Investigation of hydrolytic microorganisms and enzymes for depolymerisation of recalcitrant biomass

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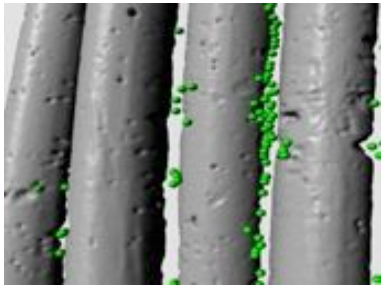
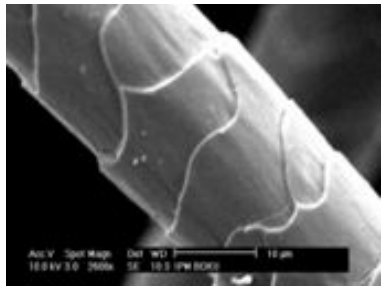
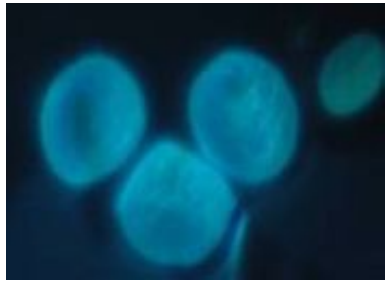
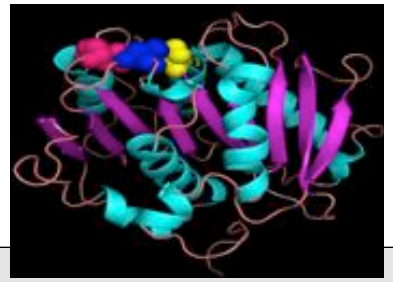
Graz University of Technology









Energy sources

Fukushima 2011

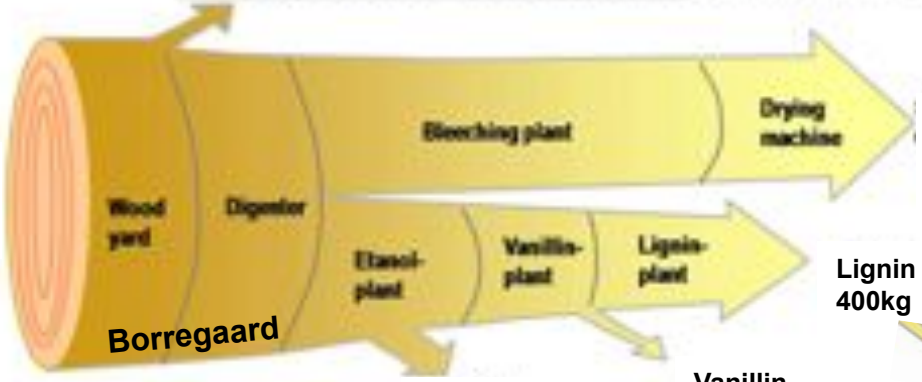
nuclear **fossil** **renewable**

The Biorefinery

Biogas etc.

Kudanga et al. *Biore Techno* 2010, 101:2793-2799.
Kudanga et al: *J Molecular Catal B:* 2009, 61:143–149
Kudanga et al: *J. Biotechnol*, 2010, in press
Kudanga et al: *Enz Microb Technol* 2010, 46:272-280.
Widsten et al: *Proc Biochem*, 2010, 45, 1072–1081
Kudanga et al: *Eng Life Sci* 2008, 8:297-302.
Prasetyo et al.. *Biores Technol* 2010, 101:5054–5062

