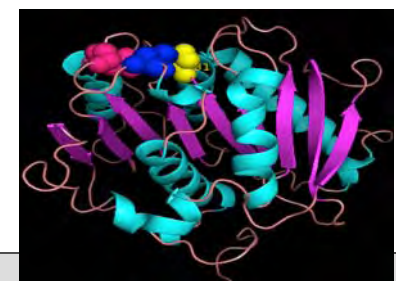
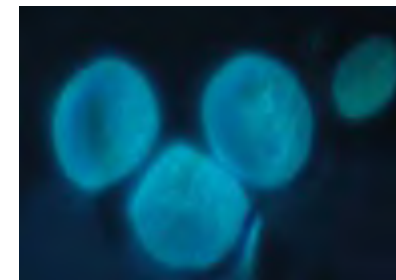
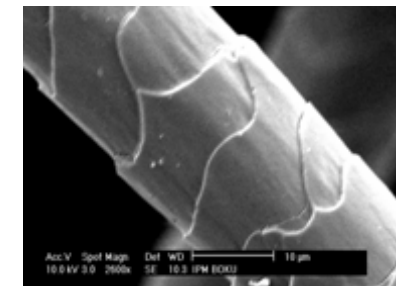
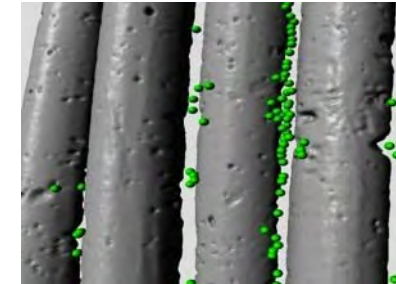


# Investigation of hydrolytic microorganisms and enzymes for depolymerisation of recalcitrant biomass

**Stefan Weiß & Georg M. Guebitz**

***Graz University of Technology***



# Energy sources



Fukushima 2011



**nuclear**



**fossil**



**renewable**

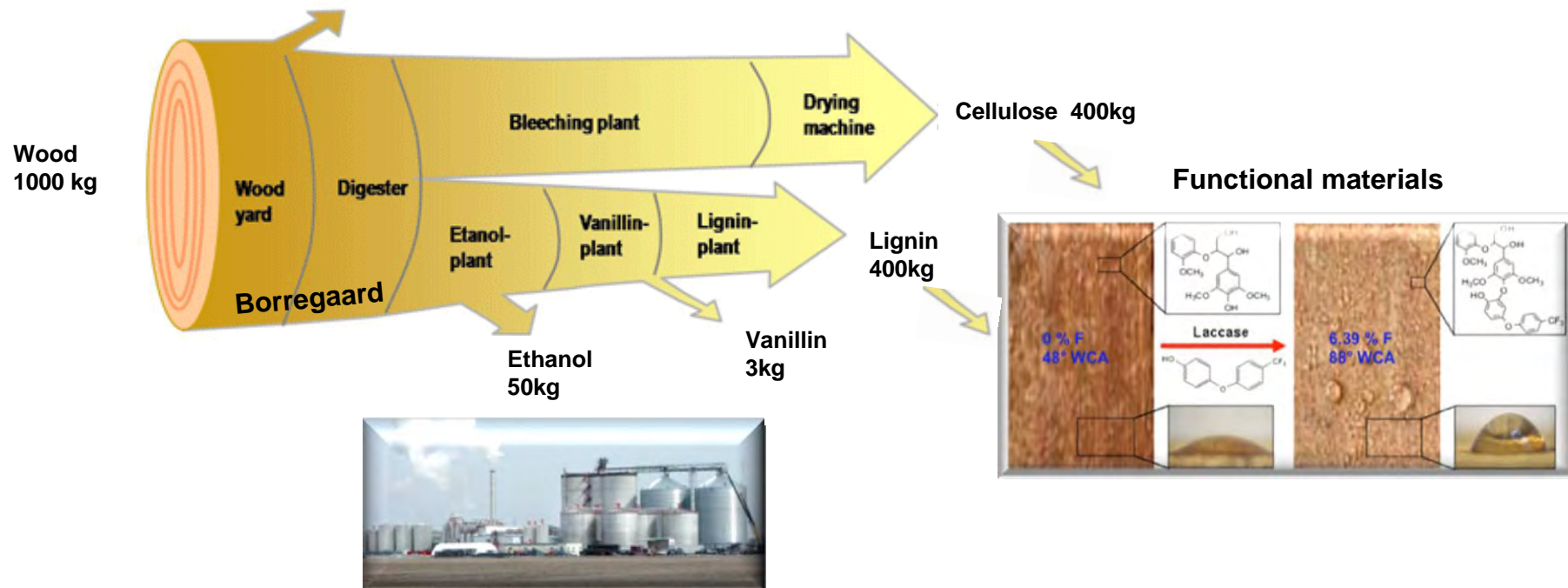


# The Biorefinery



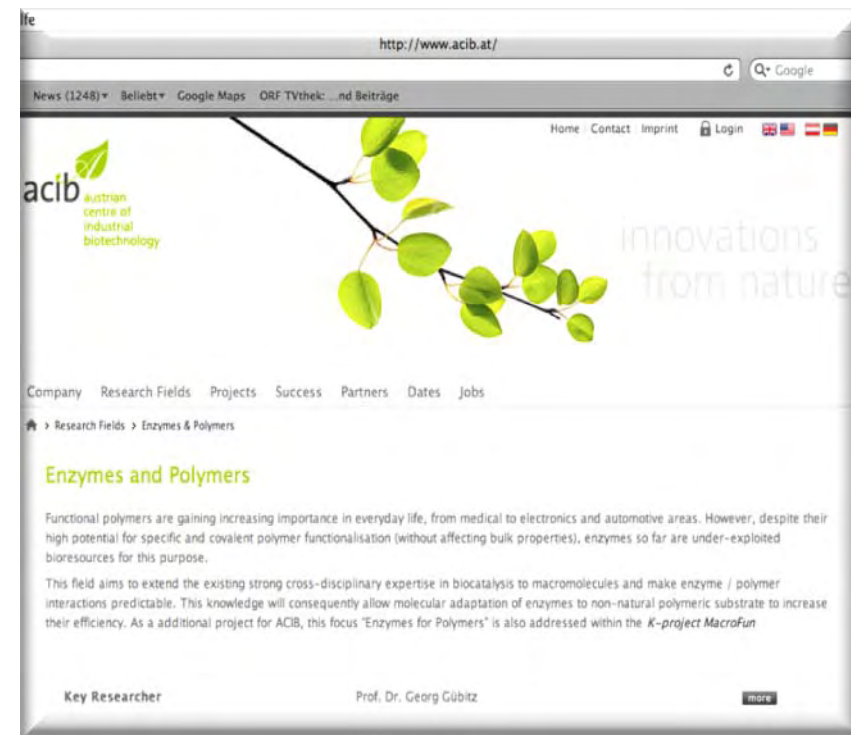
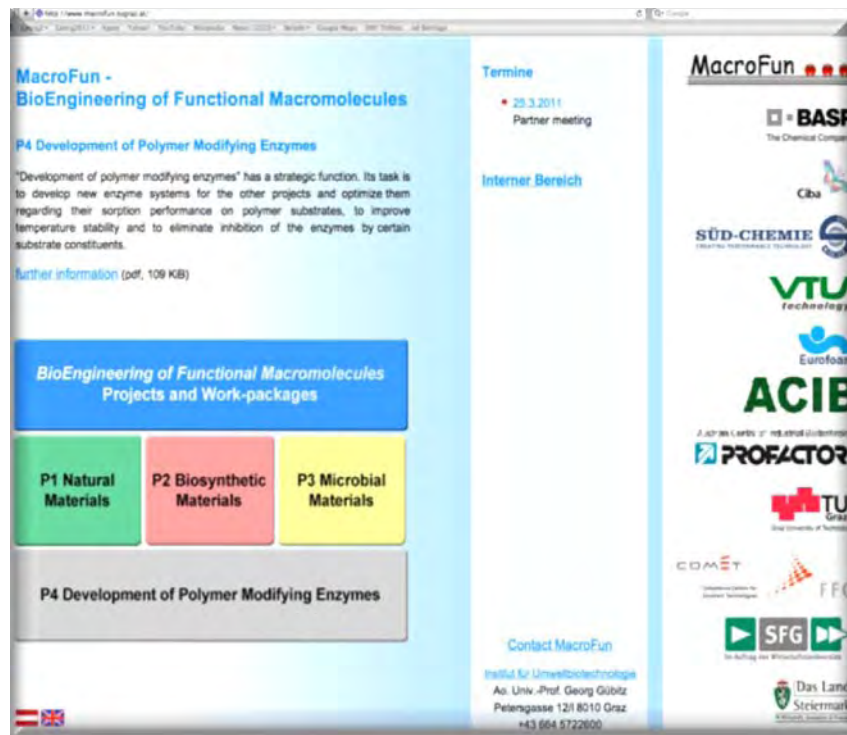
**Biogas etc.**

