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Flow Cytometry Analysis of Solventogenic Clostridia

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Why Flow Cytometry ?

FC = simultaneous multiparametric analysis of the physical and chemical characteristics of single cells

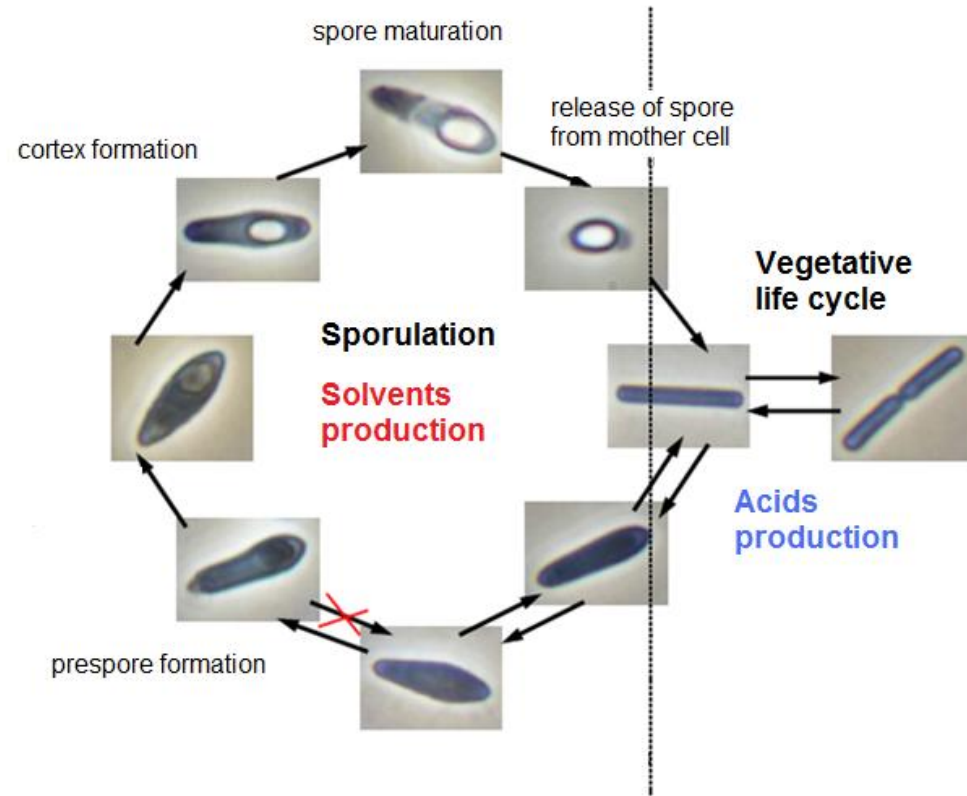
- routinely used in medicine (in 2009 only 8% of FC publications dealing with microorganisms)

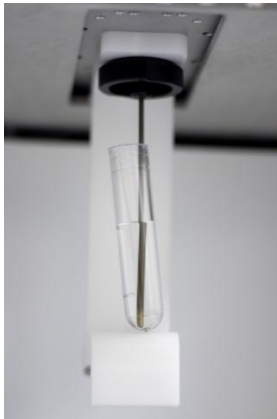
Solventogenic clostridia

- only few parameters followed during ABE process
- cell population changes during process, becomes heterogenic



FC – useful tool in ABE fermentation

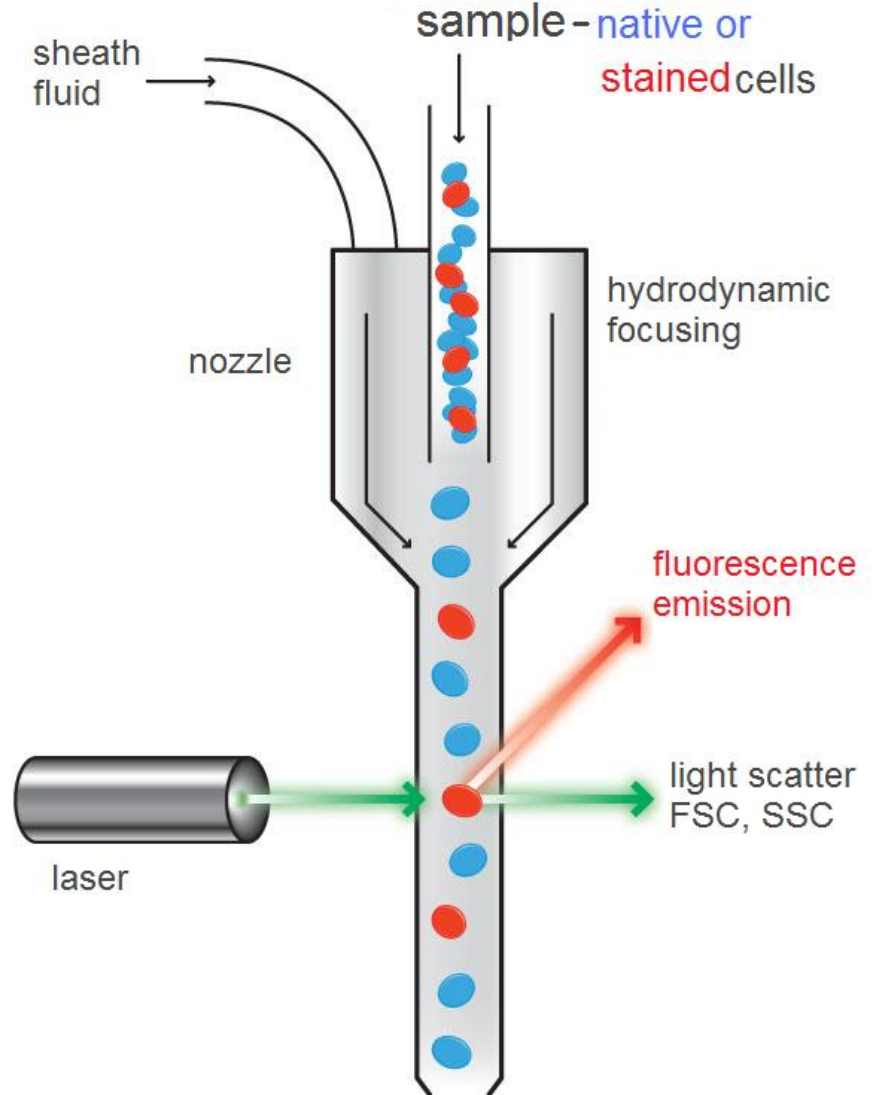




How FC works?