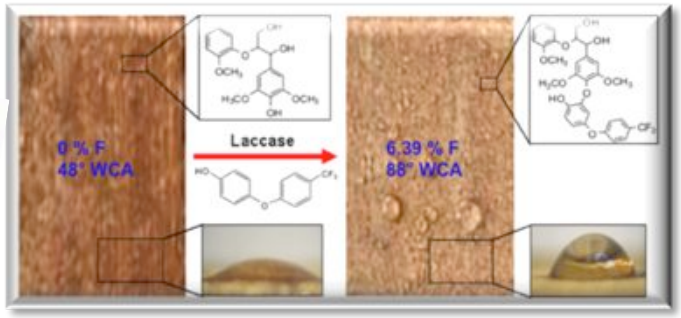
Wood
1000 kg



Cellulose 400kg

Functional materials

Lignin
400kg

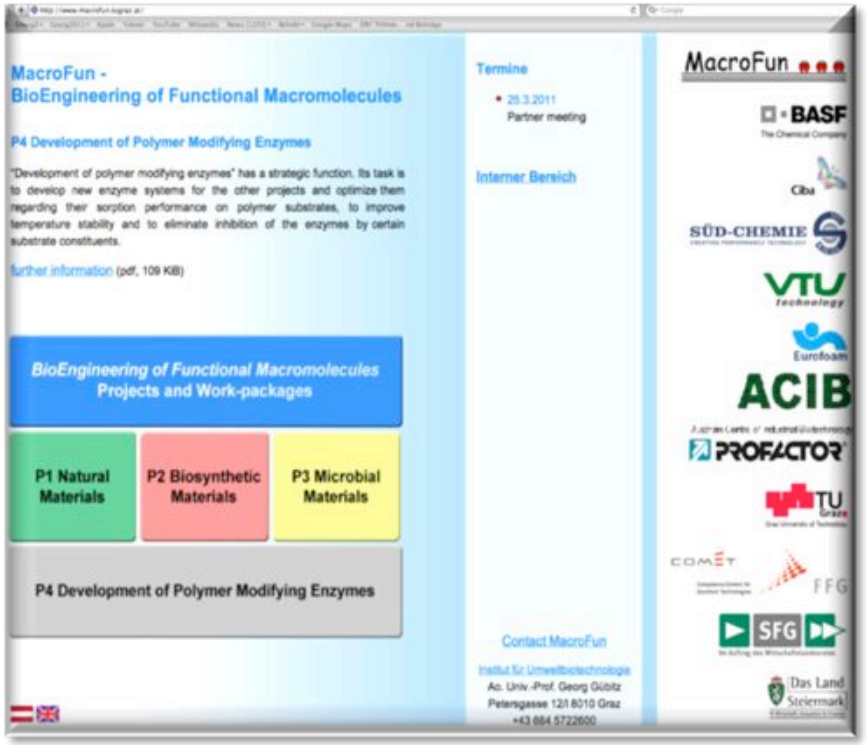


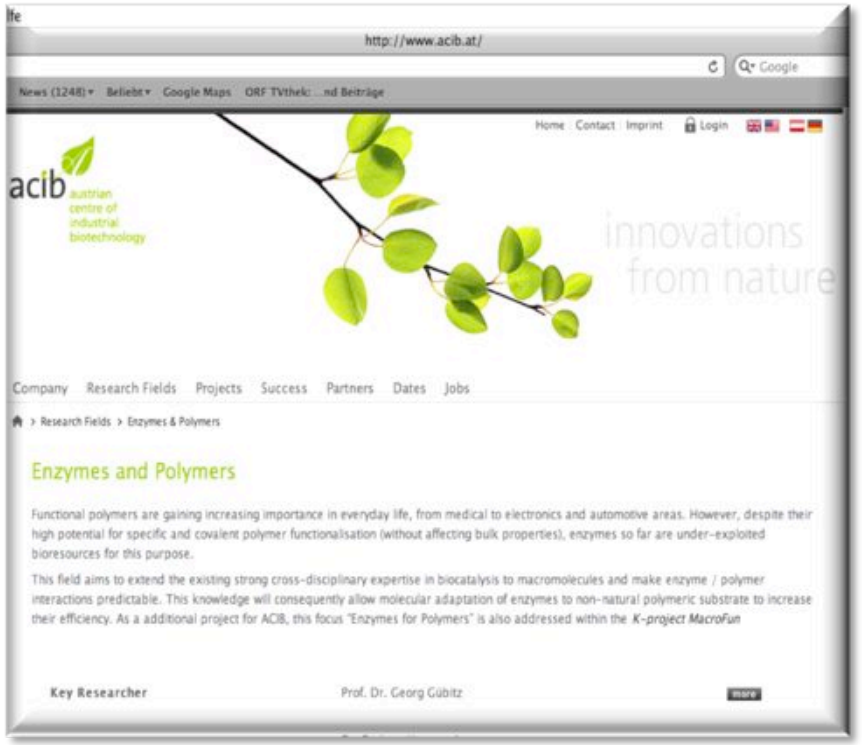
Ethanol
50kg

Vanillin
3kg

MacroFun and ACIB

K/K2 Comet Centres Industrial Biotechnology and Polymer Processing

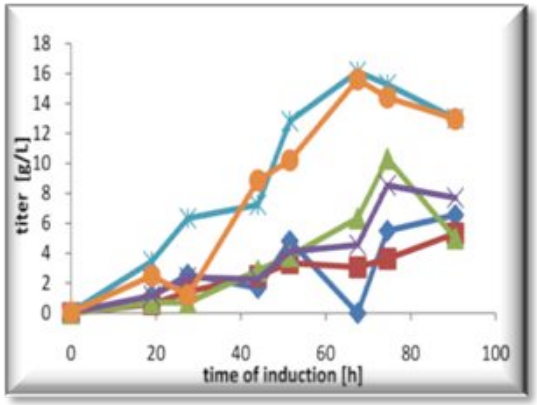


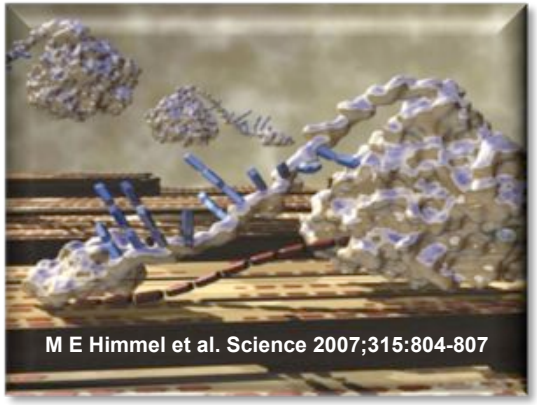


Funded project opportunities!

Progress

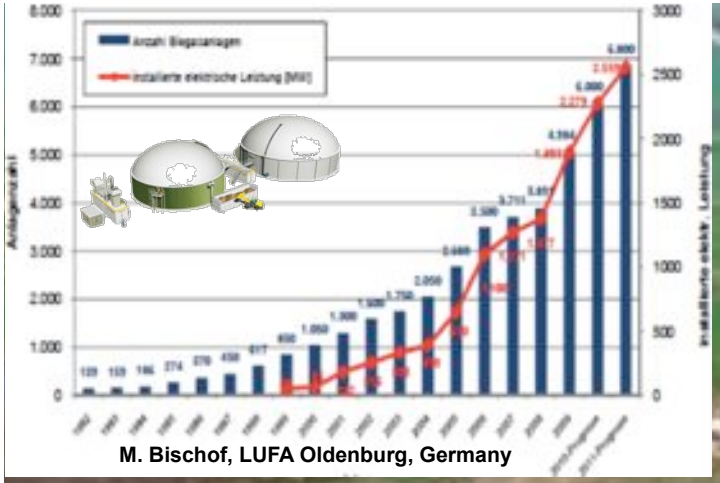
- Novel enzymes/organisms
  - Via MS sequencing of relevant proteins
- Enzymes successfully engineered
  - Towards higher stability
- Highest level of expression
  - of polymer modifying enzymes using
  - new promotor variants
  - efficient strain selection
  - activity base screening
- Factors limiting hydrolysis identified for
  - process engineering
  - enzyme engineering



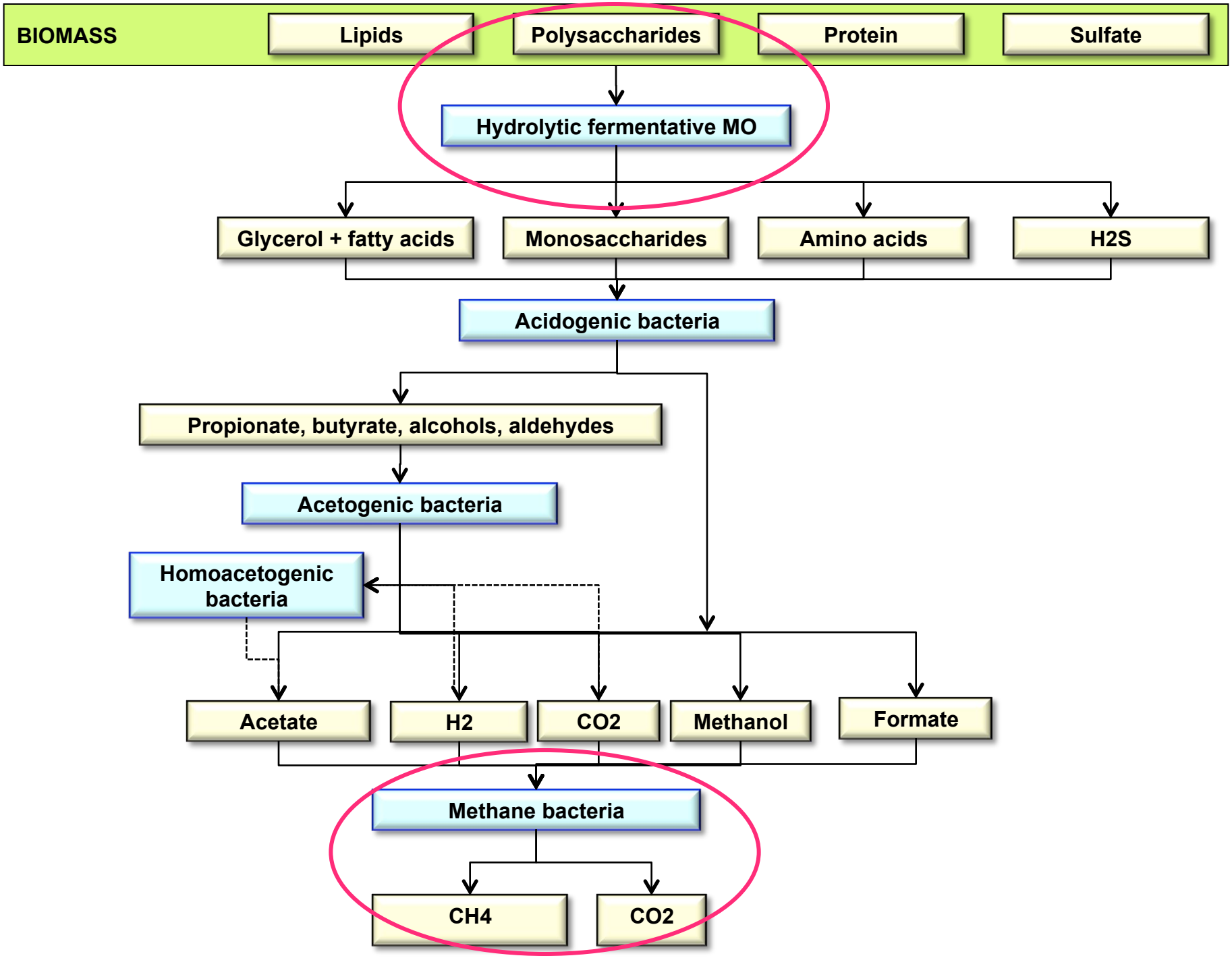


Biogas I

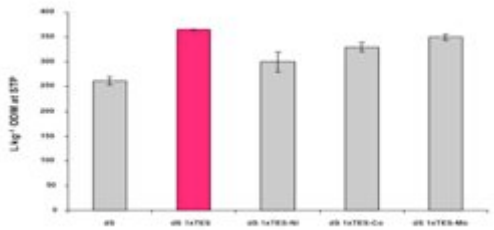
Biogas II


M. Bischof, LUFA Oldenburg, Germany

Germany 2011:
6800 plants
2600MW

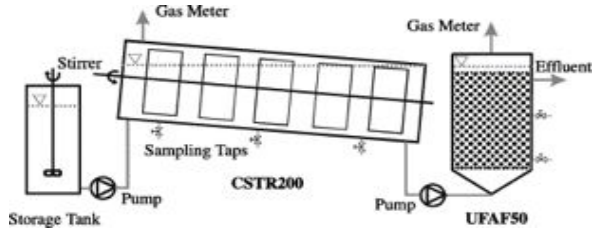

BIOMASS
Lipids
Polysaccharides
Protein
Sulfate
Hydrolytic fermentative MO
Glycerol + fatty acids
Monosaccharides
Amino acids
H2S
Acidogenic bacteria
Propionate, butyrate, alcohols, aldehydes
Acetogenic bacteria
Homoacetogenic bacteria
Acetate
H2
CO2
Methanol
Formate
Methane bacteria
CH4
CO2