Kudanga et al. *Biore Technol* 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



# MacroFun and ACIB

K/K2 Comet Centres Industrial Biotechnology and Polymer Processing



Funded project opportunities!

introduction

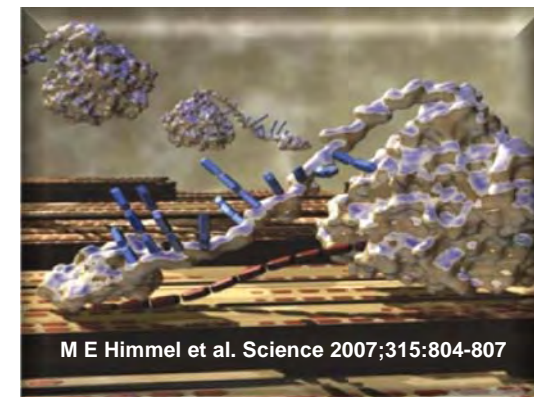
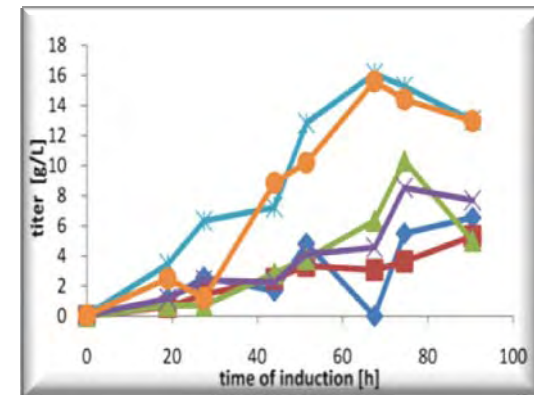
novel lignocellulolytic enzymes

hydrolysis in the biogas process

conclusions

# Progress

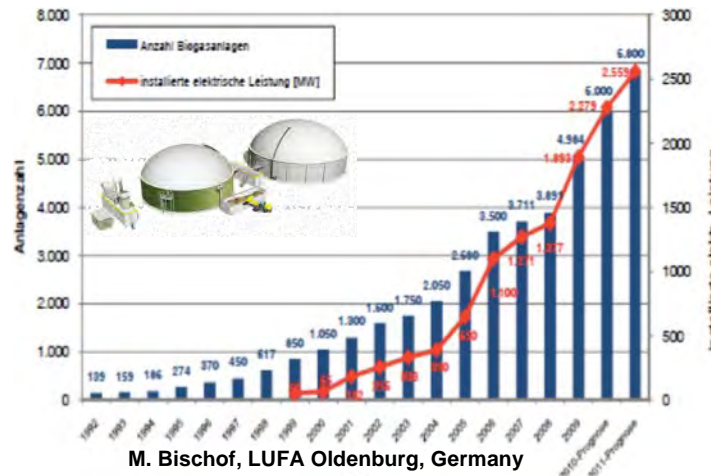
- Novel enzymes/organisms
  - Via MS sequencing of relevant proteins
- Enzymes successfully engineered
  - Towards higher stability
- Highest level of expression
  - of polymer modifying enzymes using
  - new promotor variants
  - efficient strain selection
  - activity base screening
- Factors limiting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