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<http://www.abcam.com/index.html?pageconfig=resource&rid=11446>;

adapted

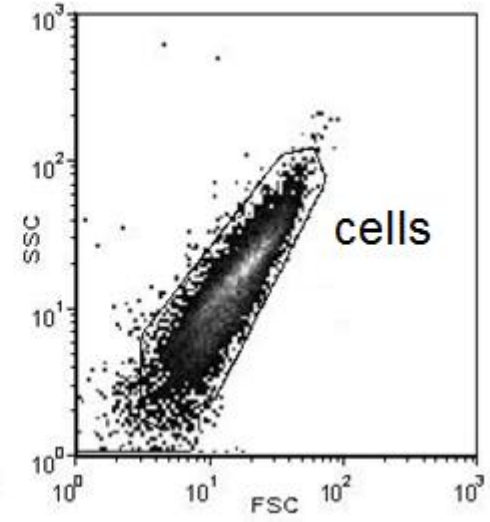
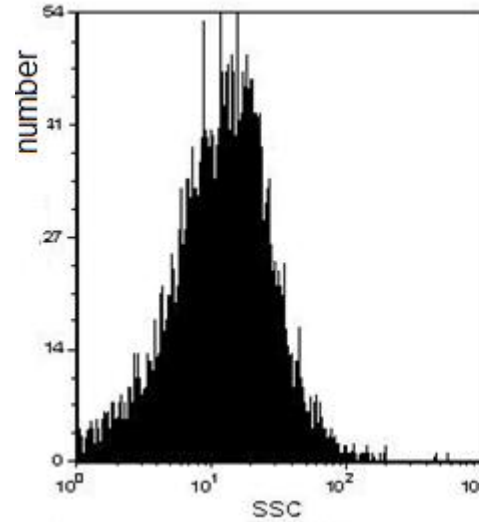
FC outputs



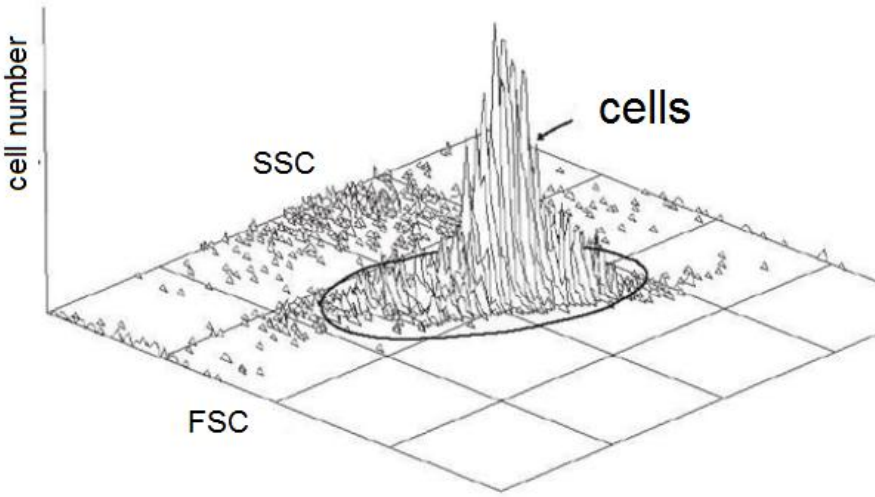
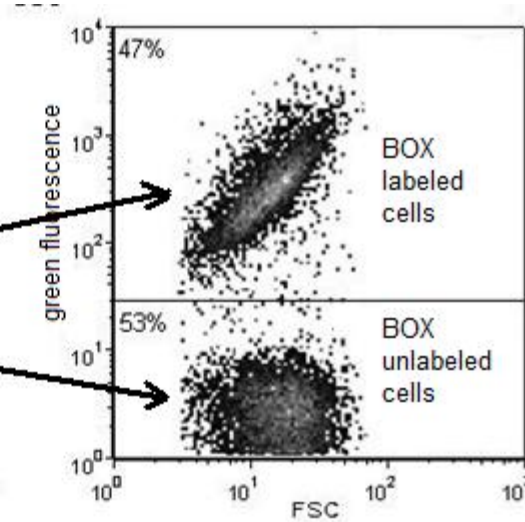
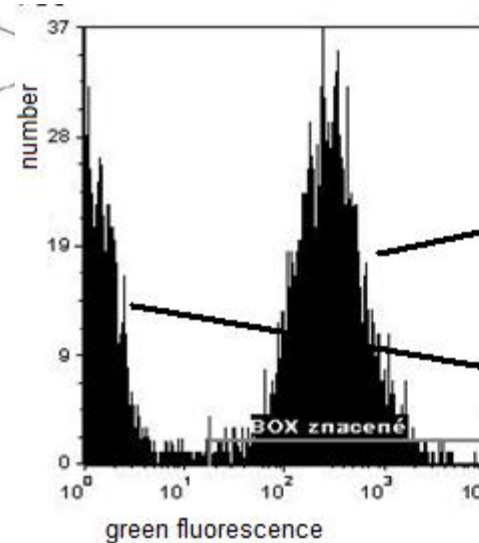
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Cells X non cells particles
(ideal state – yeast cells)