Process improvement



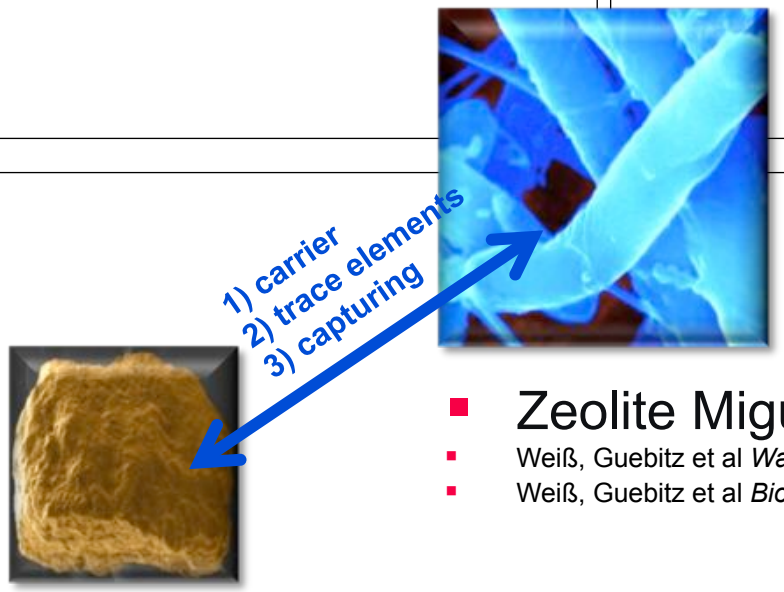
Trace elements

- Pobeheim, Guebitz et al. *Biores. Technol*. 101 (2010) 836–839
- Pobeheim, Guebitz et al, *Chemosphere* 80 (2010) 829–836
- Pobeheim, Guebitz et al, *Water Res.* 45 (2011) 781-788



Reactor design

- Staubmann, Guebitz et al. *Appl Bioch. Biotech* 63 (1997) 457
- Held, Guebitz et al. *Biores Technol* 81 (2002) 19-24



Zeolite Migulators

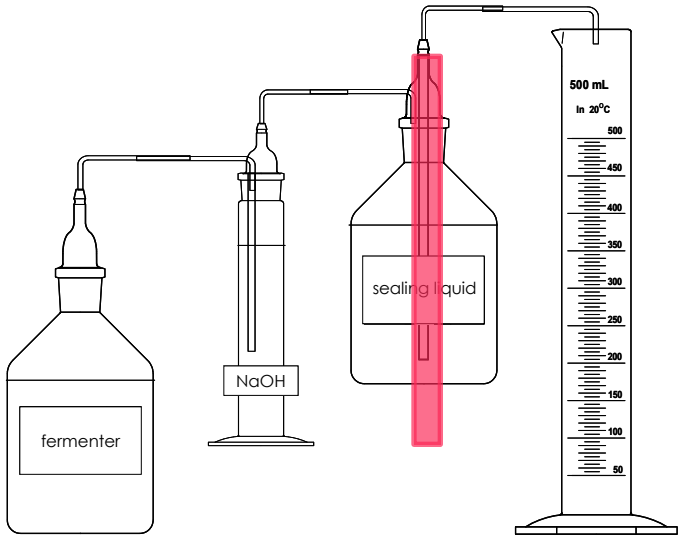
- Weiß, Guebitz et al *Water Res* 2010, 44:1970-198
- Weiß, Guebitz et al *Biores technol* (2011) in press

Experiments

Gras silage

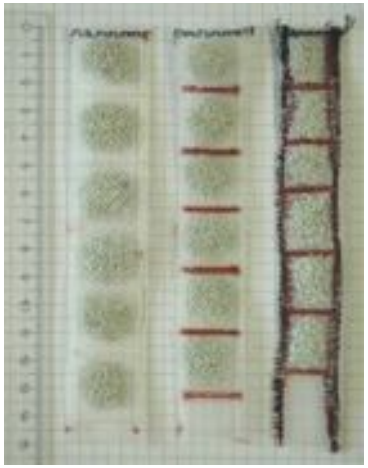
Model substrate:

SSCP/ADRA analysis: Hemicellulose hydrolysing Methanoculleus sp. (Pobeheim et al, Chemosphere 80 (2010) 829–836)



compounds	composition [%]	amount per 1 g	amount per 5 g
cellulose	16.7	0.334	1.67
xylan	25.8	0.516	2.58
lignin	7.2	0.144	0.72
pectin	0.5	0.01	0.05

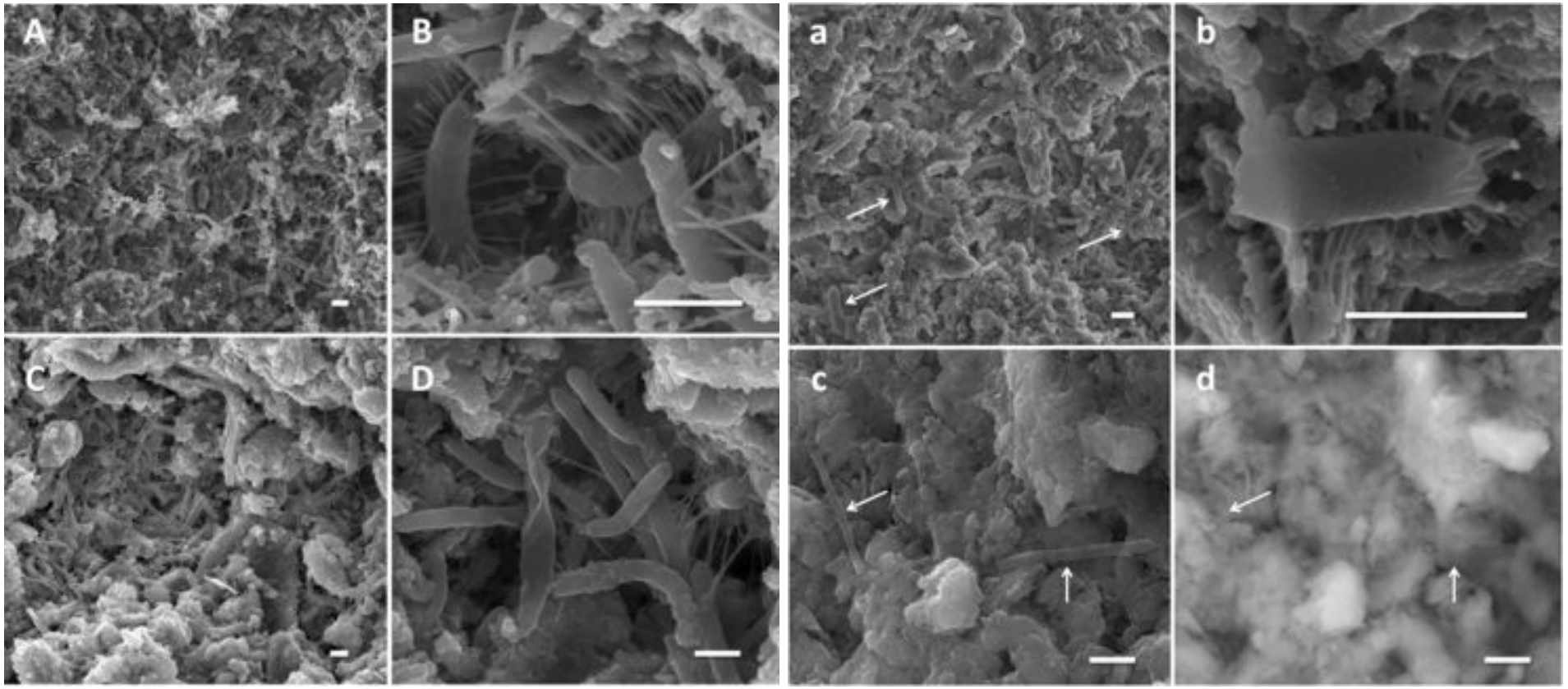
***in sacco* technique** for **continuously operated bioreactors** (LfL Institute)