# Biogas I



# Biogas II



Germany 2011:  
6800 plants  
2600MW

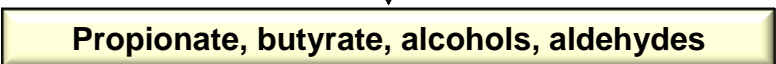




Hydrolytic fermentative MO

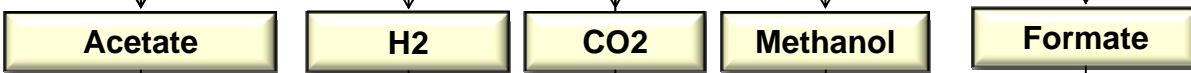


Acidogenic bacteria



Acetogenic bacteria

Homoacetogenic bacteria

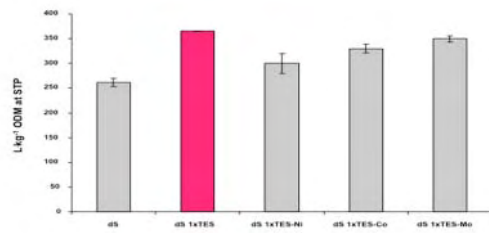


Methane bacteria



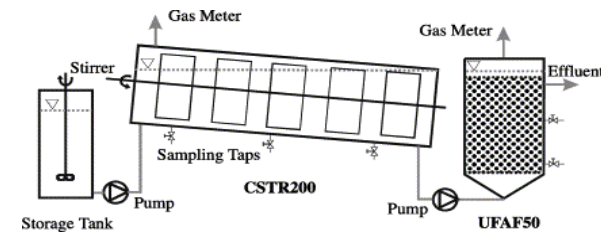


# Process improvement



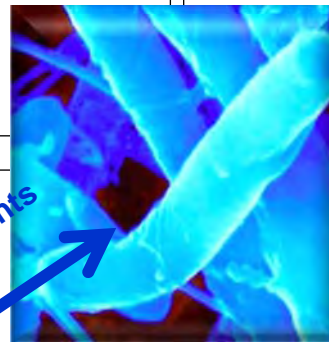
## Trace elements

- 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



1) carrier  
2) trace elements  
3) capturing

## Zeolite Migulators

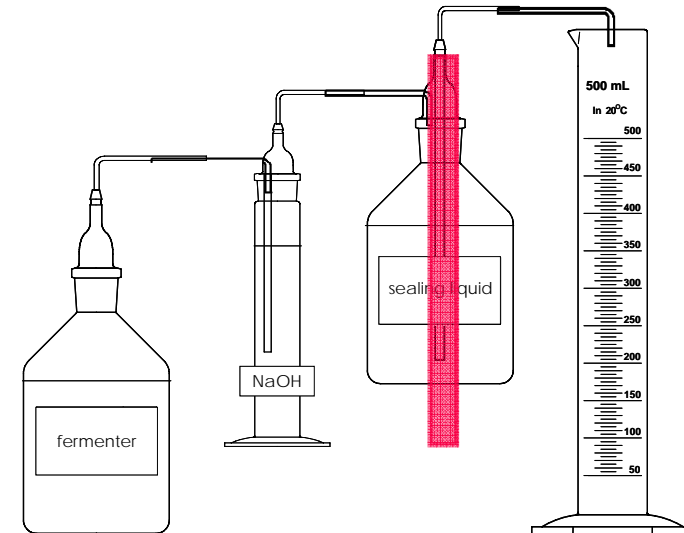
- Weiß, Guebitz et al *Water Res* 2010, 44:1970-198
- Weiß, Guebitz et al *Biores technol* (2011) in press

# Experiments

## Gras silage

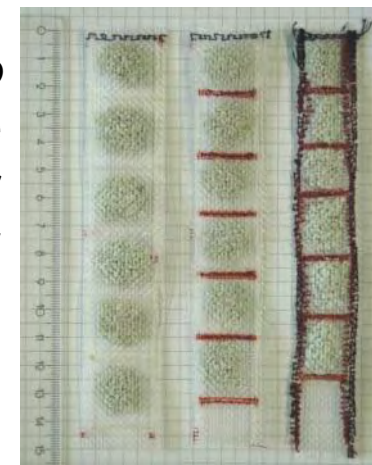
### Model substrate:

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

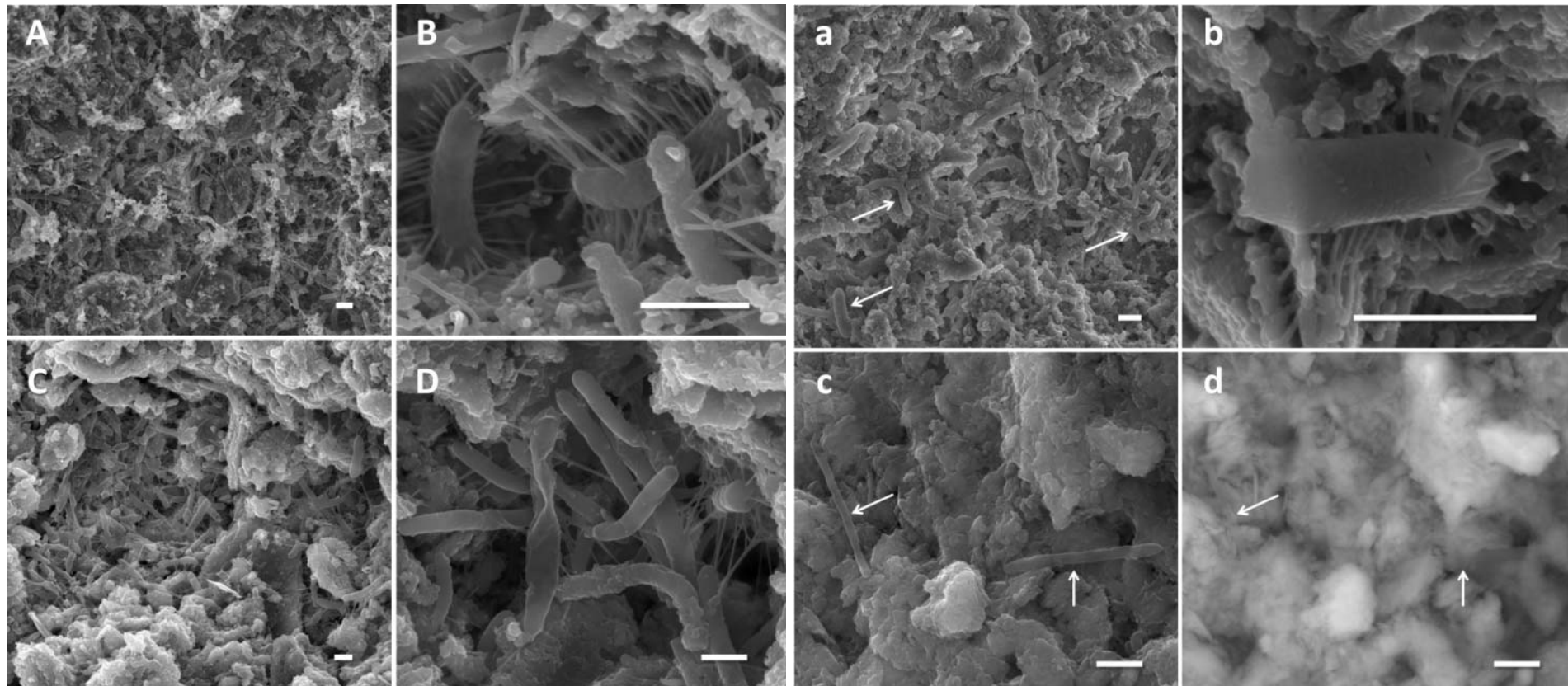
***in sacco***  
**technique**  
 for  
**continuously**  
**operated**  
**bioreactors**  
 (LfL Institute)