Native population



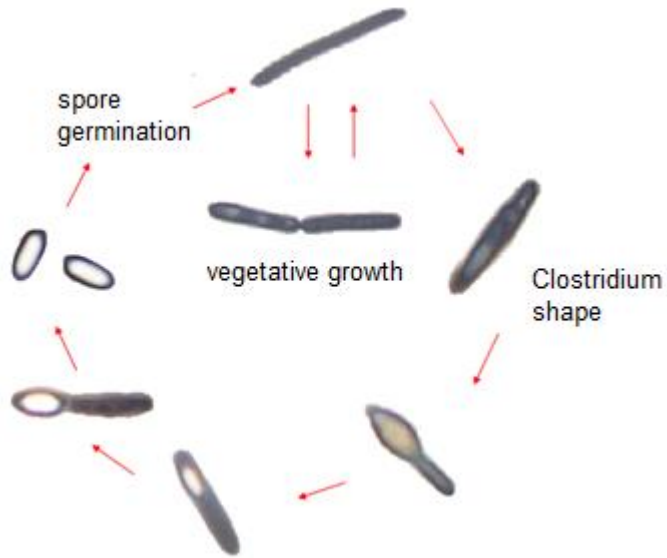
Labeled population



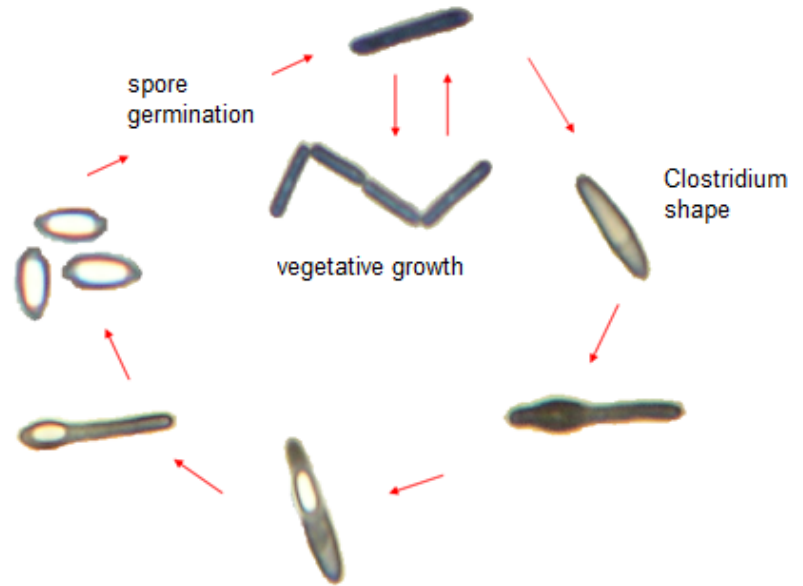
Our favourite Clostridia



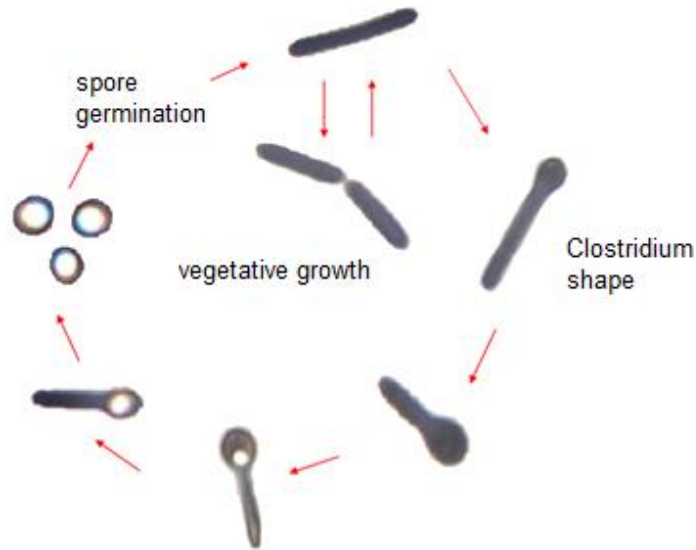
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C.pasteurianum
NRRL B-598



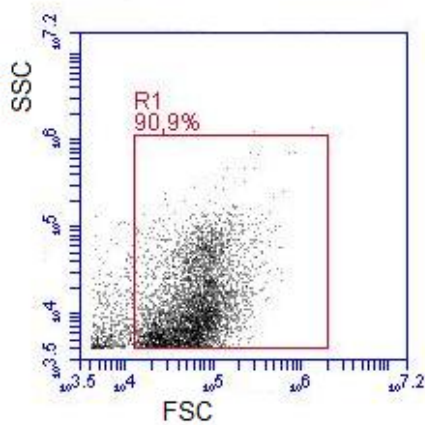
C.beijerinckii CCM 6218



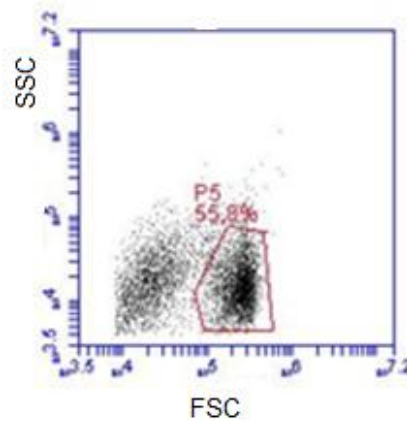
C.tetanomorphum DSM 4473



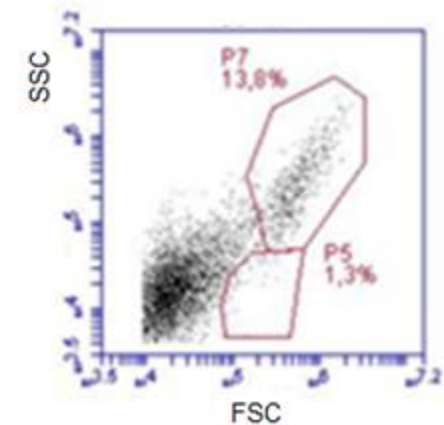
FC analysis of native population - spore number estimation (*C.beijerinckii*)



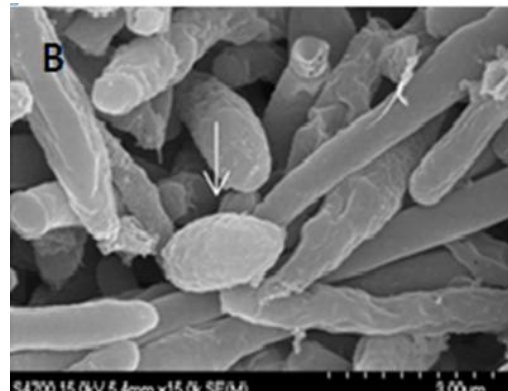
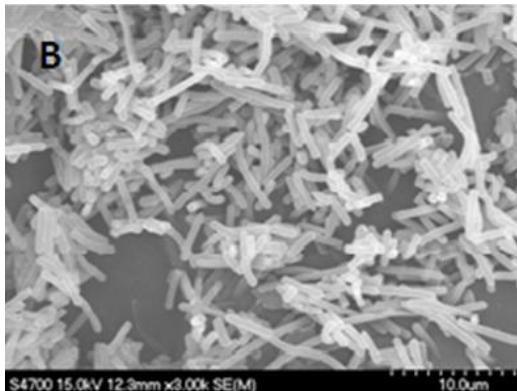
non-sporulating
gate R1 – all cells



sporulating
gate R1 – invisible
gate P5 - spores



formation of cell
aggregates- gate P7

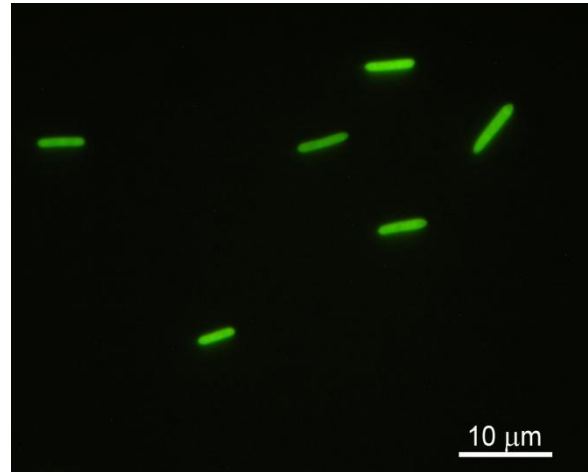
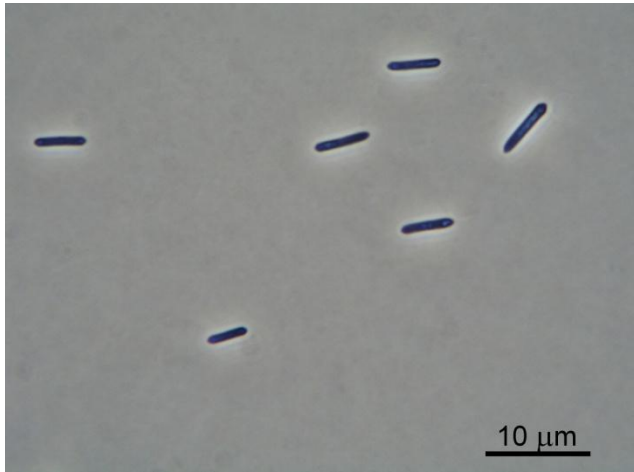




