 & 0 & 0 & 0 & 0 & -2 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix}$$

$$Ec = \begin{pmatrix} 0 & 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1 & -1 & 0 & -1 & 1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 1 & -1 & 0 & -1 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1 & 0 & 0 \\ -1 & 0 & 0 & 1 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & -1 & 0 & 1 & 0 & 0 & 0 & 1 & 1 & 0 & 0 & 0 \\ 0 & 0 & 2 & 0 & 0 & -2 & 0 & 0 & 0 & 0 & 1 & 0 \\ 0 & 0 & 2 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 2 & 0 & 0 & 0 & 1 & 0 & 0 & 1 & 0 & -1 \end{pmatrix}$$

$$r_m = [r_{glucose},\ r_{biomass},\ r_{acetate}, r_{acetone}, r_{butyrate}, r_{butanol}, r_{acetoin}, r_{EtOH}]^T$$

$$r_c = [r_{H2},\ r_{CO2},\ r_{glycolysis},\ r_{pyr\text{-}acCoA},\ r_{acCoA\text{-}aceto},\ r_{aceto\text{-}buCoA},\ r_{acCoA\text{-}ac},\ r_{aceto\text{-}acetone(ac)},\ r_{acetoCoA\text{-}acetone(bu)},\ r_{buCoA\text{-}bu},\ ,\ r_{ATP}]^T$$

7. Weiterführende Unterlagen

Nr.	Dok.-ID	Beschreibung
1	DA von Lars Blank	

8. Abkürzungen und Definitionen

Ausdruck	Bedeutung
ENG	engineo GmbH