# 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  $\mu\text{m}$ )



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

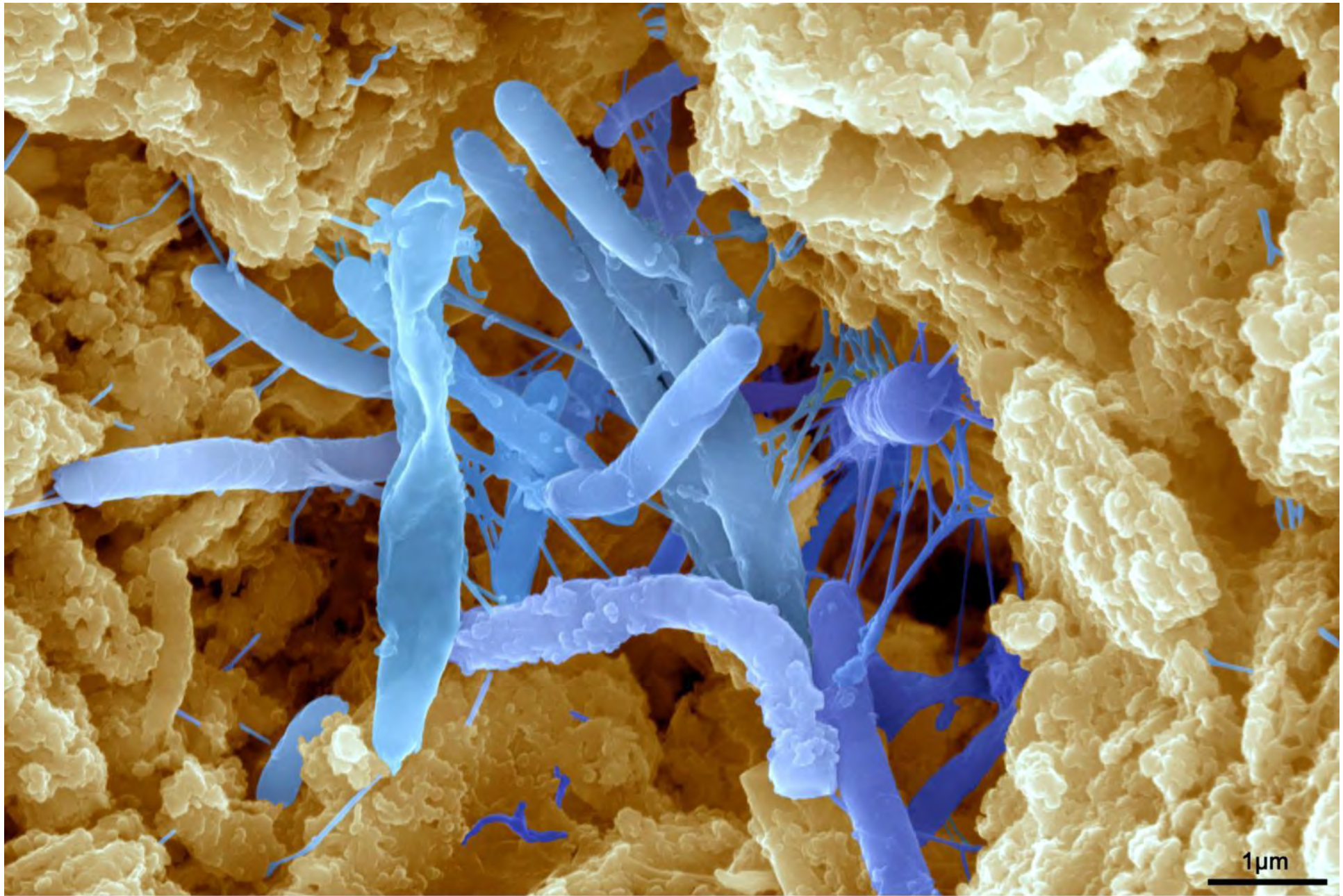
84 d *in sacco* incubation in continuously operated bioreactors (28 l) fed with grass silage at 45 °C

introduction

novel lignocellulolytic enzymes

hydrolysis in the biogas process

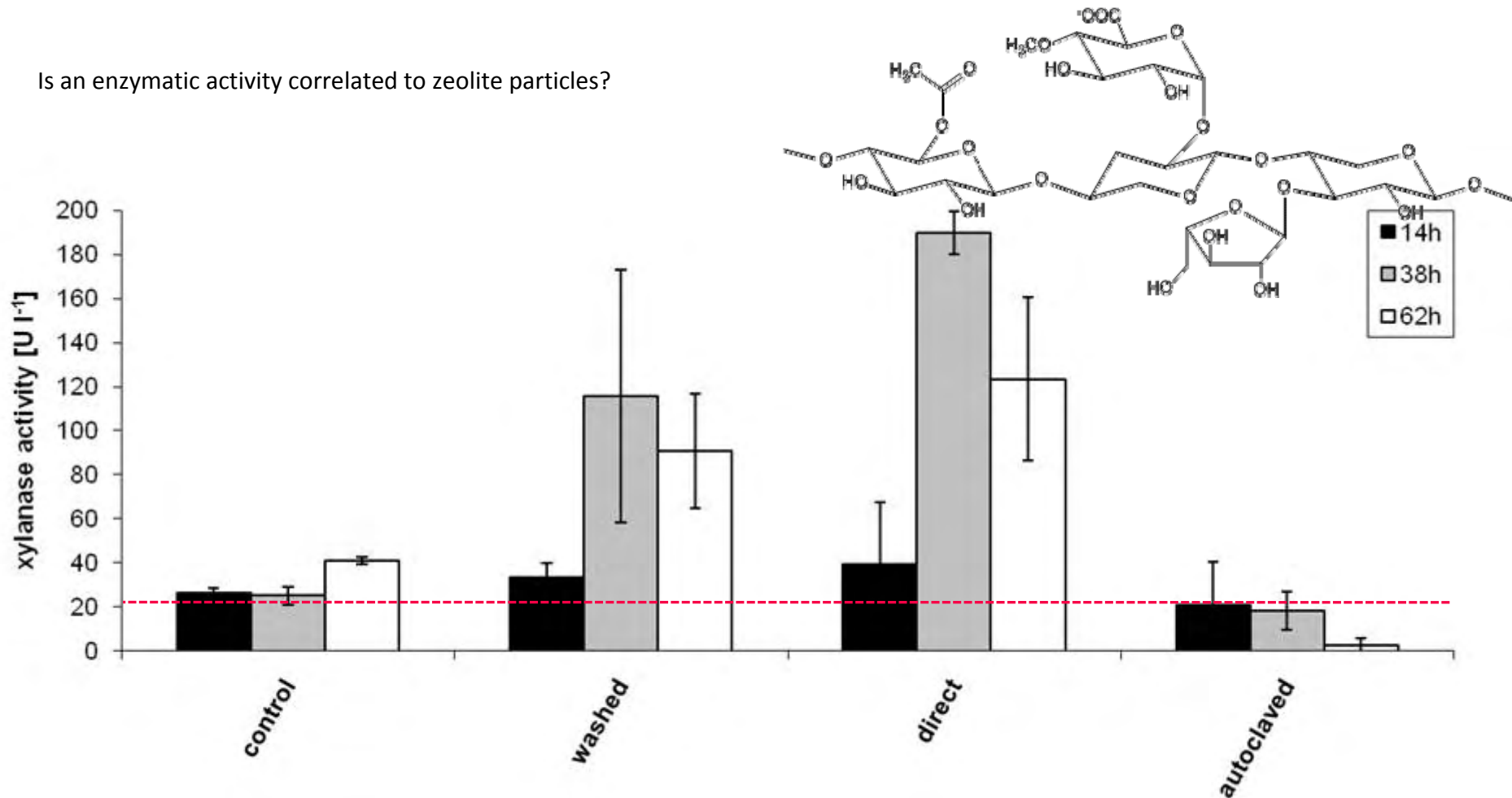
conclusions



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)