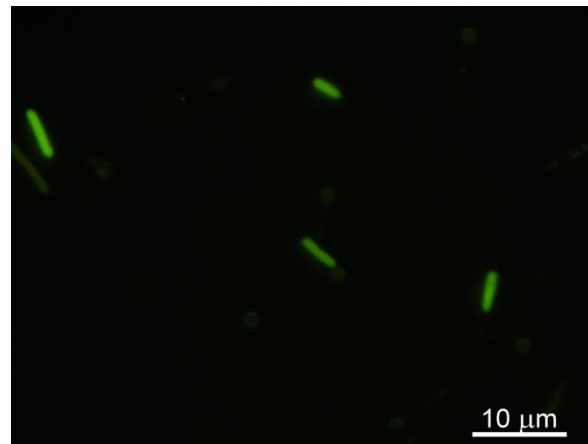
Viability estimation by fluorescent analysis

Fluorescent probe	Application	Principle	fluorescence
Propidium iodide (PI)	Membrane integrity	Nucleic acids in cells with permeabilized membrane	Red
Sytox [®]	Membrane integrity	Nucleic acids in cells with permeabilized membrane	Green, red, orange, blue
Carboxy fluorescein diacetate (CFDA)	Esterase activity (intracellular pH)	Non-fluorescent stain is converted to fluorescent product	green
Bis-oxonol (BOX)	Transmembrane potential	Anionic probe cumulated by cells with depolarized membranes	green

Development of method for estimation of metabolically active cells – *C.tetanomorphum*, propidium iodide



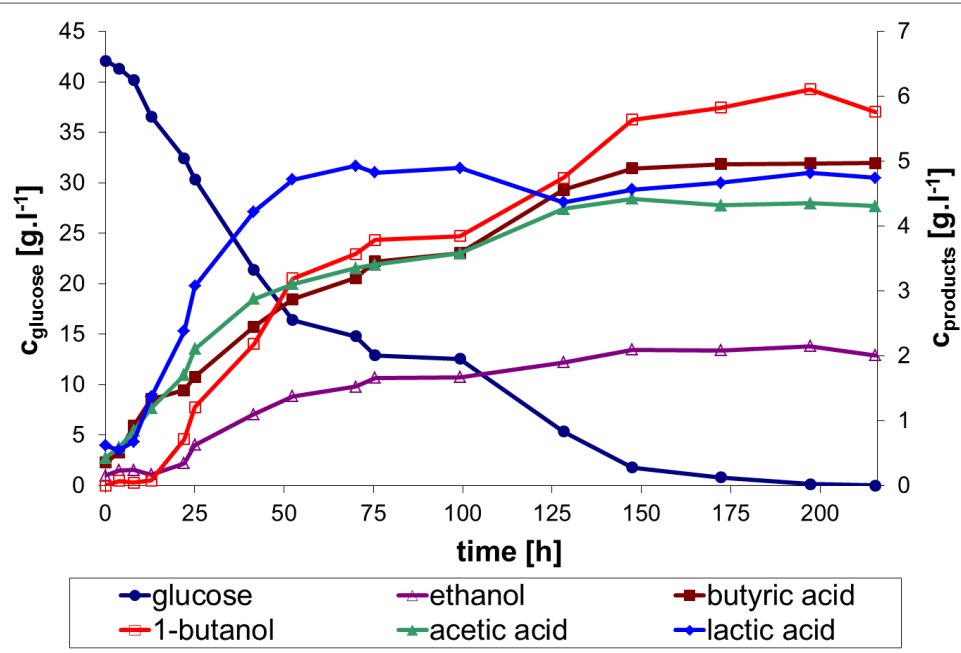
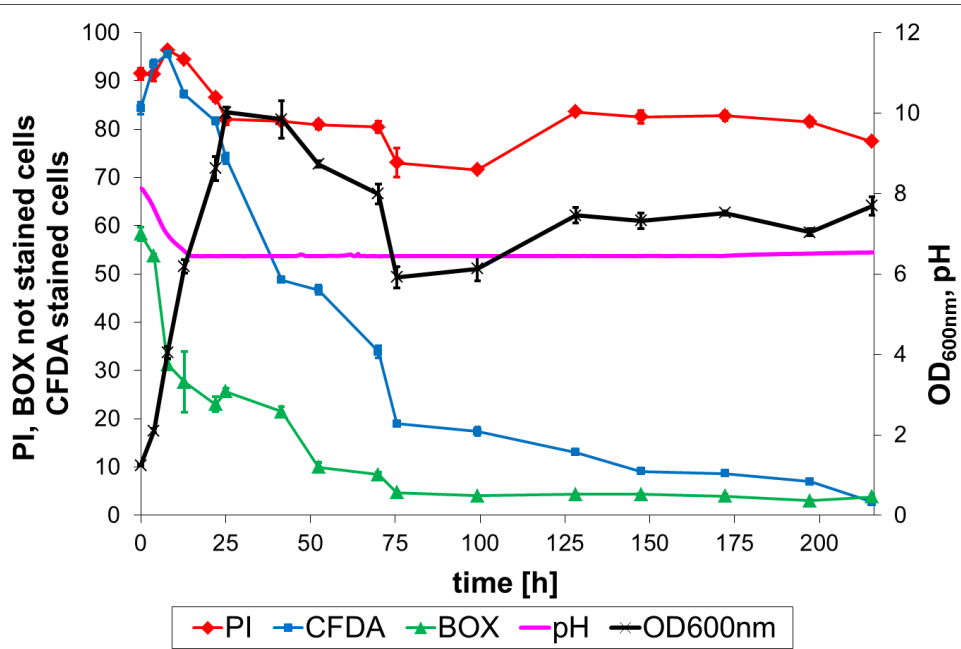
Exponential growth phase



