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# Experiences in the production and use of butanol as biofuel

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# INSTITUTE OF CHEMICAL TECHNOLOGY, PRAGUE

## Faculty of Food and Biochemical Technology

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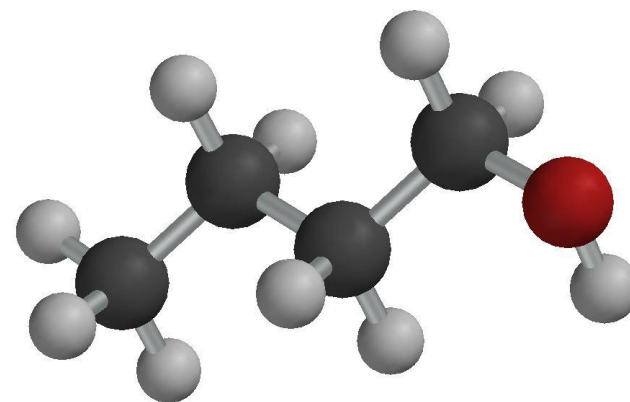


# Why biobutanol?

## Advantages over bioethanol:

- higher energy content
- lower volatility
- hydrophobity
- lesser corrosivity
- possibility to use the same pipe system as in case of conventional liquid fuel

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- x** lower octane number
  - x** lower vapour pressure



1-butanol



# History and present of acetone-butanol-ethanol (ABE) fermentation

1861 - discovery of ABE fermentation – L. Pasteur

1909 - Ch. Weizmann – isolation of *Clostridium acetobutylicum*

**1913 - origination of industrial acetone production from corn for gun-powder, jet fuel... in U.K.**

1916 - production of solvents in Canada and U.S.A.

1935 – first solvent industrial production from molasses

after 1936 – industrial ABE production in Soviet Union, Japan, China, South Africa, Egypt....

**1950s-1960s – ABE production in Czechoslovakia**

after 1960s – closing of fermentative industrial ABE

productions because of cheap oil (last factory operated till 1986 in South Africa)

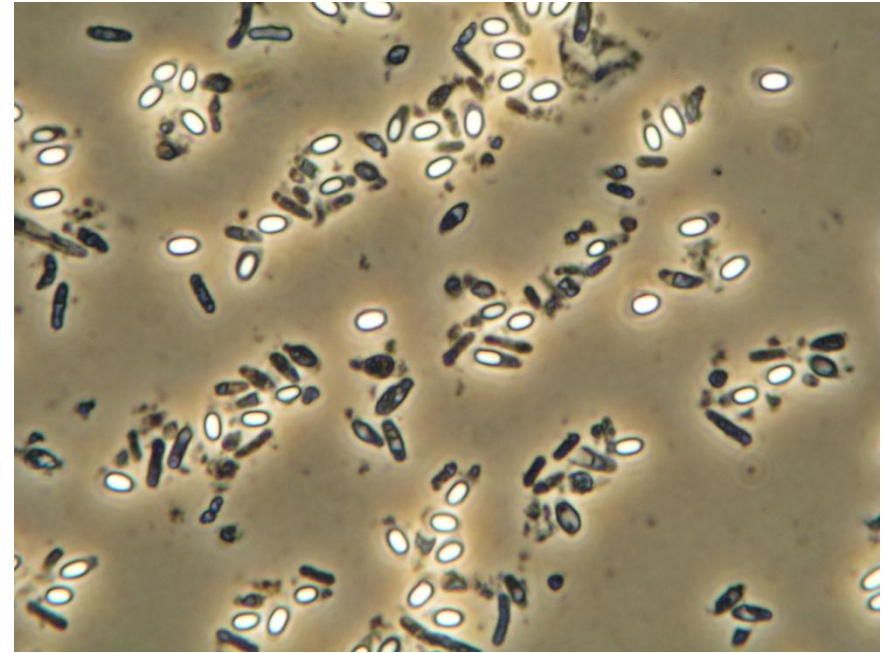
**nowadays – industrial production in China, small pilot plant in Brazil, many plans for plants in different stages of preparation in U.S.A., Slovakia, France, U.K. ...**



**Production strains** – *Clostridium acetobutylicum*,  
*C.beijerinckii*, *C. saccharoperbutylacetonicum*,  
*C.pasteurianum*, ...