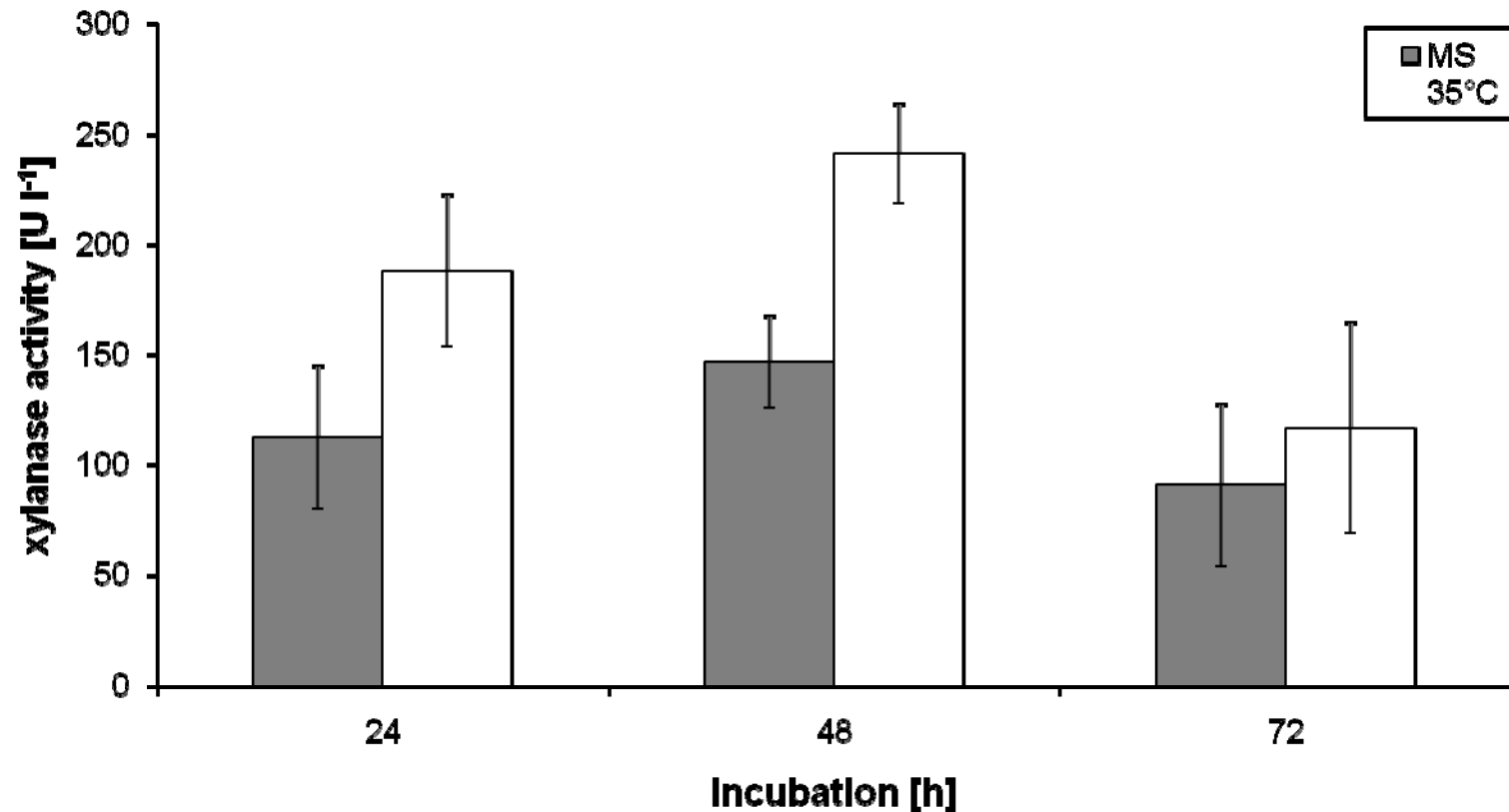
# xylanase re-activation on zeolites

Is an enzymatic activity correlated to zeolite particles?



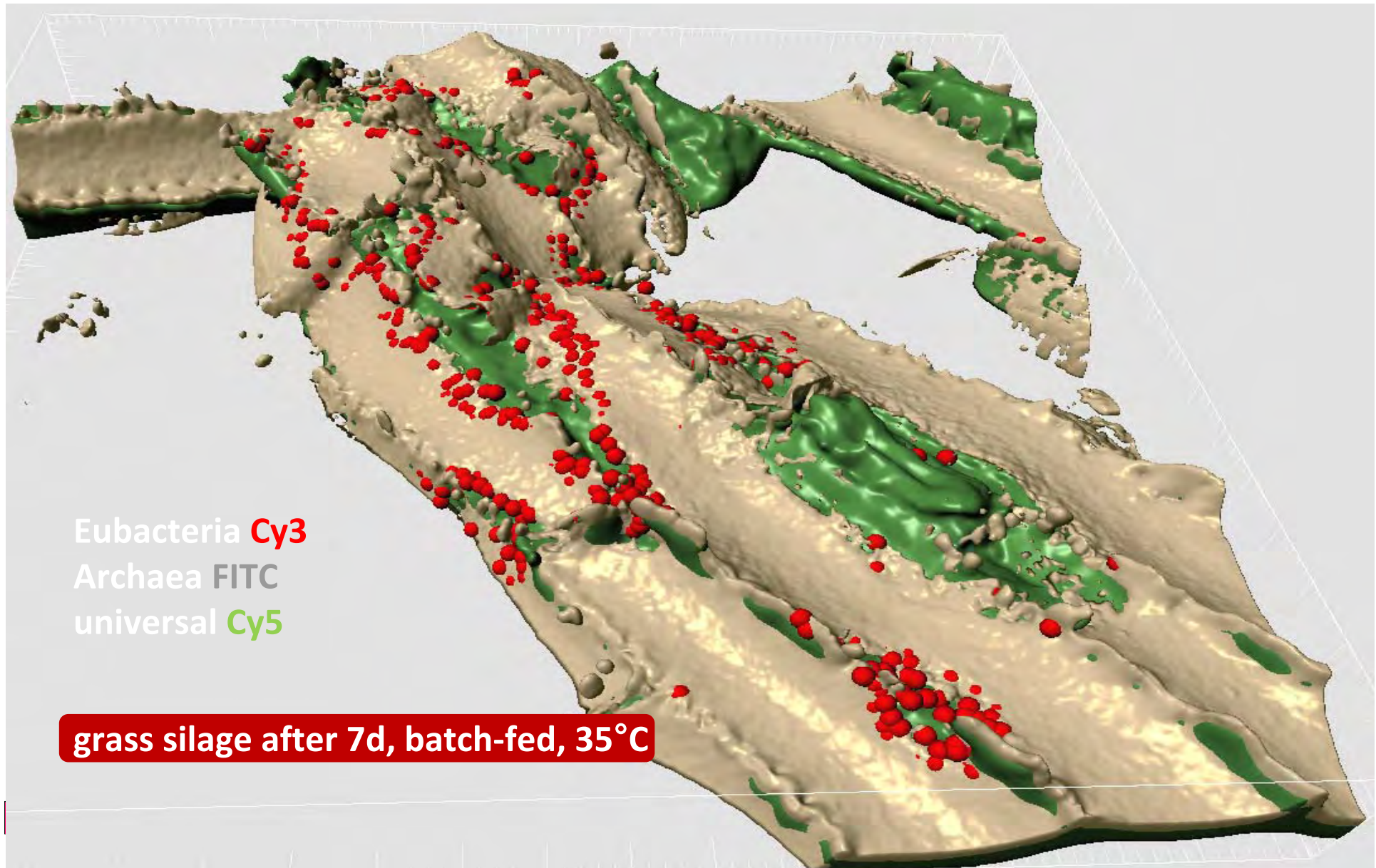
zeolite particle size 1.0-2.5mm / batch-cultivation on model substrate 14d, 35°C