Stationary growth phase

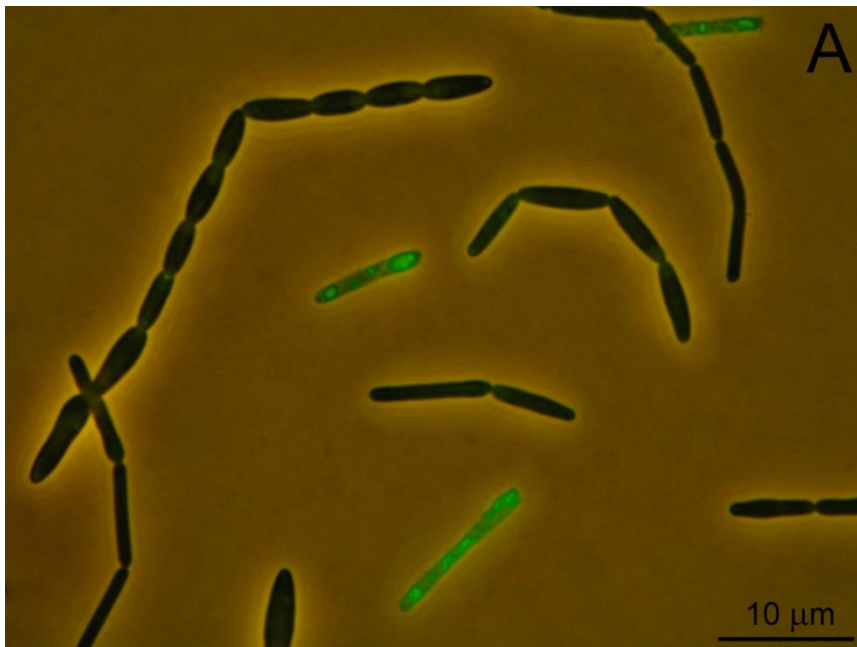


Monitoring metabolic activity of *C.tetanomorphum*

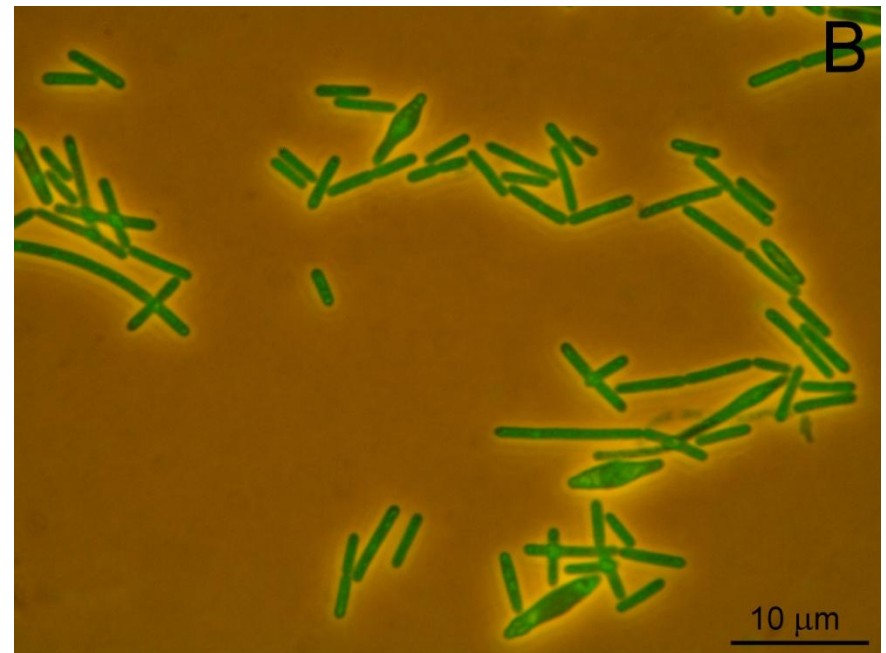


Development of method for estimation of metabolically active cells

Bisoxonol (BOX) was chosen from seven selected fluorescent probes, BOX stains depolarized (non-viable) cells with destroyed membrane potential