SEM analysis

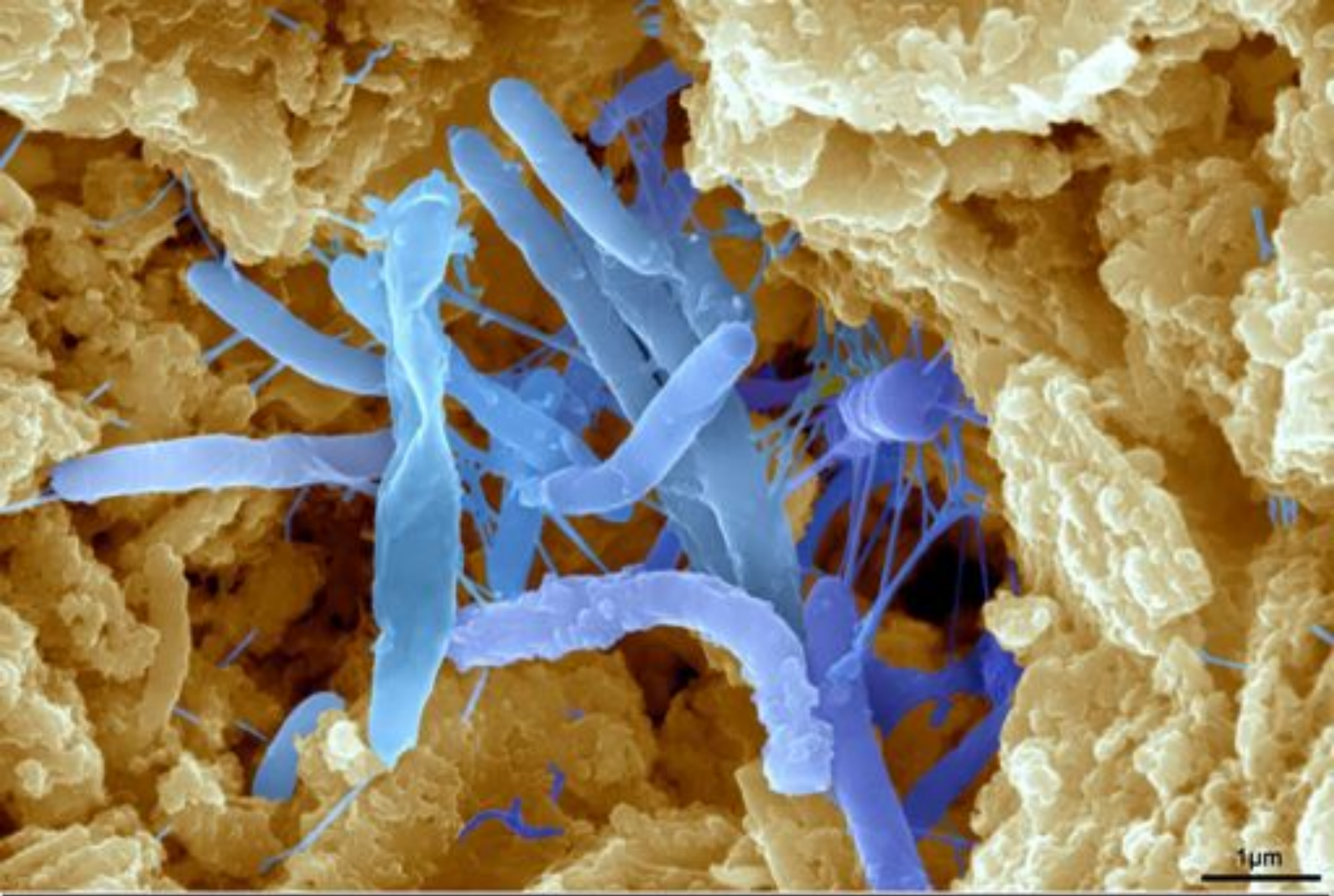
A CPD dried zeolite with area-wide biofilm formation
B CPD dried zeolite, cells forming fibrous structures
C Lyophilised zeolite with biofilm formation mainly in a pit
D Lyophilised zeolite, microorganisms colonising a pit

a Zeolite with biofilm formation
b Microorganism with fibrous structures on the surface of a zeolite
c, d Two rod-shaped cells, vanishing when AsB detector is used
(Bar length: 1 µm)



5 d batch-wise cultivation on a model substrate for grass silage at 45°C

84 d *in sacco* incubation in continuously operated bioreactors (28 l) fed with grass silage at 45 °C



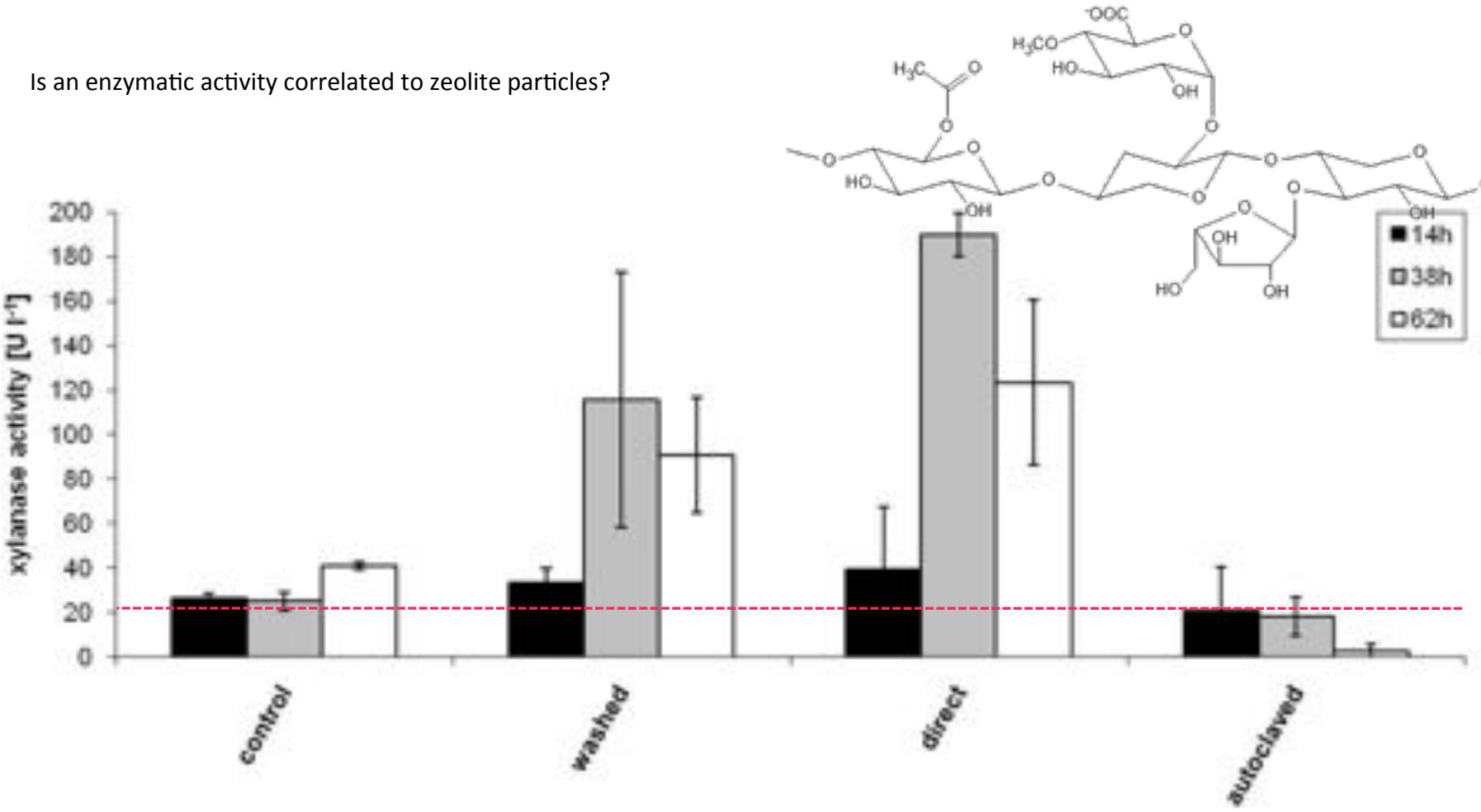
Scanning electron microscopic image of a zeolite particle (clinoptilolite) colonized by bacteria
from: *Investigation of activated zeolites as carriers in anaerobic biogas production processes*
Weiß S., Zankel A., Petrak S., Somitsch W., Guebitz G.M. (2010)