Growth phase – acids production



Sporulation – solvents production

**usually wild strains or strains obtained after chemical undirected mutagenesis were used**



# Solventogenic clostridia

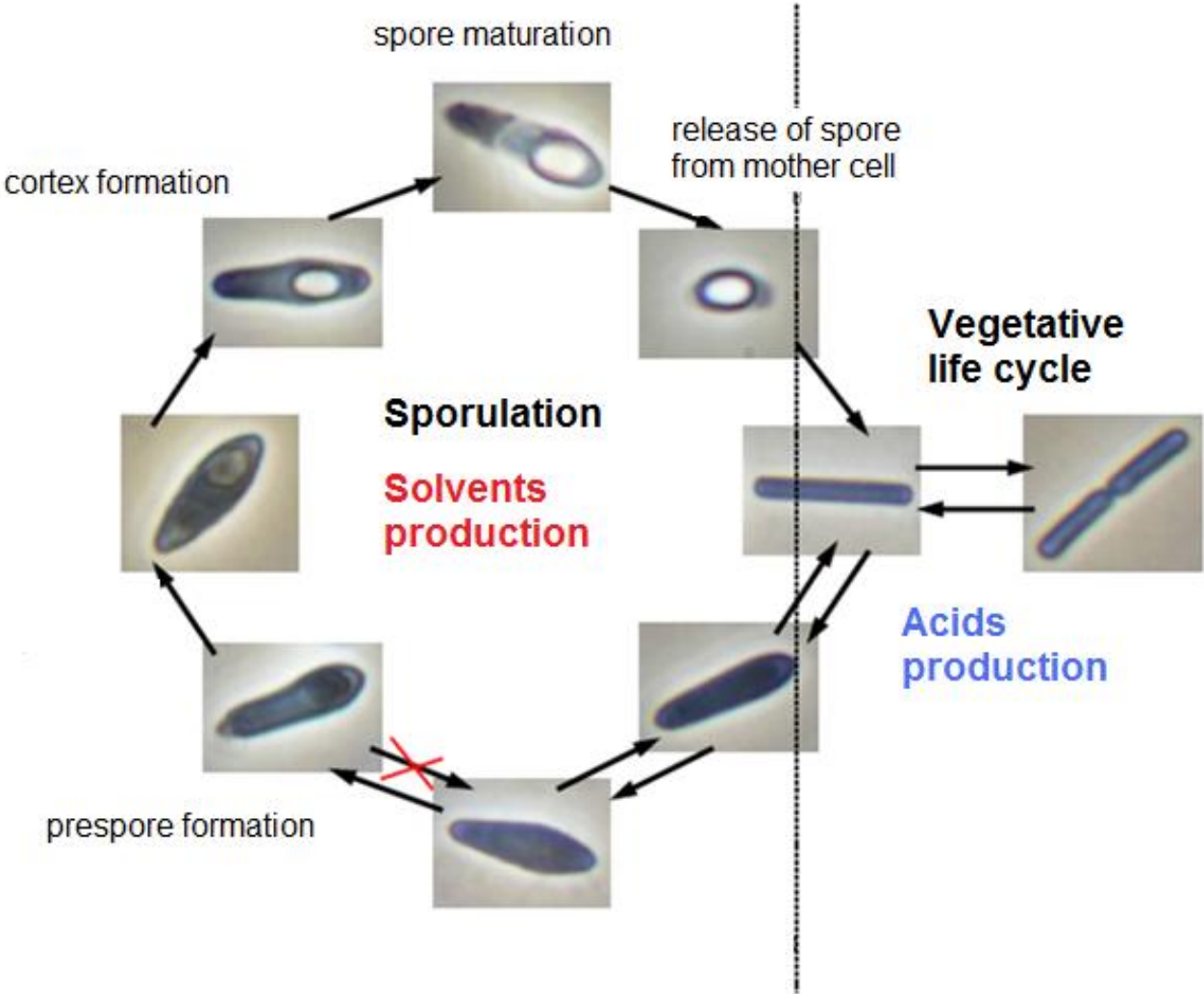
## – common features

- rod shaped, motile bacteria
- young cells – typically  $G^+$ , older (sporulating) cells –  $G^-$
- anaerobic
- heterofermentative
- endospore formation induced by pH drop (in bioreactor) or oxygen presence (in nature)
- utilization of different sugars – glucose, sucrose, lactose, starch, inulin... (*X not cellulose*)





# Clostridial life cycle versus metabolites formation





# Main bottlenecks of butanol production

- 1. Low final butanol concentration** – max. 20 g/l caused by butanol toxicity – not easy to overcome – butanol affects activities of about 200 genes, complex toxic effect on cell membrane, synergic stress effect of butyric and acetic acids; butanol tolerance is about the same for bacteria and yeasts
- 2. Low butanol productivity** in comparison with ethanol formation
- 3. Many side-products**

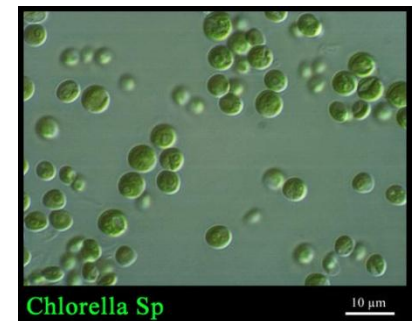
So far no GM *Clostridium* or other microorganism with technologically more convenient properties exceeding wild or mutant strains used in large scale.