# zeolite correlated enzyme activities

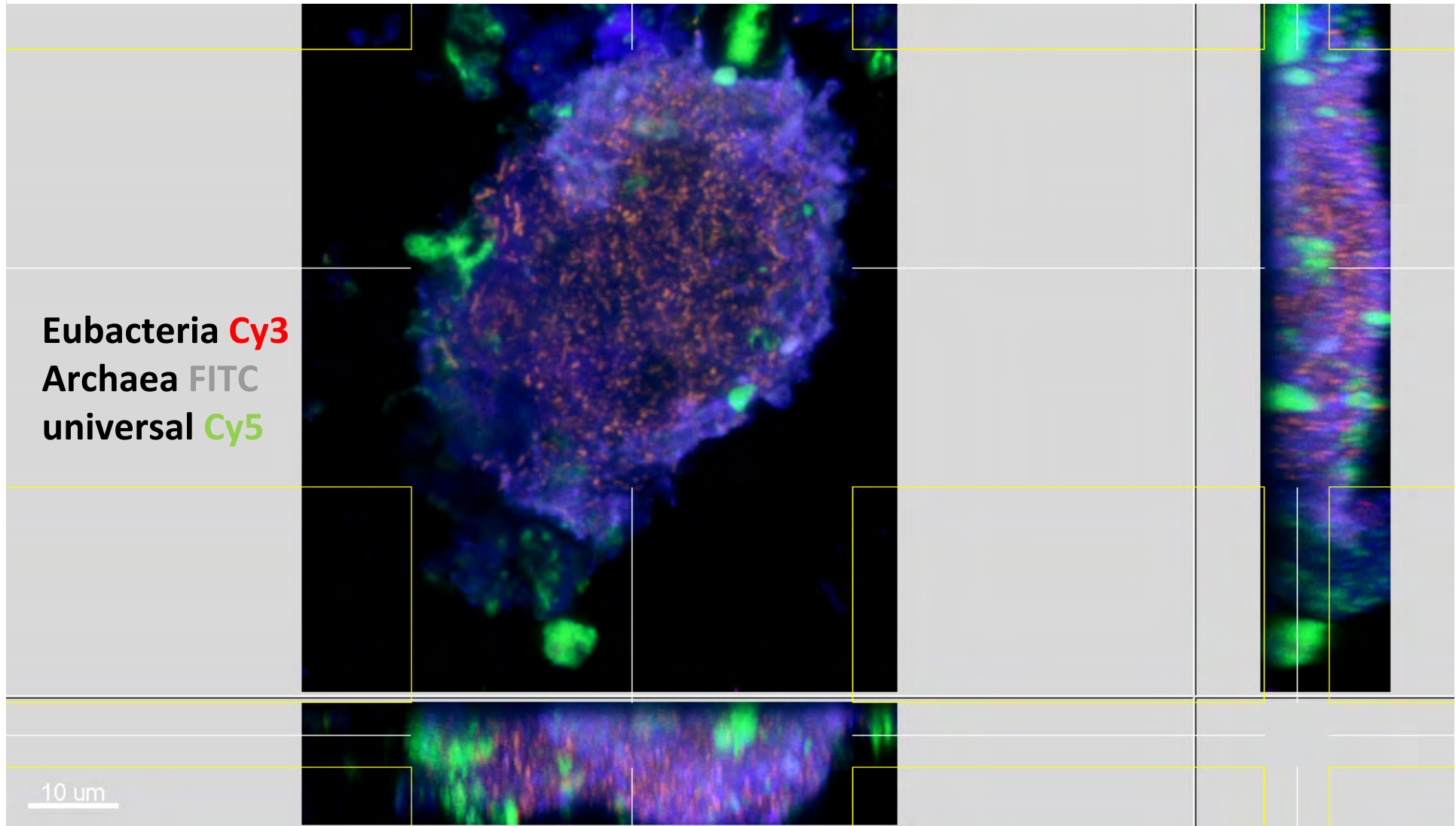


zeolite particle size <100 $\mu$ m / batch-cultivation on model substrate, 35°C/45°C