TU Graz

emze

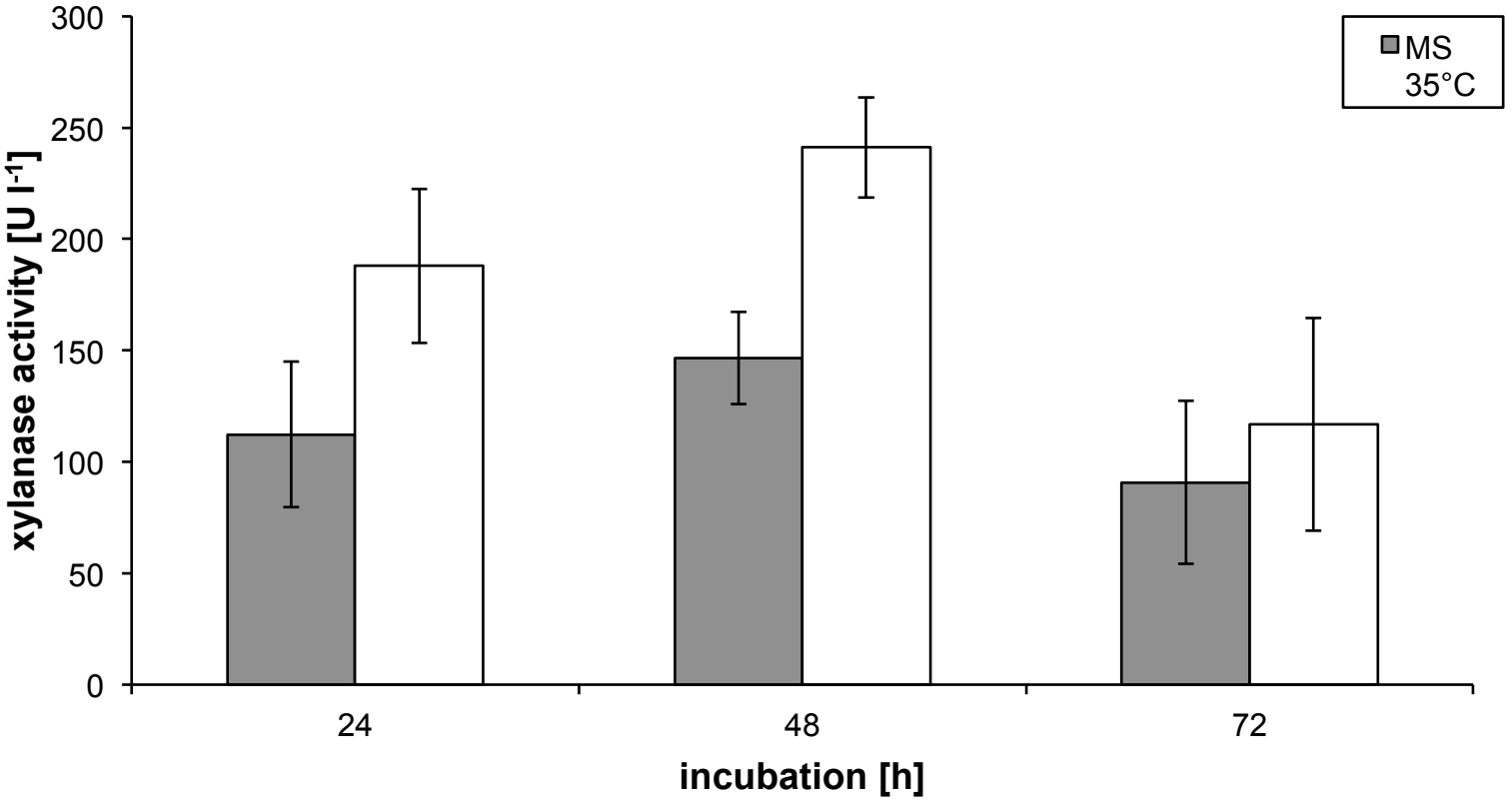
xylanase re-activation on zeolites

Is an enzymatic activity correlated to zeolite particles?



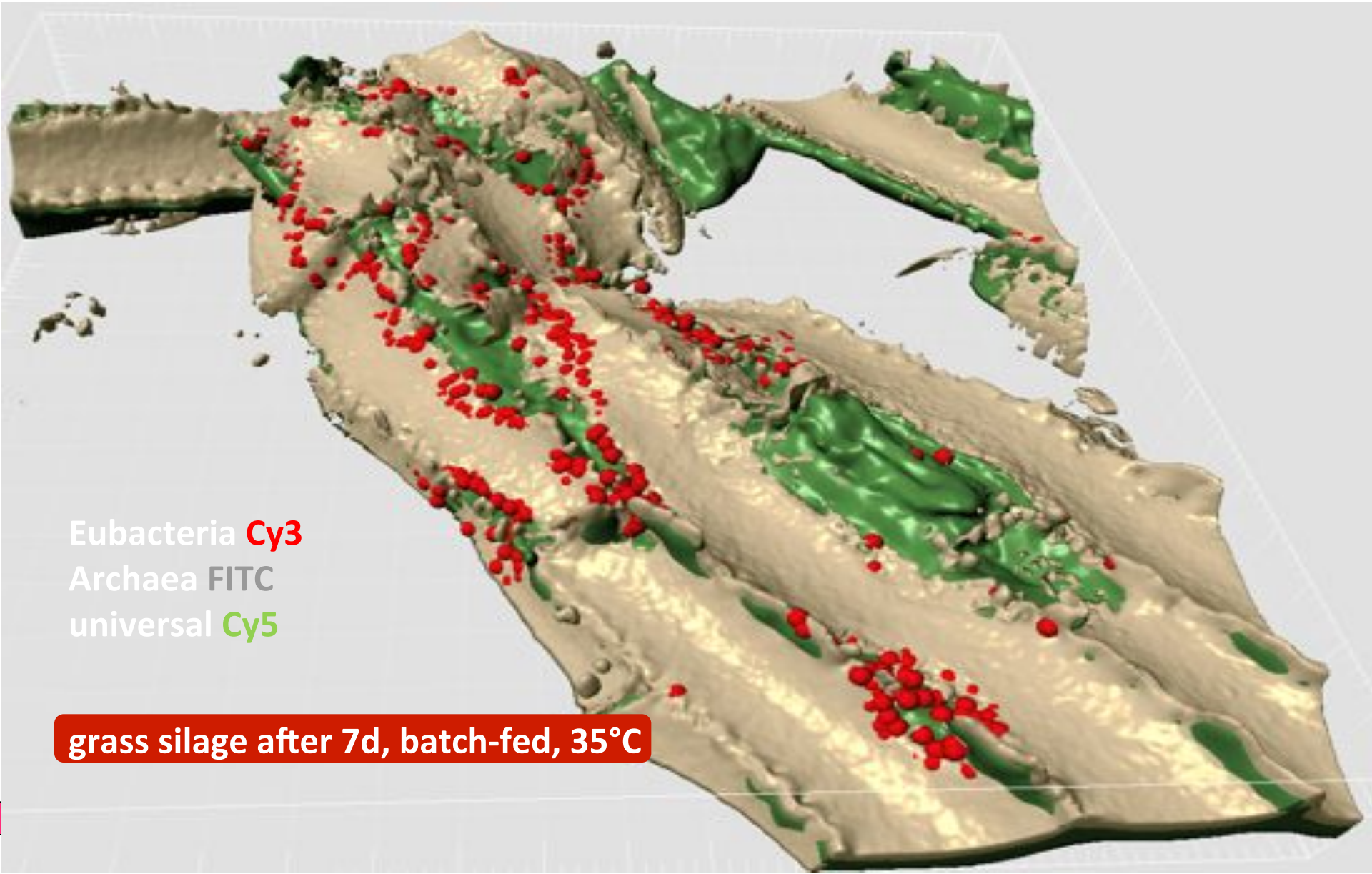
zeolite particle size 1.0-2.5mm / batch-cultivation on model substrate 14d, 35°C