# Feedstock for biobutanol production



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1. Traditional sugar sources – starch crops (corn), sugar cane or sugar beet
2. Feedstock of 2<sup>nd</sup> generation – hydrolysates of lignocellulosic materials
3. Alternative carbon sources – algae, whey, glycerol

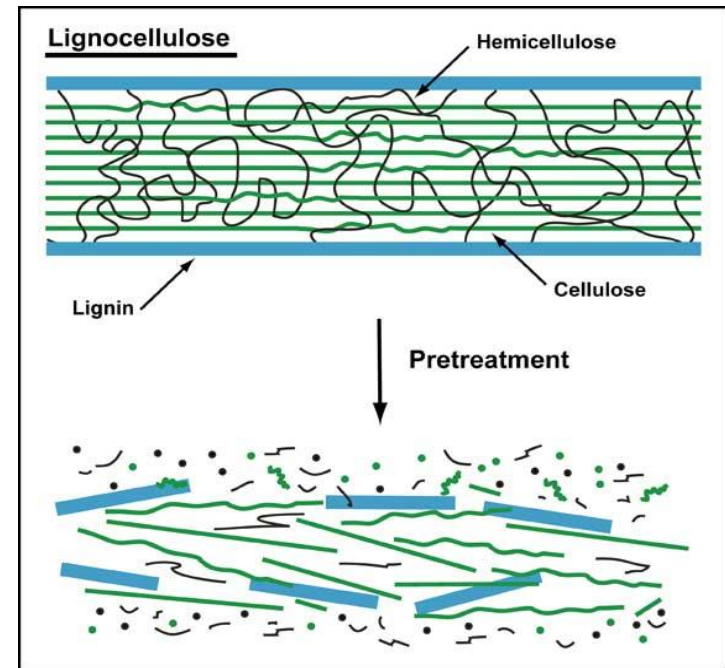



+ nitrogen source – both ammonium ions and amino acids (yeast extract, vitamins solutions...) – strains usually auxotrophic

# Lignocellulosic hydrolysates

Nowadays – probably the best option – two steps process

1. **Lignocellulose pretreatment** – acid or alkaline hydrolysis, steam explosion, extrusion, ammonium expansion...
2. **Enzyme hydrolysis** (X enzyme price, time of hydrolysis, conditions...)



X necessity of material pretreatment before enzyme hydrolysis  inhibitors like furfural, HMF, ferulic and coumaric acids are formed

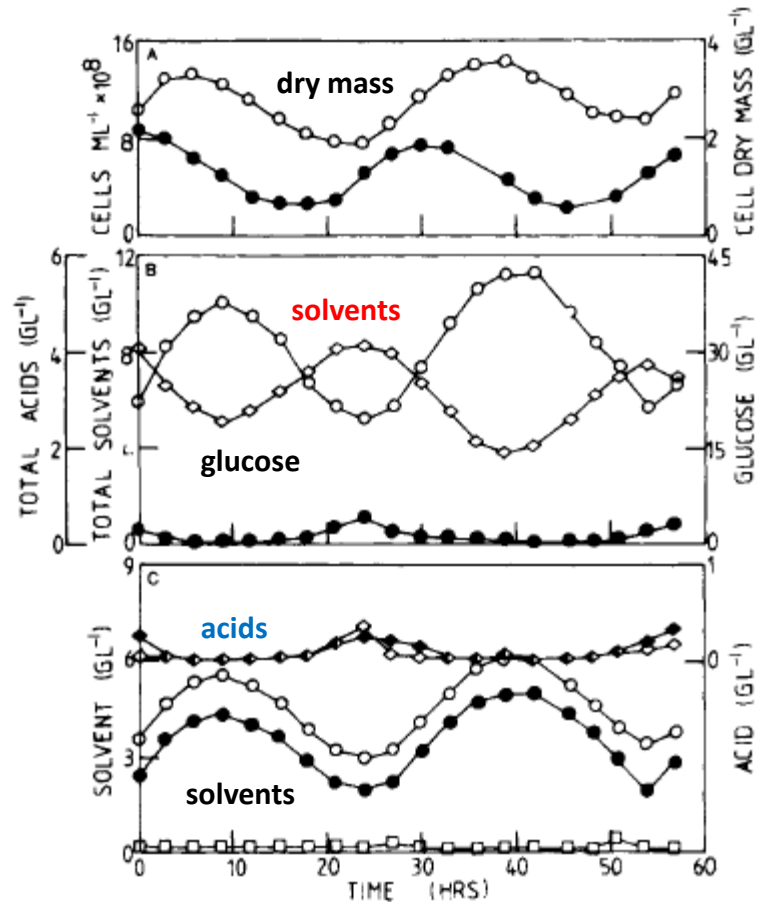
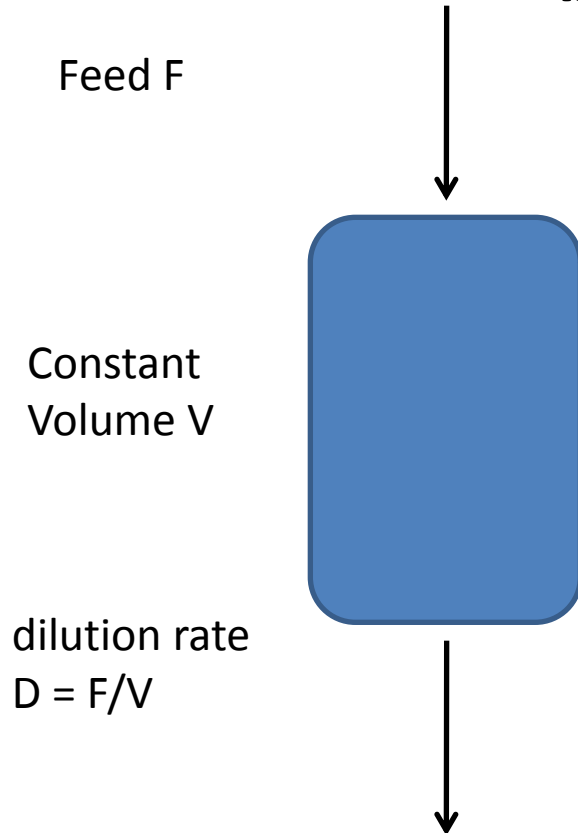