# CLSM of (hemi)cellulolytic bacteria



# CLSM of zeolite particle <math><100\mu\text{m}</math> $\varnothing$



introduction

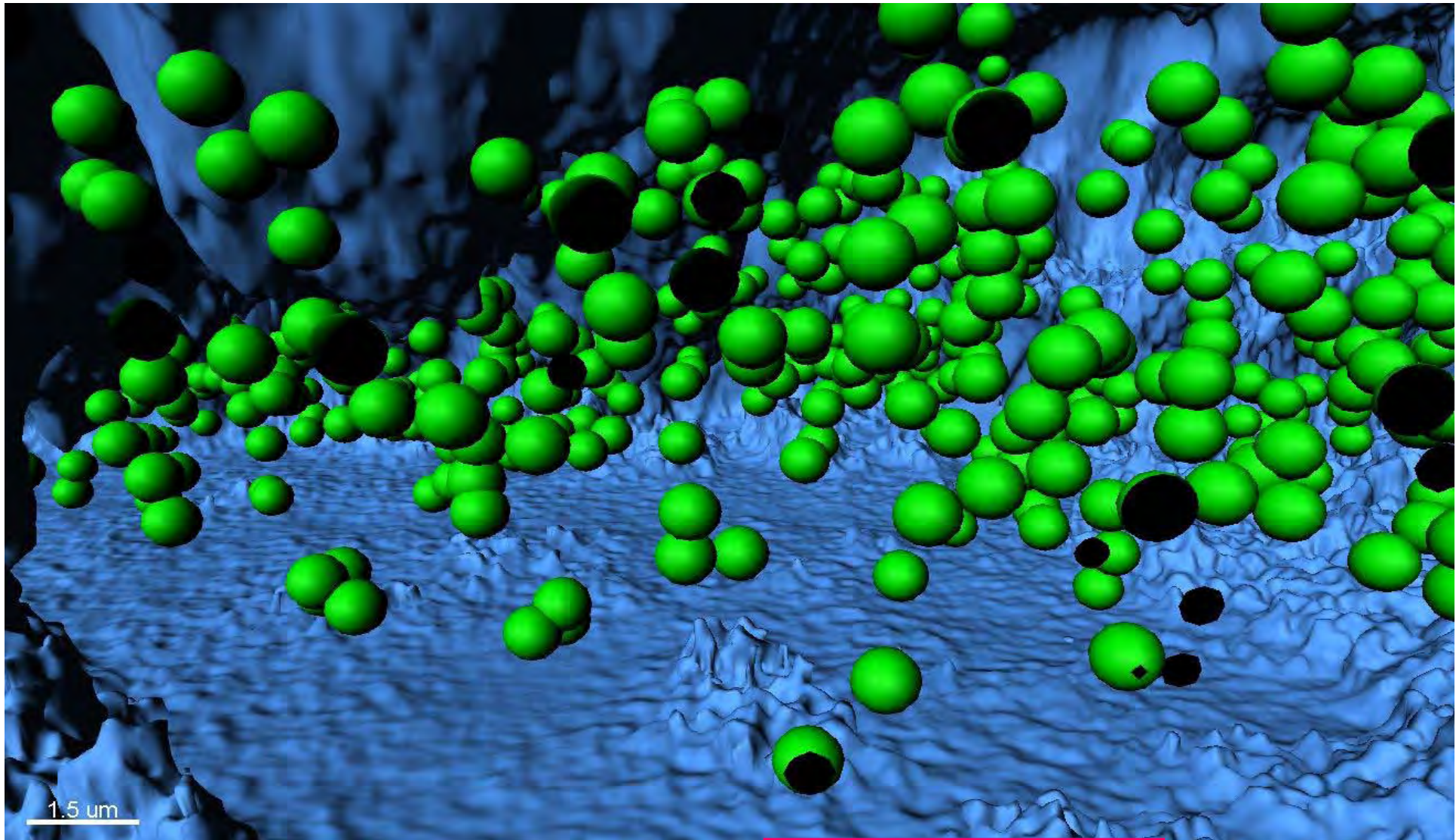
novel lignocellulolytic enzymes

hydrolysis in the biogas process

conclusions



# zeolite particle+bacteria



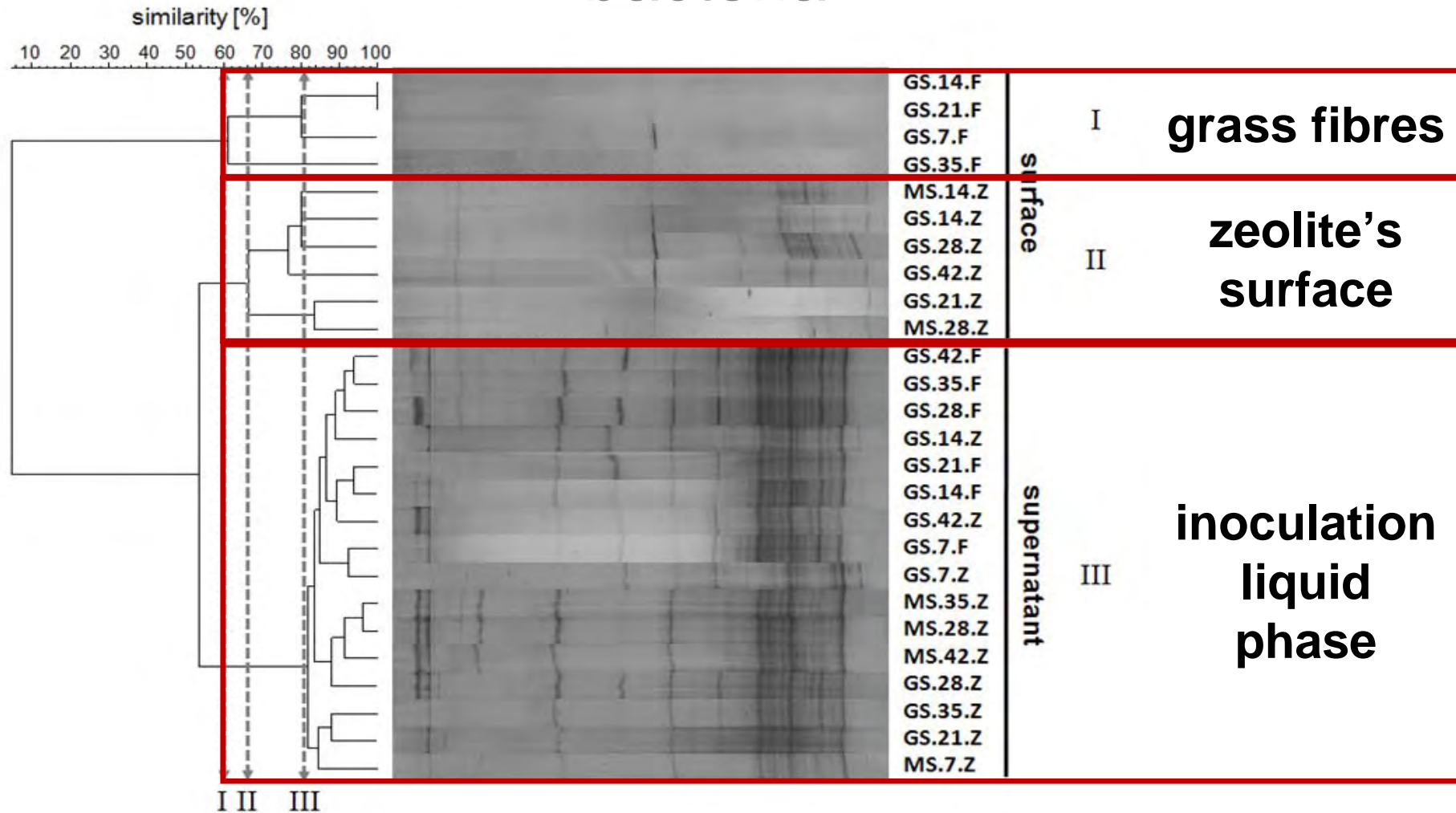
introduction

novel lignocellulolytic enzymes

hydrolysis in the biogas process

conclusions