zeolite correlated enzyme activities

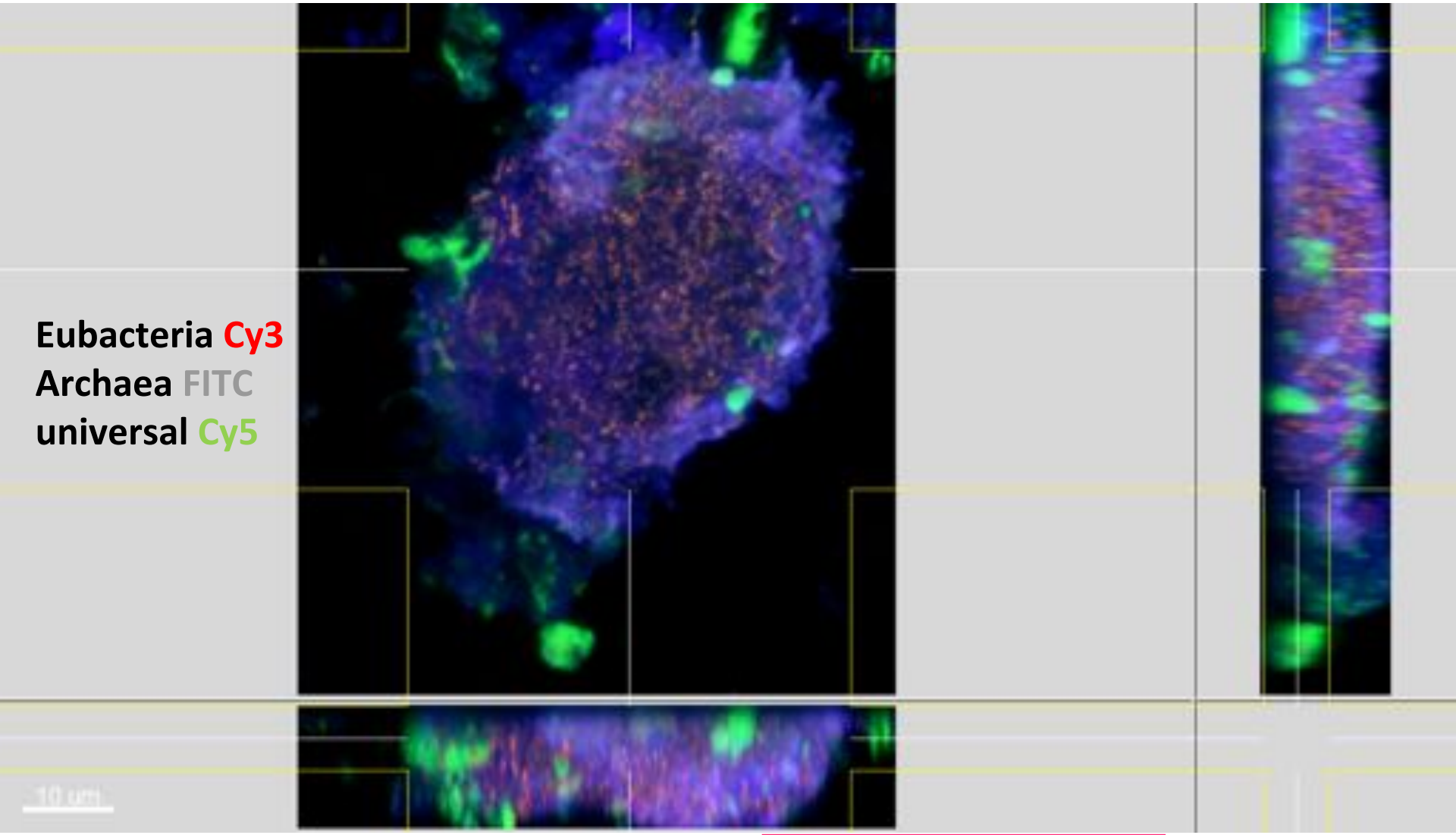


zeolite particle size <100µm / batch-cultivation on model substrate, 35°C/45°C

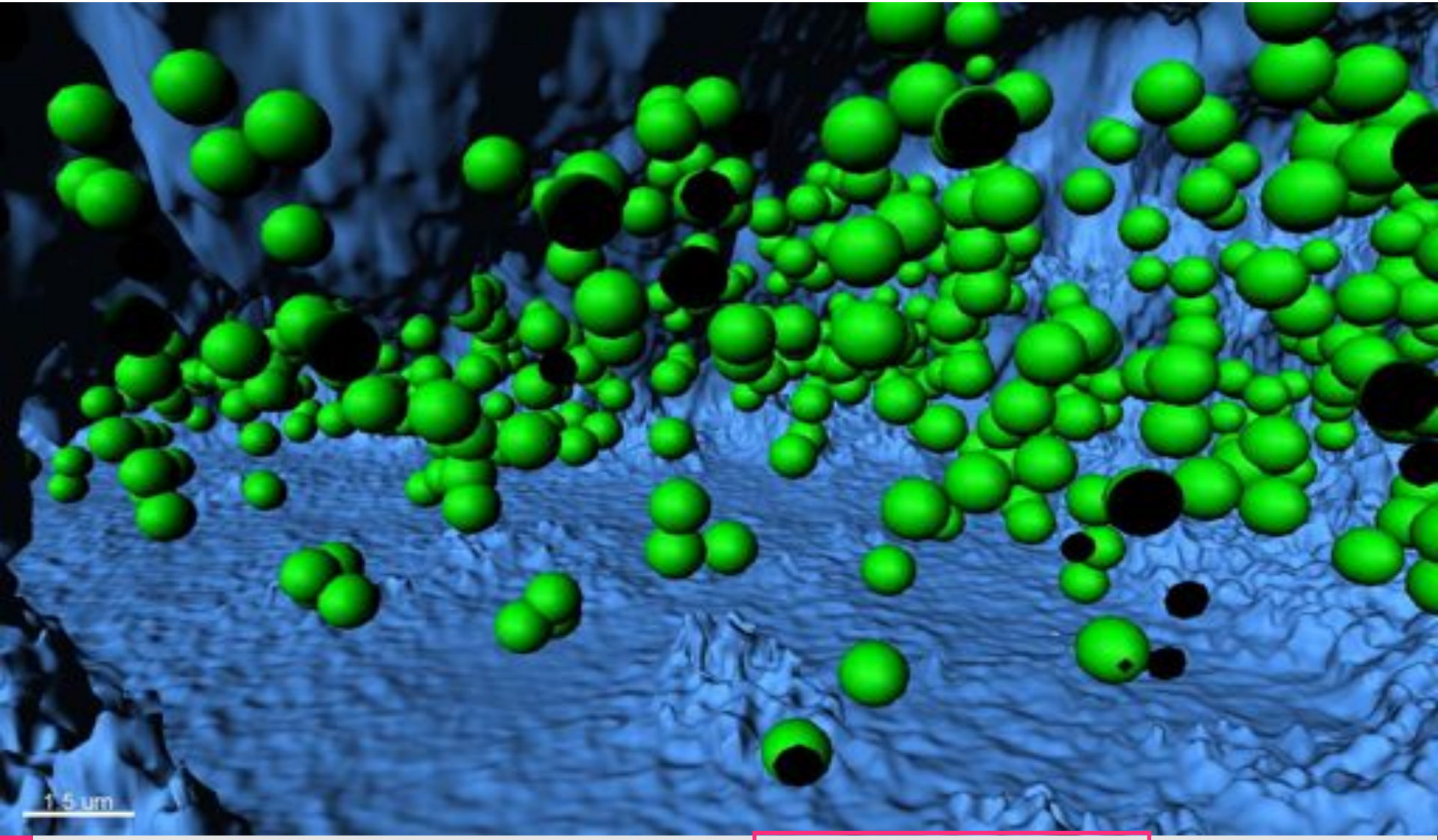
CLSM of (hemi)cellulolytic bacteria



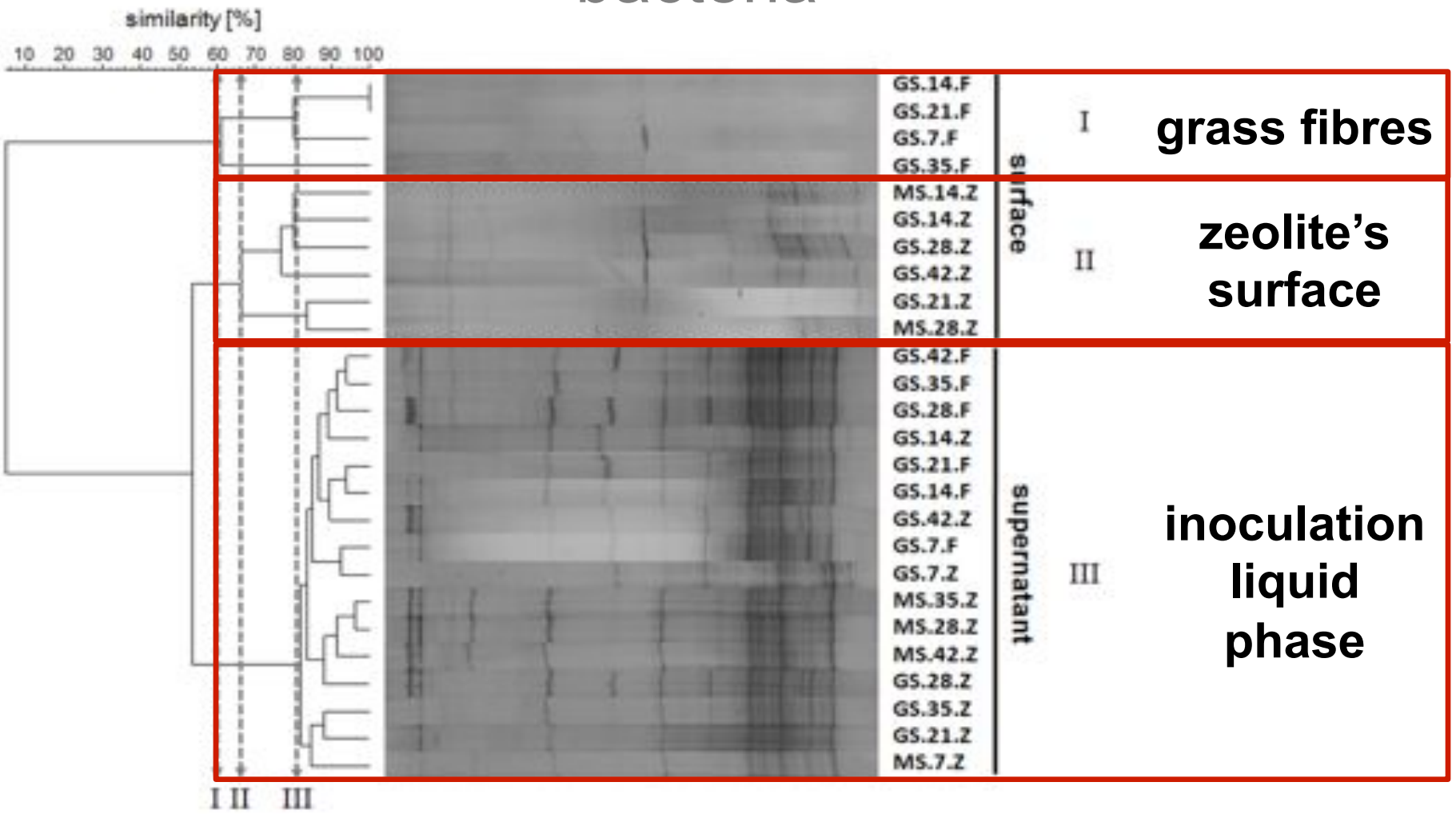
CLSM of zeolite particle <100µm ø



zeolite particle+bacteria



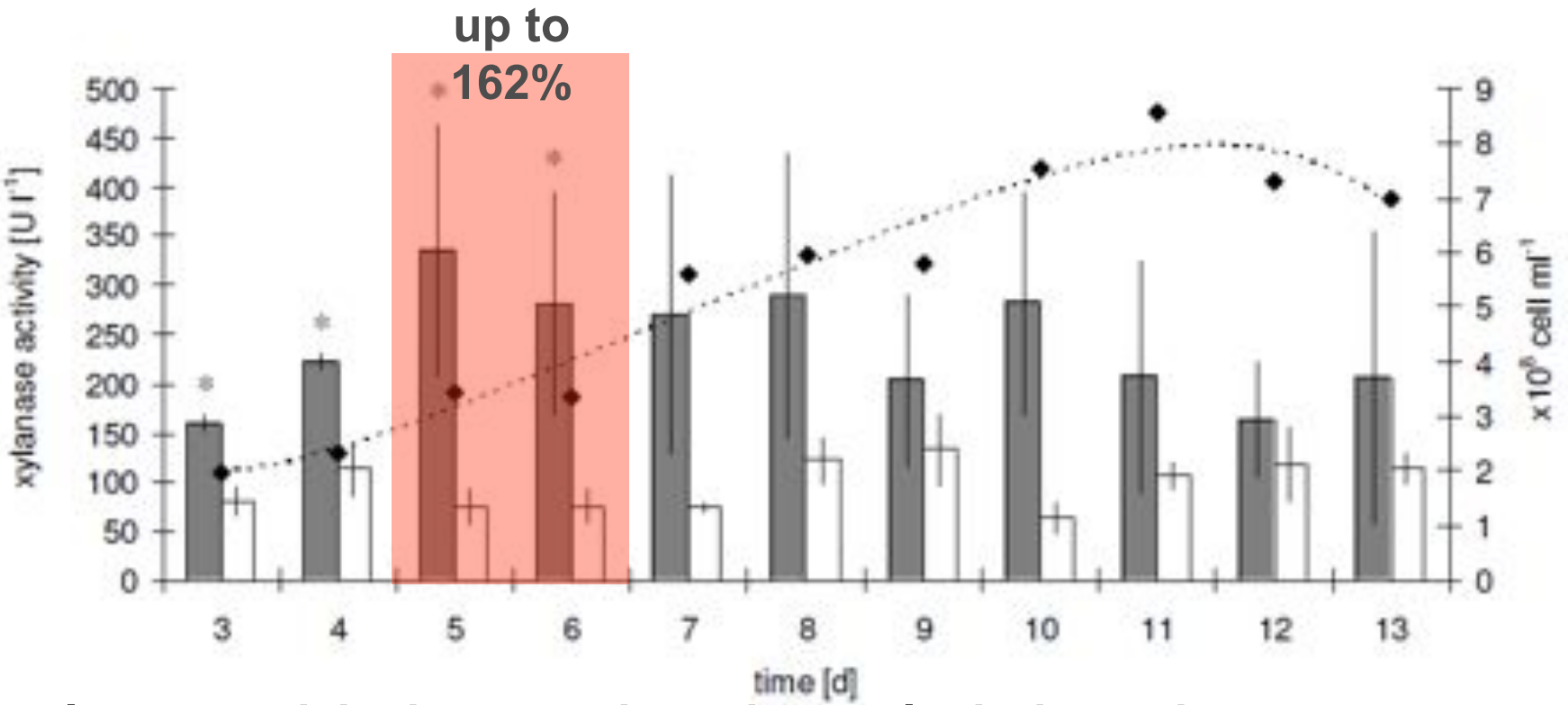
PCR-SSCP-Clustering : *rrs* bacteria



Sequencing : *rrs* bacteria/archaea

sample site	inoculation time [d]	organism	phylum/order*	sequence identity [%]	accession no.
zeolite's surface grass fibres	14-42	*Ruminofilibacter xylanolyticum*	Bacteroidetes	98-99	DQ141183 EU551120
zeolite's surface grass fibres supernatant	14-42	uncultured bacterium	Thermotogae	98	CU924654 CU919517
supernatant	28	*Bacillus* sp.	Firmicutes	97	AF548884
zeolite's surface	7-42	*Methanocarcina barkeri*	*Methanosarcinales	89	AF028692
zeolite's surface	7-42	uncultured archaeon	*Methanomicrobiales	94	AB479397
zeolite's suface supernatant	21	*Methanoculleus* sp.	*Methanomicrobiales	91	AF107105 AJ550158
zeolite's suface	21	*Methanoculleus bourgensis*	*Methanomicrobiales	91	AY196674 DQ150254