# Options for cultivation arrangement



1. **Batch cultivation** - max. end butanol concentration 12-20 g/l, yield 20% and productivity 0.3-1.2 g/l/h
2. **Fed-batch cultivation** – moderate increase of productivity (overcome of substrate inhibition)
3. **Continuous cultivation** – difficult to perform due to biphasic metabolites formation

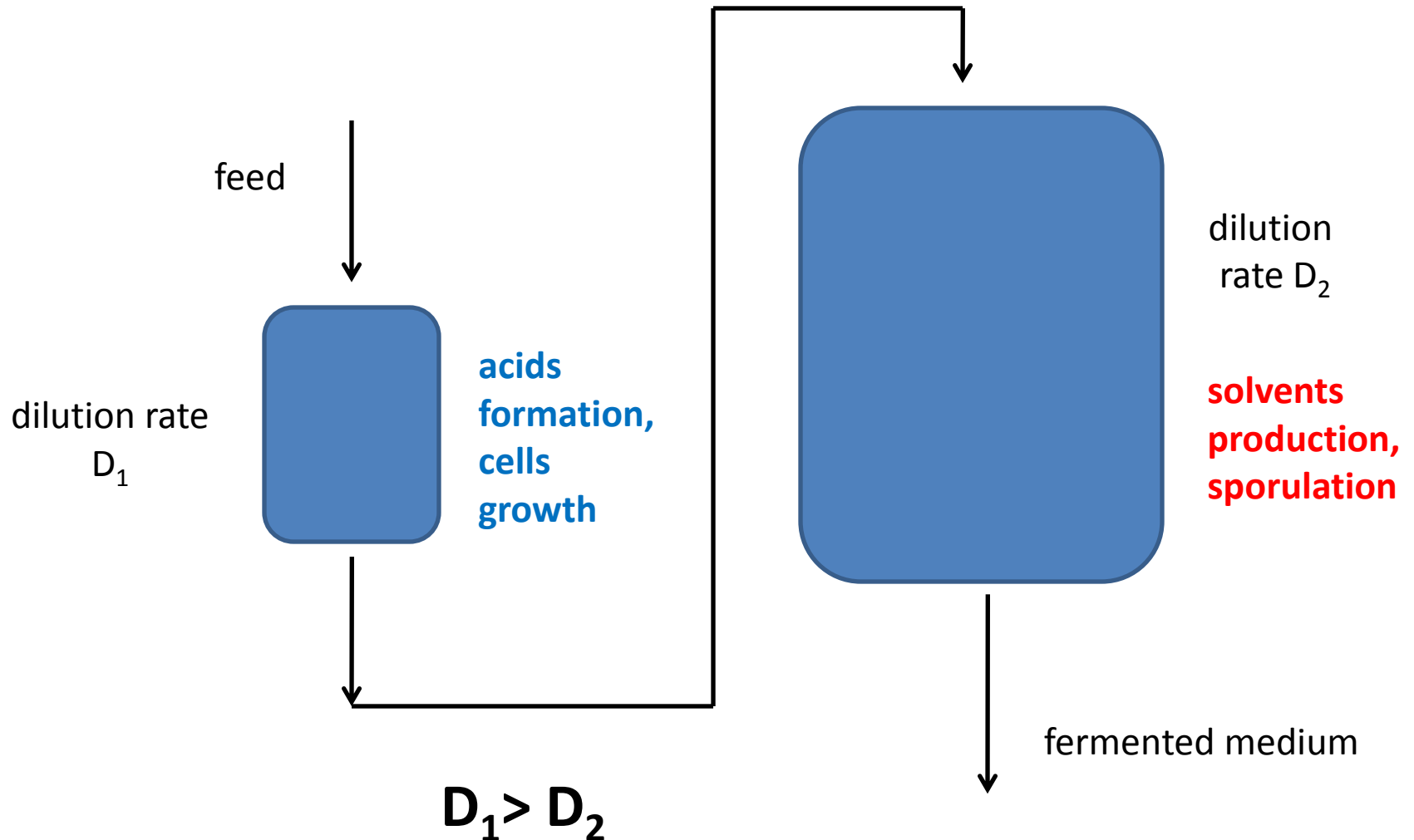
# Continuous cultivation in one bioreactor