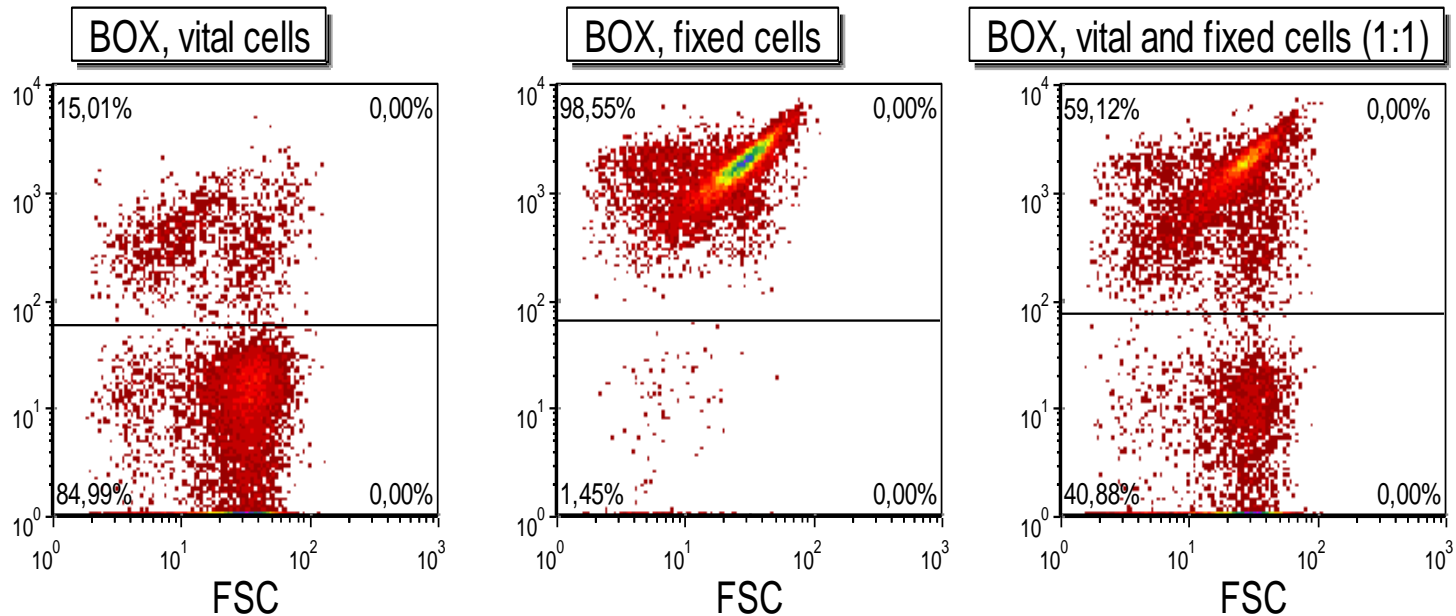


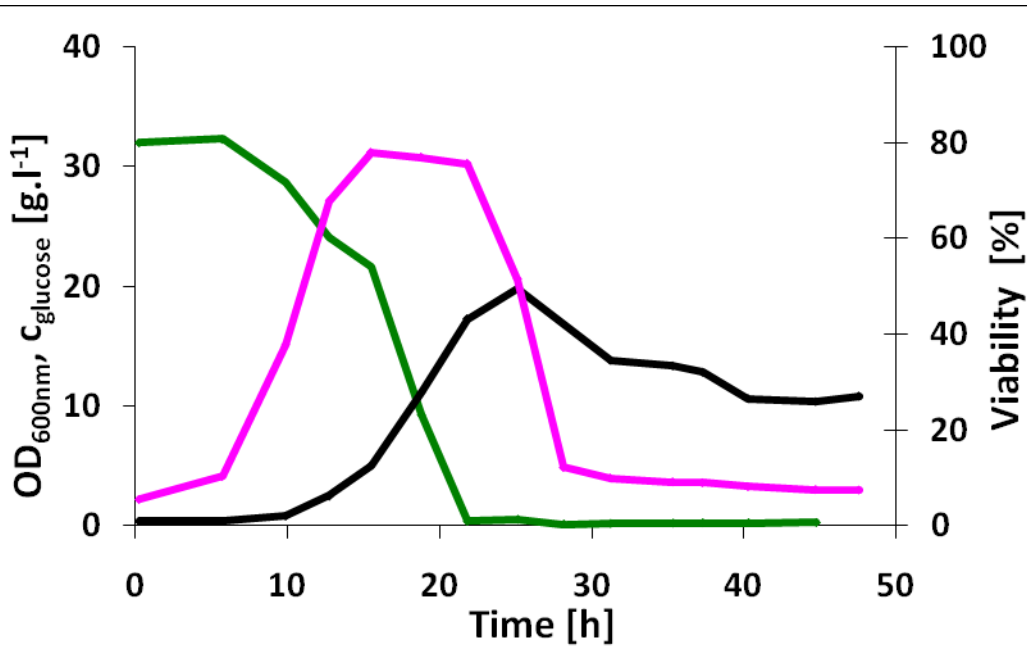
C.pasteurianum active cells



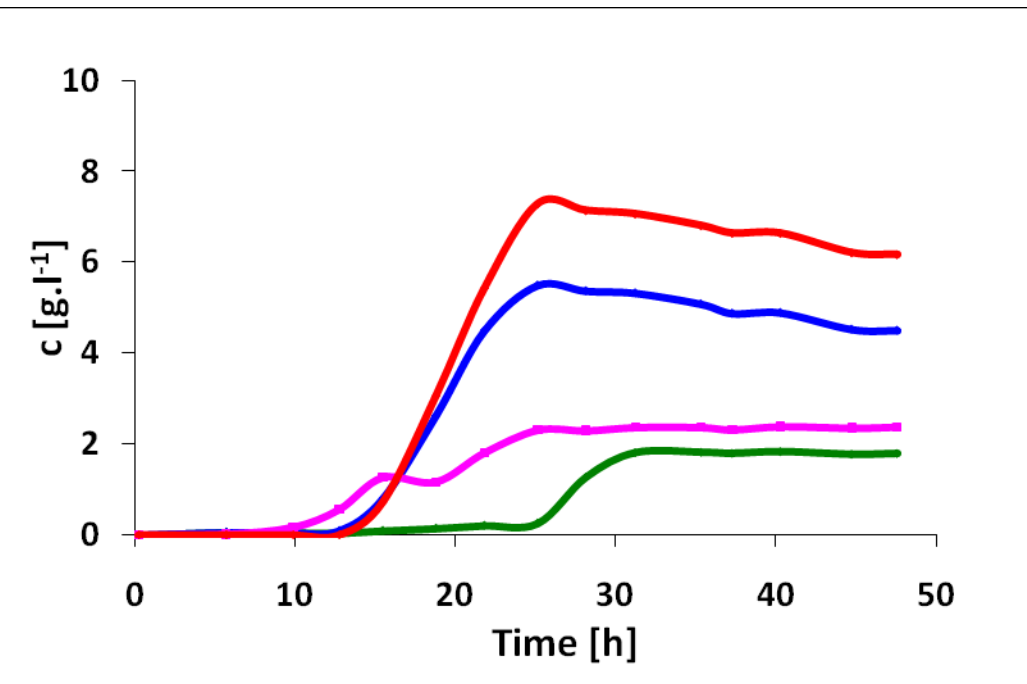
C.pasteurianum fixed (non-viable) cells

Dot-plot diagrams after BOX labelling of *C.pasteurianum* populations of active (1), fixed (2) and mixture of active and fixed cells (3)





Estimation of metabolically active cells in *C.pasteurianum* population during batch cultivation



- 1-butanol
- Acetone
- Ethanol
- Butyric acid

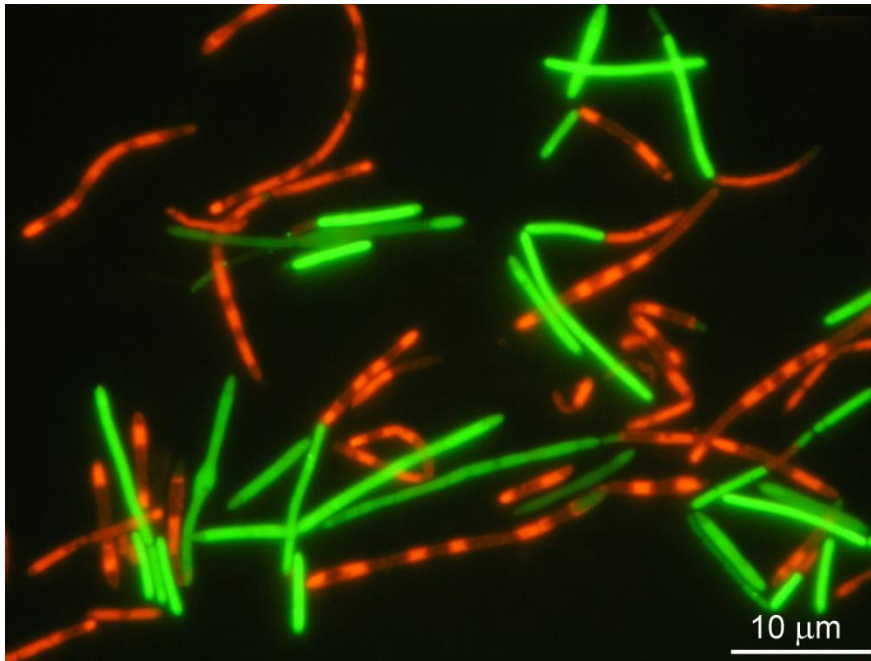
**Use of combination of dyes (propidium iodide (PI) +
carboxy fluorescein diacetate(CFDA))**