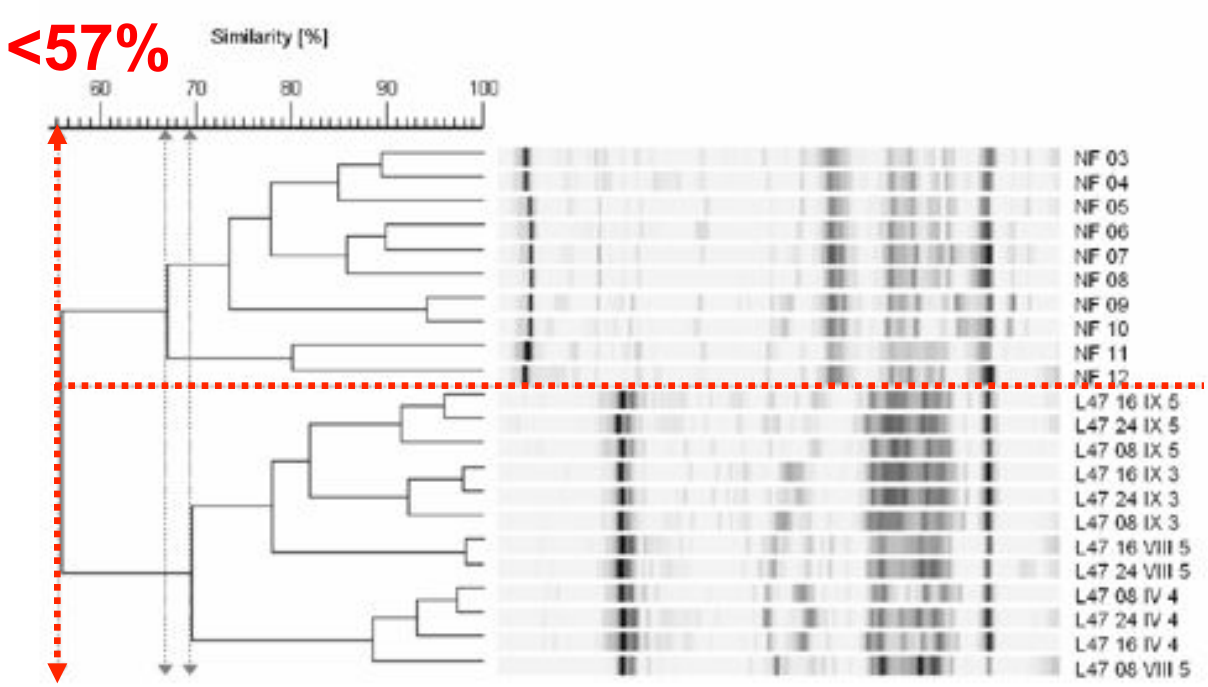
Enrichment of enzyme producers

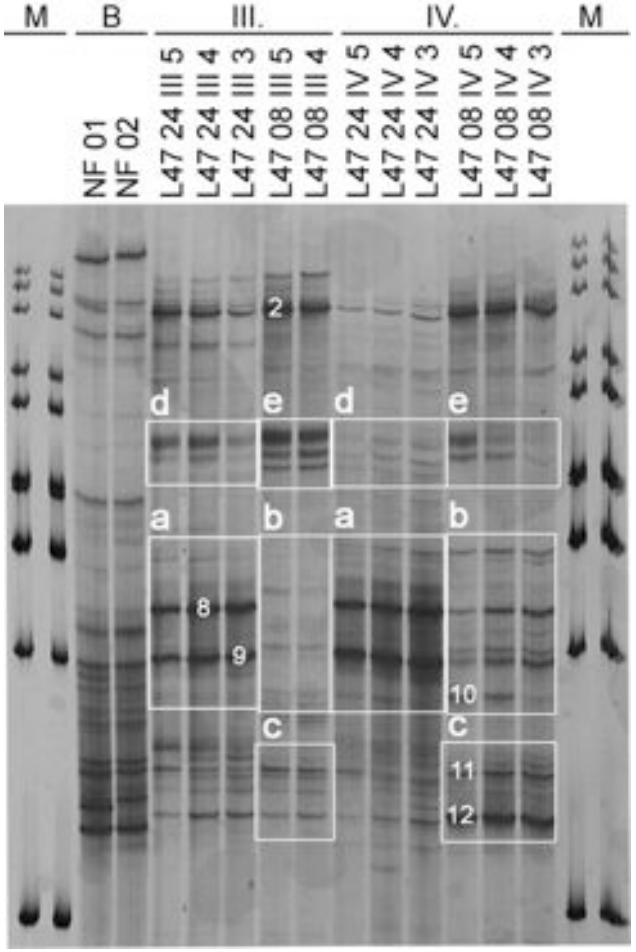


xylanase activity increased continuously during subsequent enrichment cycles significantly higher ($P < 0.05$) when compared to the origin seeding sludge

•Weiß et al *Water Research* 2010, 44:1970-1980.

Enriched strains

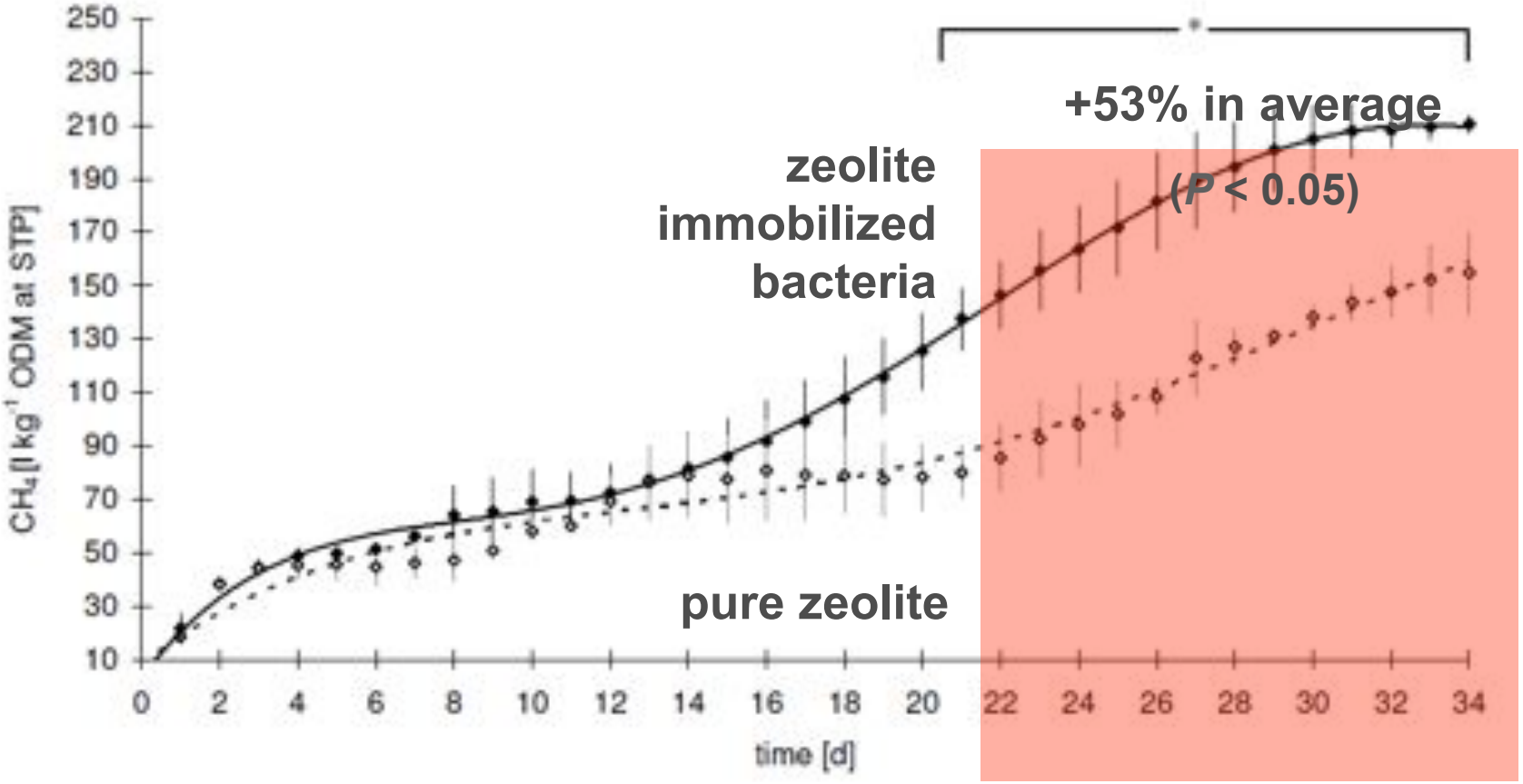




***Bacteroides sp.* (*B. succinogenes*, *B. ruminicola*)**
***Clostridium sp.* (*C. beijerinkii*, *C. diolis*, *C. butyricum*)**
Azospira oryzae

•Weiß et al *Water Research* 2010, 44:1970-1980.

CH4-yield in batch-experiments



•Weiß et al *Water Research* 2010, 44:1970-1980.

Conclusions

- More efficient (hemi)cellulolytic enzymes via
  - Screening
  - Protein engineering
  - Novel expression systems
  - Improved process conditions
- Specific populations develop on zeolite (SSCP, CLSM)
- (Hemi)cellulase producers enriched
- (Hemi)cellulase producers immobilsed on zeolite


Thanks to
FFG IPUS, LfL
and the microbes...
1µm