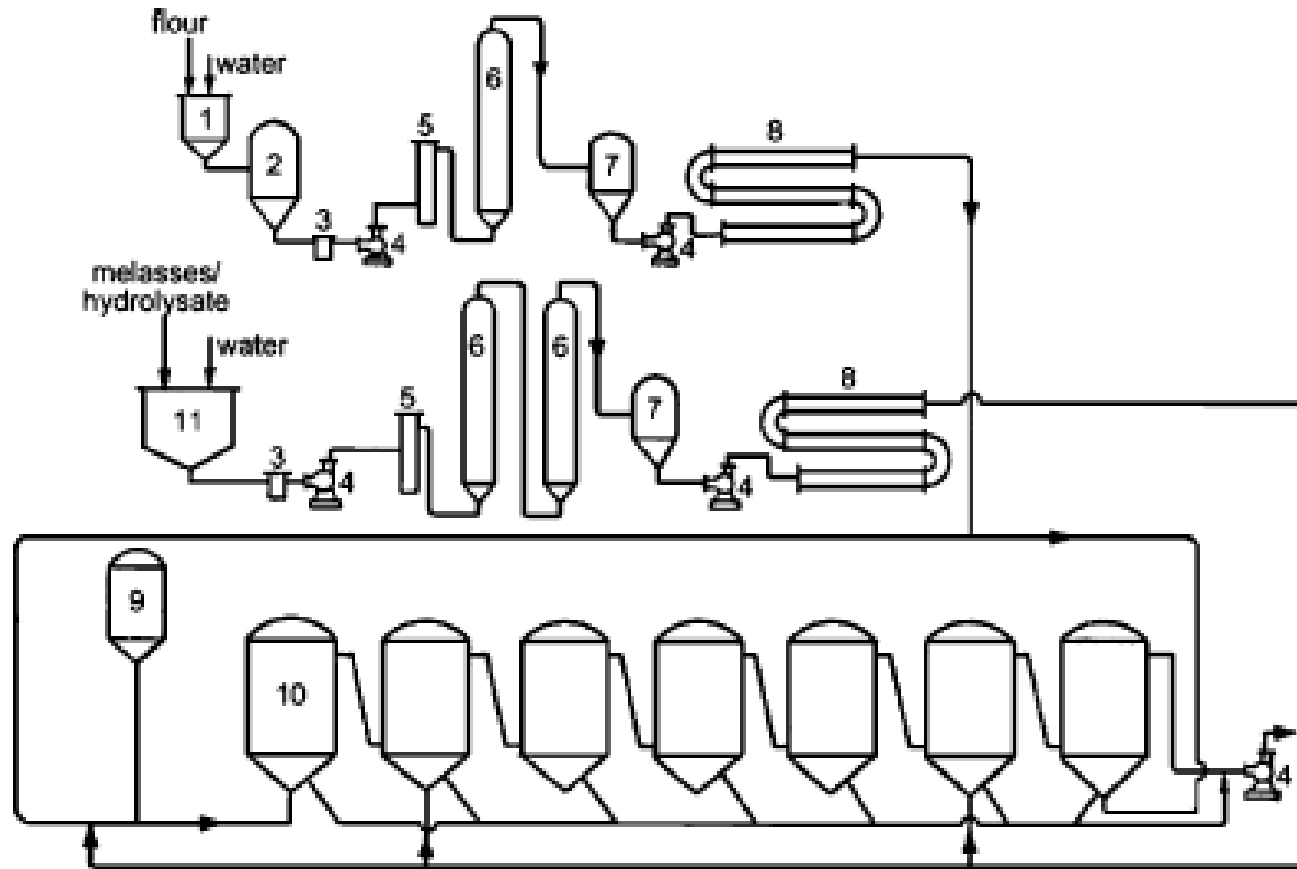
Typical – oscillatory behaviour  
with irregular oscillation



# Continuous cultivation in two bioreactors



# Continualization of ABE fermentation in more vessels



Zverlov et al. 2006 – battery of bioreactors working in Dukshukino plant in Soviet Union



# Continuous ABE fermentation

## pros

- no inoculum preparation
- reduction of operation times necessary for cleaning and sterilization
- increase of productivity and yield ?

## cons

- danger of cells wash-out
- pressure on cells growth - risk of strain degeneration
- stringently aseptic conditions
- lower end product concentrations
- sterilization of bigger medium volume – higher amount of waste



# Other cultivation options

- cells immobilization in the bioreactor  
(lowering of products toxicity)
- **integration of cultivation with products separation** – solution for products inhibition during batch and fed-batch cultivations
- cells recycle during continuous cultivation

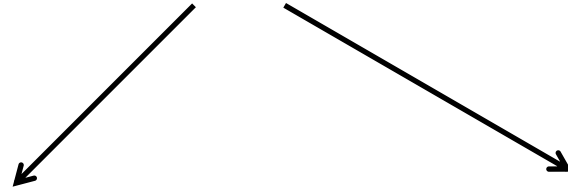
# Distillation as final separation step for biobutanol



- in the past - direct distillation from fermented medium – great energy consumption due to big medium volumes
- azeotropic mixture butanol – water formation (b.p. 93 C, 55% butanol)  
➔ extractive distillation,  
distillation with decantation
- necessary step for final solvents separation



# Separation of solvents during fermentation or products concentration before distillation



## Membrane techniques

Pervaporation

Perstraction

Reverse osmosis

## Non-membrane techniques

Gas stripping

Liquid-liquid extraction

Sorption

Gas stripping – simple method, possible butanol concentration up to 172 g/l

Sorption – the most convenient from the view of energy requirement

# Our research

- screening of butanol production by different strains on available substrates
- bioreactor cultivations in batch, fed-batch and continuous regimes using glucose as a model substrate
- batch bioreactor cultivations on sugar beet juice and milled corn
- gas stripping as a method for reducing product inhibition and/or distillation cost
- behaviour of biobutanol-petrol blends during storage and distribution
- test car fueled with the biobutanol-gasoline blends operated on-the-road traffic
- affect on fuel emissions and engine oil degradation