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PI - membrane integrity probe, stains non-viable cells

**CFDA labelling - esterases activity of cells - originally nonfluorescent stain
converted to fluorescent product**



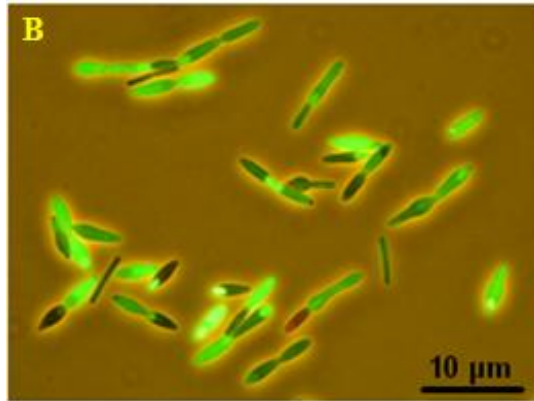
C.pasteurianum

PI stained (red) cells - non-viable, CFDA stained (green) cells – metabolically active

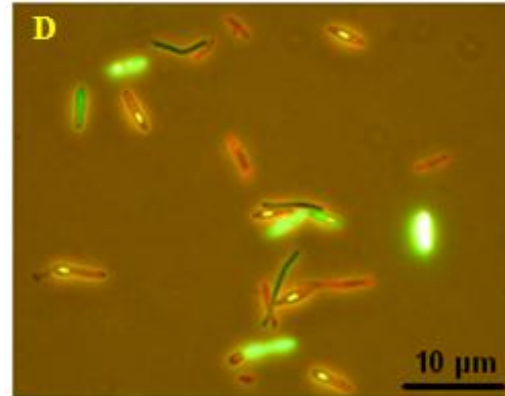
Combination of PI + CFDA for monitoring of metabolic activity of *C.beijerinckii* cells during batch fermentation



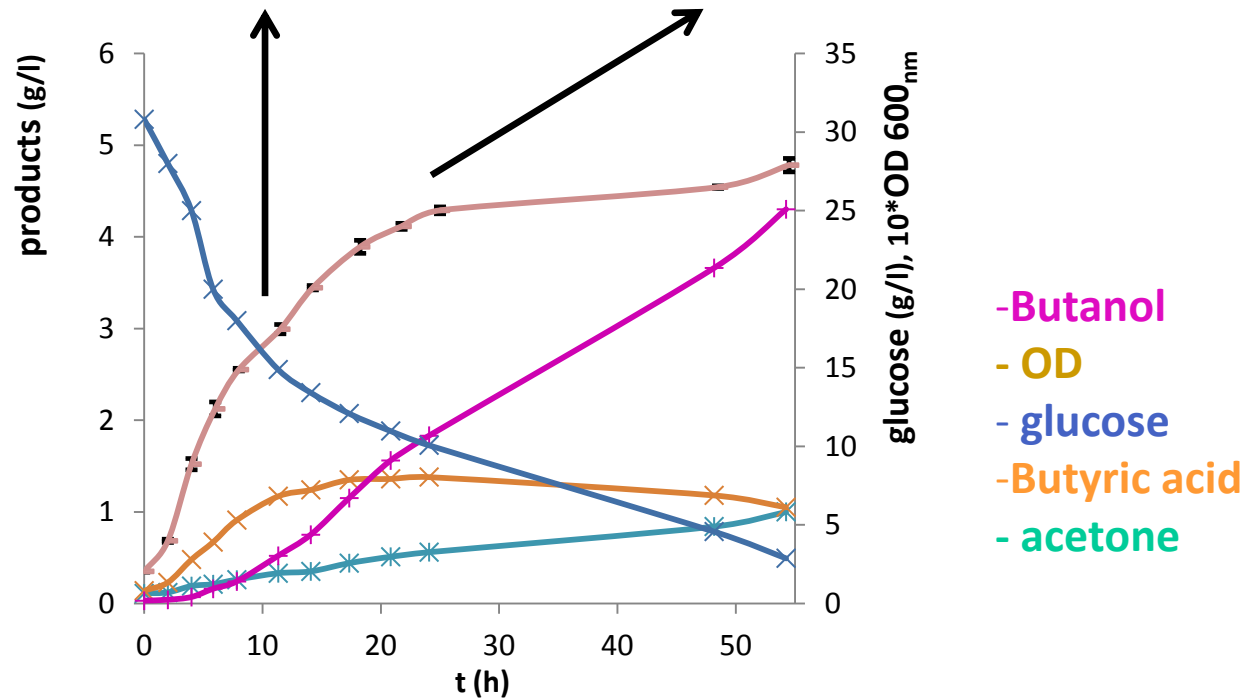
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High proportion of metabolically active cells



Low proportion of metabolically active cells



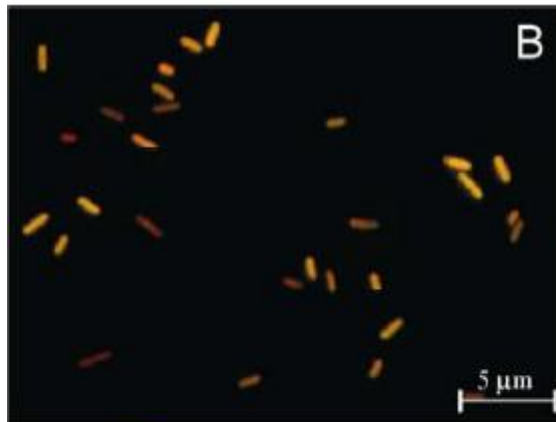
Fluorescent alternative of Gram staining (hexidium iodide + SYTO13)

Goals – to recognize metabolic phase, to monitor physiological state of bacteria

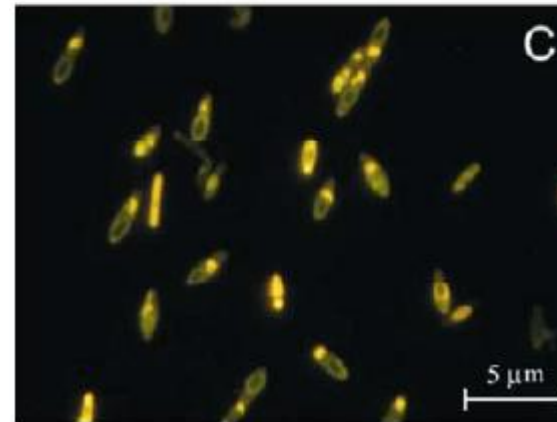
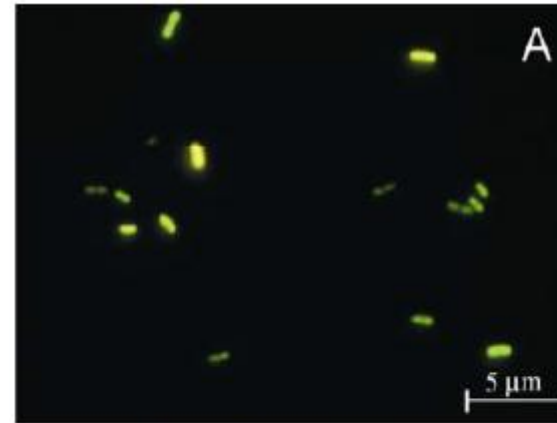
Gram positive