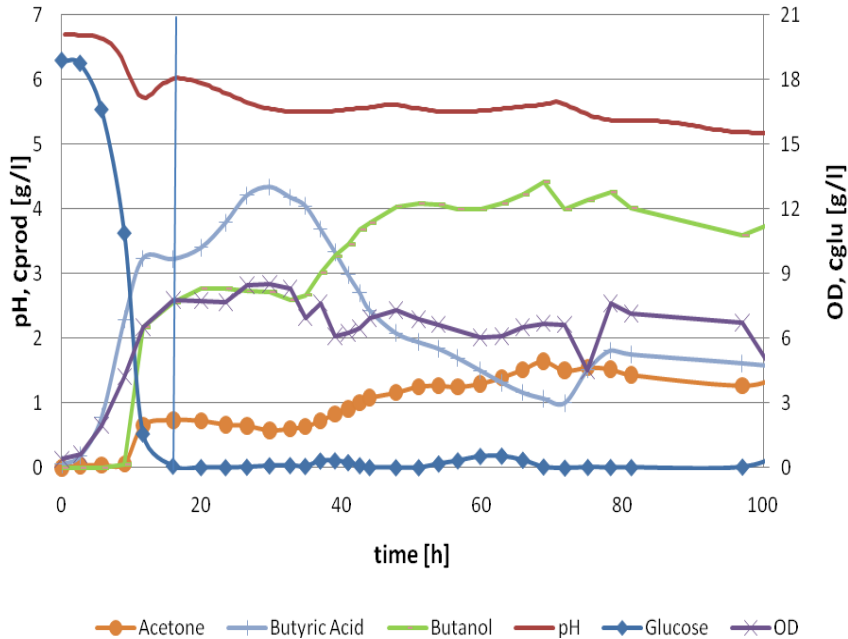
# Parameters of batch, fed-batch and continuous cultivations

<b>cultivation</b>	max. butanol conc. (g/l)	max. total ABE conc. (g/l)	yield of total ABE $Y_{ABE/S}$ (%)	ABE productivity $P_{ABE}$ (g/l/h)
<b>batch</b>	<b>7.2</b>	<b>11.6</b>	<b>30</b>	<b>0.23</b>
<b>fed-batch</b>	<b>8.3</b>	<b>12.3</b>	<b>23</b>	<b>0.25</b>
<b>continuous<sup>#</sup></b>	<b>4.4</b>	<b>6.2</b>	<b>23<sup>*</sup></b>	<b>0.11<sup>*</sup></b>

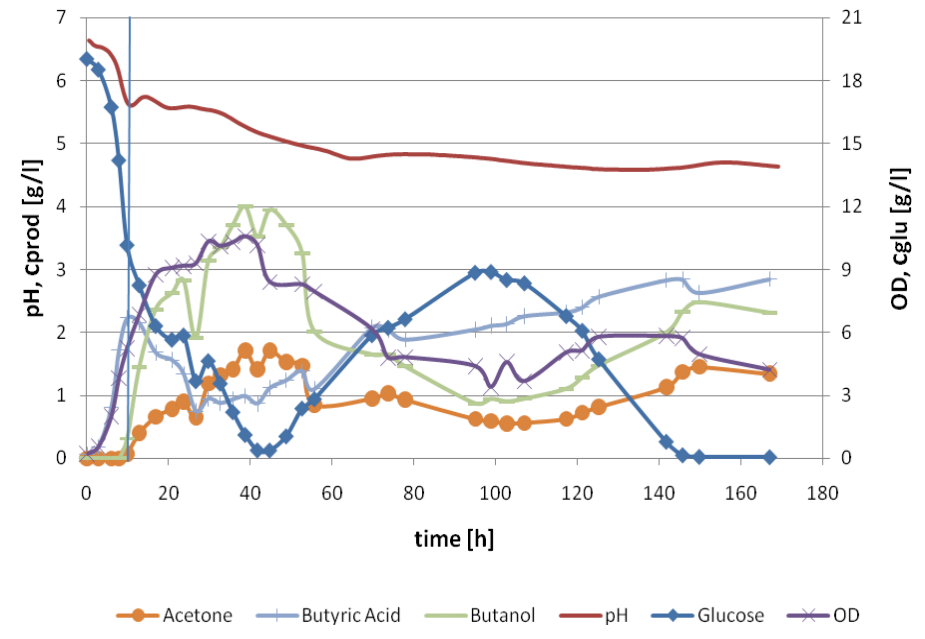
<sup>#</sup>glucose limited chemostat, <sup>\*</sup> calculated in pseudo steady-state

Model strain *C.pasteurianum* - with stable growth and medium solvents production, robust response regarding minor changes in cultivation conditions, on glucose semidefined medium