Gram negative

Bacillus megatherium



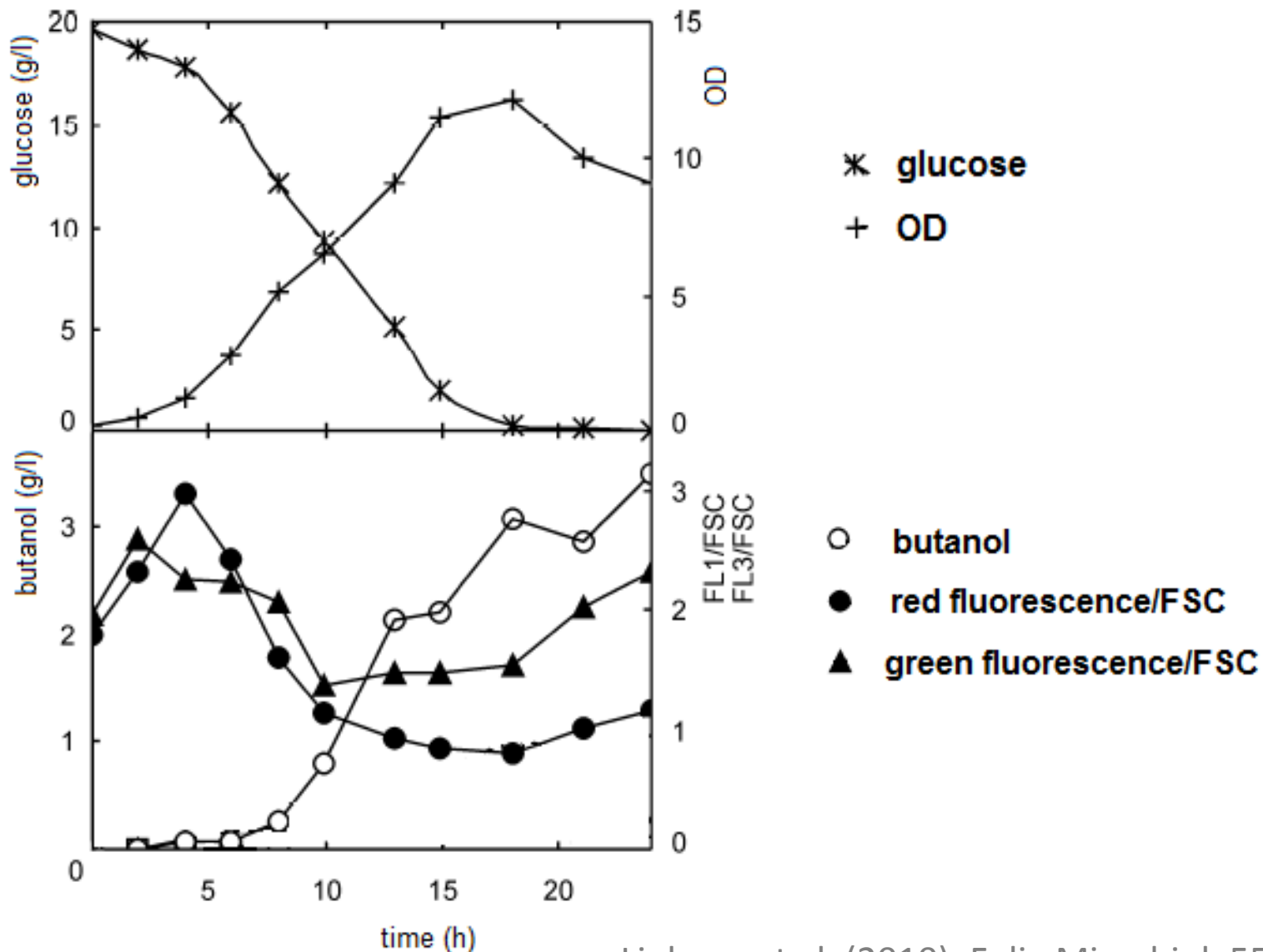
Escherichia coli



Clostridium pasteurianum

Batch fermentation with FC analysis (Gram staining)

C.pasteurianum



Conclusion

FC enables interesting insight to
clostridial population

but



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- ✓ Staining protocol must be tailored for particular solventogenic *Clostridium* strains.
- ✓ FC results must be evaluated carefully together with fluorescent microscopy and other characteristics.

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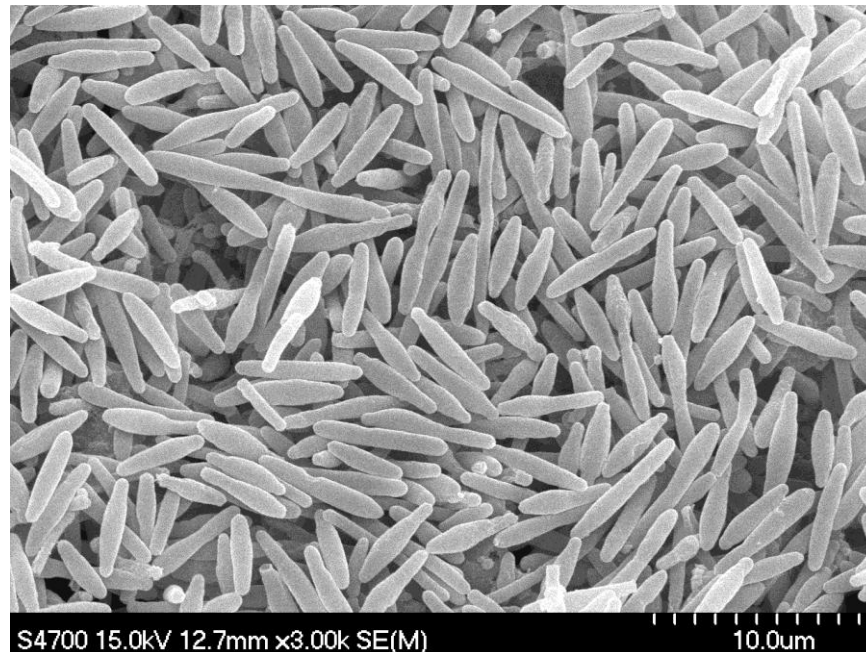
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Thank you for your attention

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Clostridium beijerinckii cells accumulating granulose