# Glucose limited chemostat X glucose unlimited continuous cultivation



pseudo steady state achieved



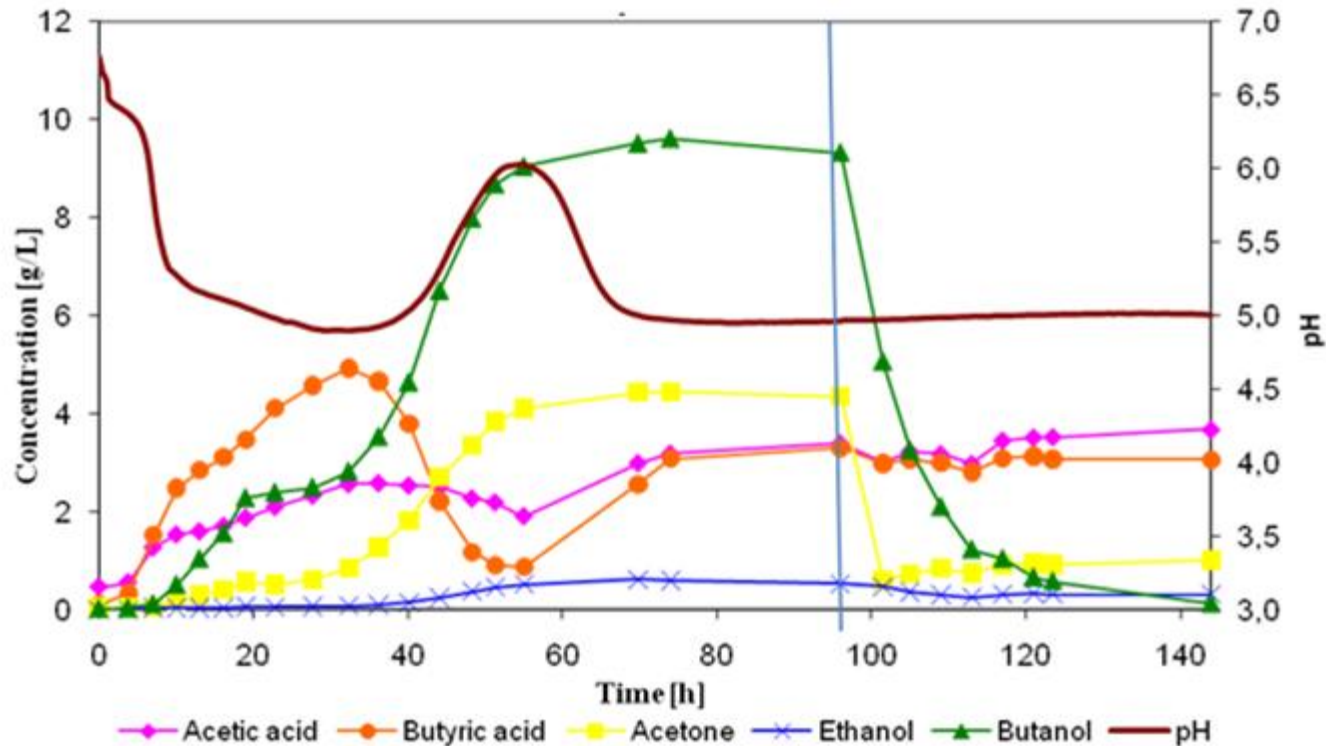
oscillatory behaviour



# Batch bioreactor cultivation using sugar beet juice or corn

species	substrate	max. butanol conc. (g/l)	max. total ABE conc. (g/l)	yield of total ABE $Y_{ABE/S}$ (%)	ABE productivity $P_{ABE}$ (g/l/h)
<i>C.beijerinckii</i>	sugar beet juice	<b>10.0</b>	<b>14.2</b>	<b>37</b>	<b>0.40</b>
<i>C.acetobutylicum</i>	corn	9.6	14.4	27	0.20

# Production of ABE solvents using corn medium with subsequent solvents separation by gas stripping



*C.acetobutylicum* used; nitrogen at 3VVM for gas stripping



# Fuel properties

Parameter	Bioethanol	Biobutanol	Gasoline
Boiling Point ( C)	78	117	30 – 215
Specific Gravity at 15 C (kg/m <sup>3</sup> )	794	814	720 – 775
Kin. Viscosity at 20 C (mm <sup>2</sup> /s)	1.52	3.64	0.4 – 0,8
Heating Volume (MJ/L)	21	27	32 – 33
Vaporization Heat (MJ/kg)	0,92	0,43	0,36
Vapour Pressure - RVP (kPa)	19.3	18.6	60 – 90
Octane Number - RON	106 – 130	94	95
MON	89 – 103	80 – 81	85
Oxygen content (% wt.)	34.7	21.6	<2.7

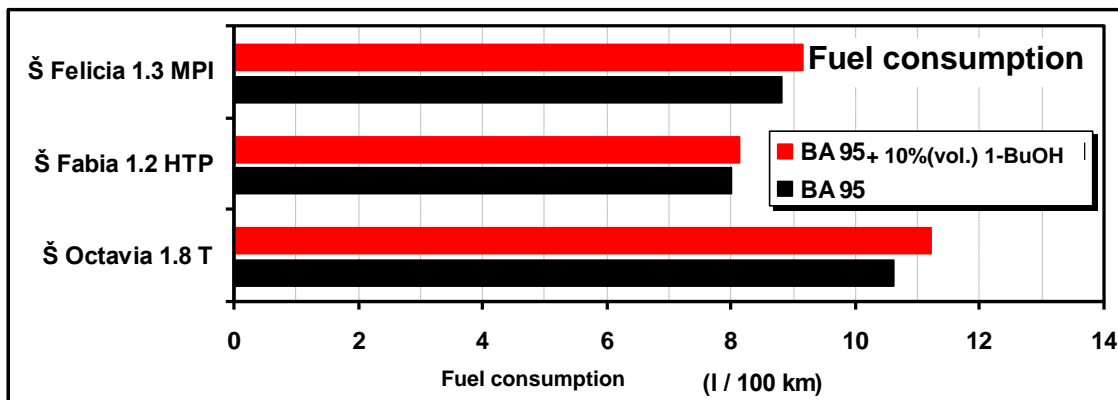
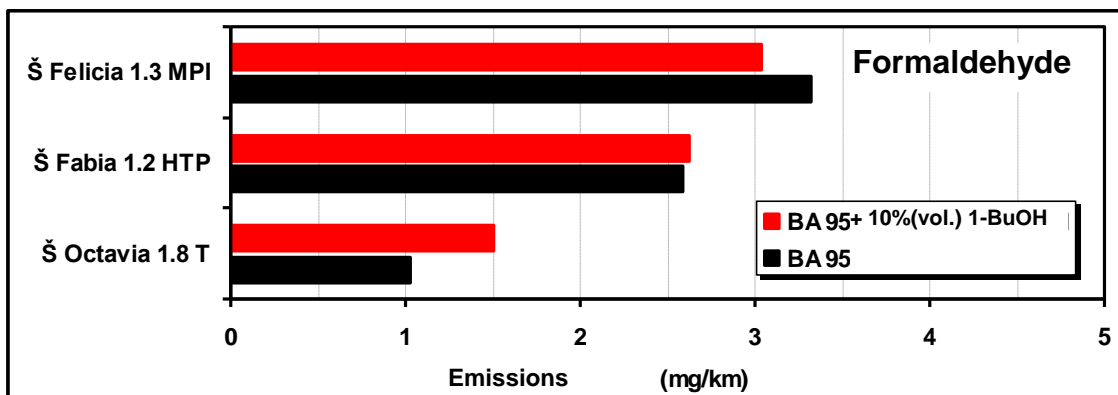
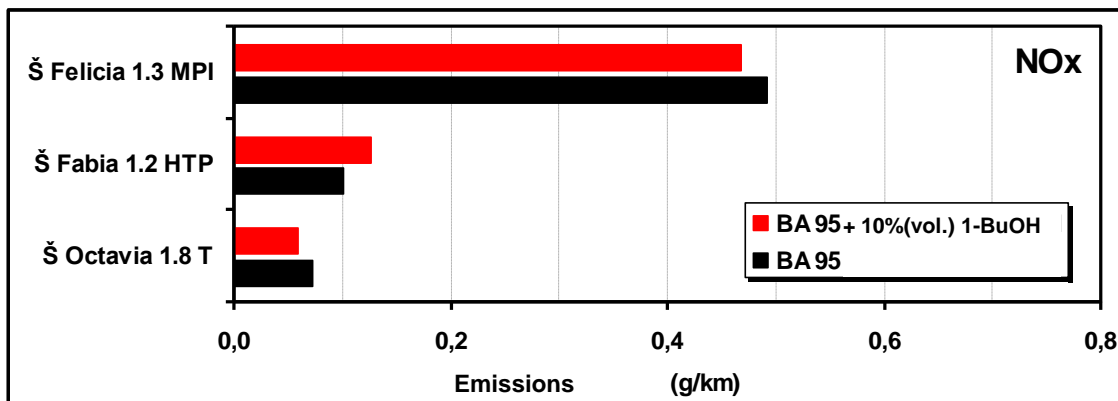
# ŠKODA Auto



# Biobutanol

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On-the-road traffic tests with cars fueled with butanol-gasoline blend





# Conclusion

## – how to succeed with butanol as biofuel

- stable strain convenient for given substrate
- cheap feedstock
- fed-batch cultivation with integrated solvents removal or continuous cultivation
- minimization of side-products
- energy efficient distillation
- valorization of all products - butanol, acetone, hydrogen, ethanol, distillation slops



# Conclusion

## – how to succeed with 1-butanol as biofuel

- 1-butanol to become superior biocomponent of motor fuel mixtures
- Biobutanol has a vapour pressure synergy with bioethanol in gasoline blends
- Addition of 1-butanol to gasoline up to 10 % vol.
- No affect adversely CO, VOC and NO<sub>x</sub> fuel emissions

**Thank you very much  
for your attention**

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**Acknowledgements:**

The work was performed with financial support from TIP Project No. FR-TI1/218, NAZV No QH81323/2008, FRVŠ supported by the Ministry of Education No. 546/2010, the research project MŠM6046137305 and targeted support for specific university research Ministry of Education No. 21/2010.



Prague panorama - Castle and Charles Bridge