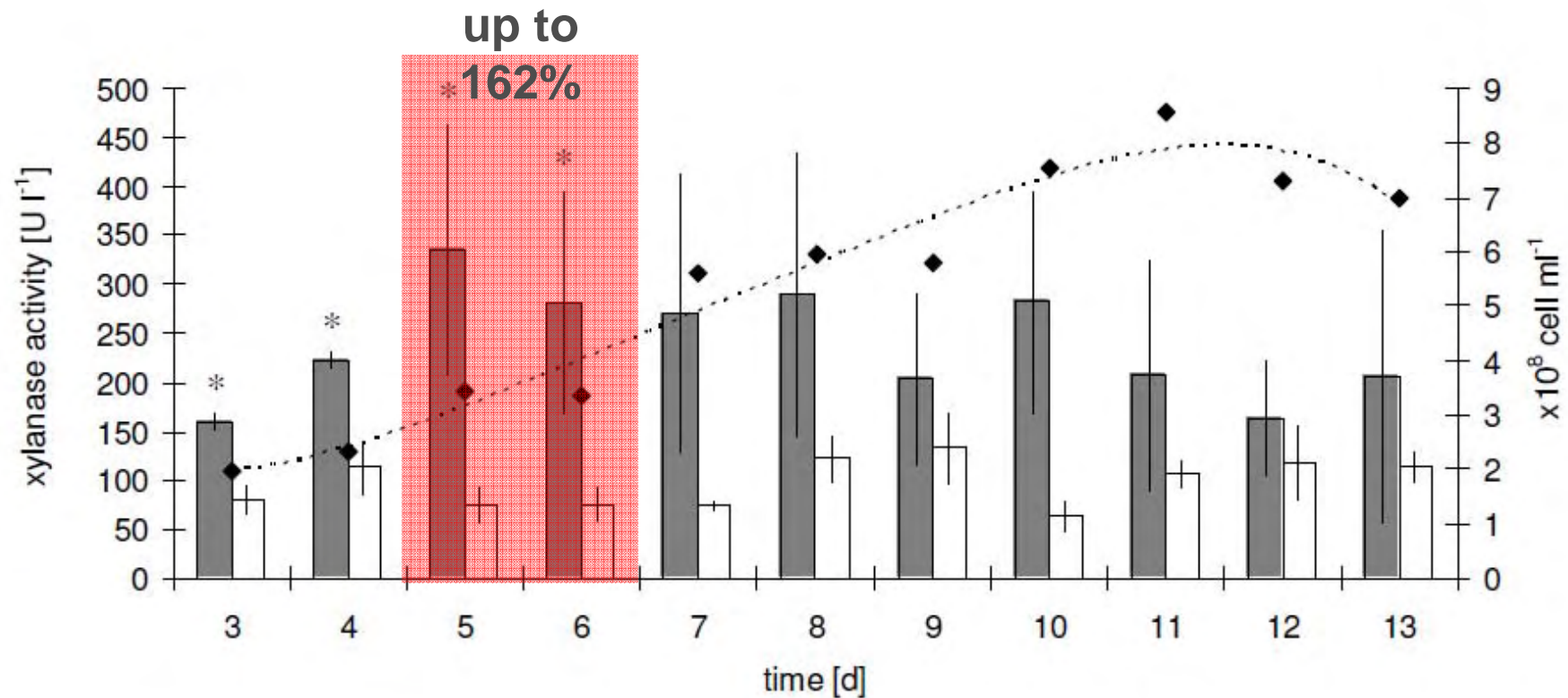
# PCR-SSCP-Clustering : *rrs* bacteria



# Sequencing : *rrs* bacteria/archaea

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

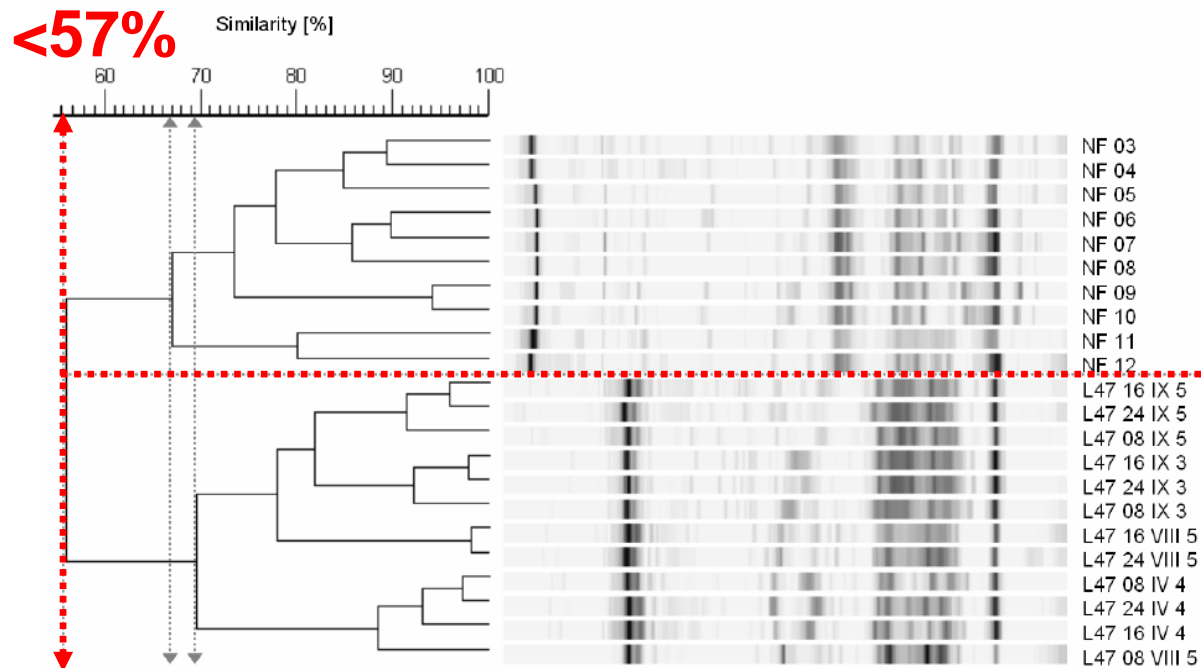
# Enrichment of enzyme producers



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.

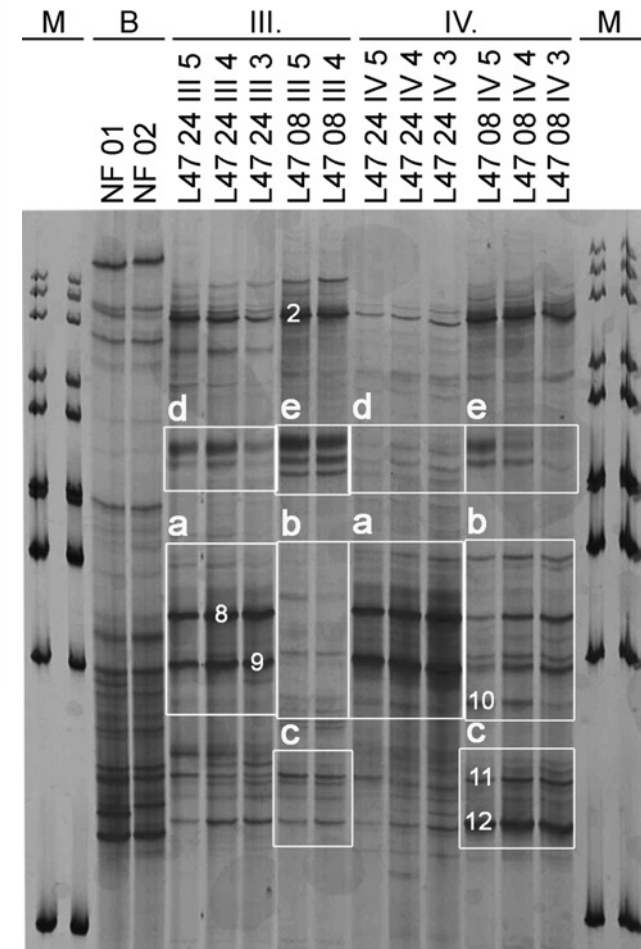
# Enriched strains



*Bacteroides* sp. (*B. succinogenes*, *B. ruminicola*)

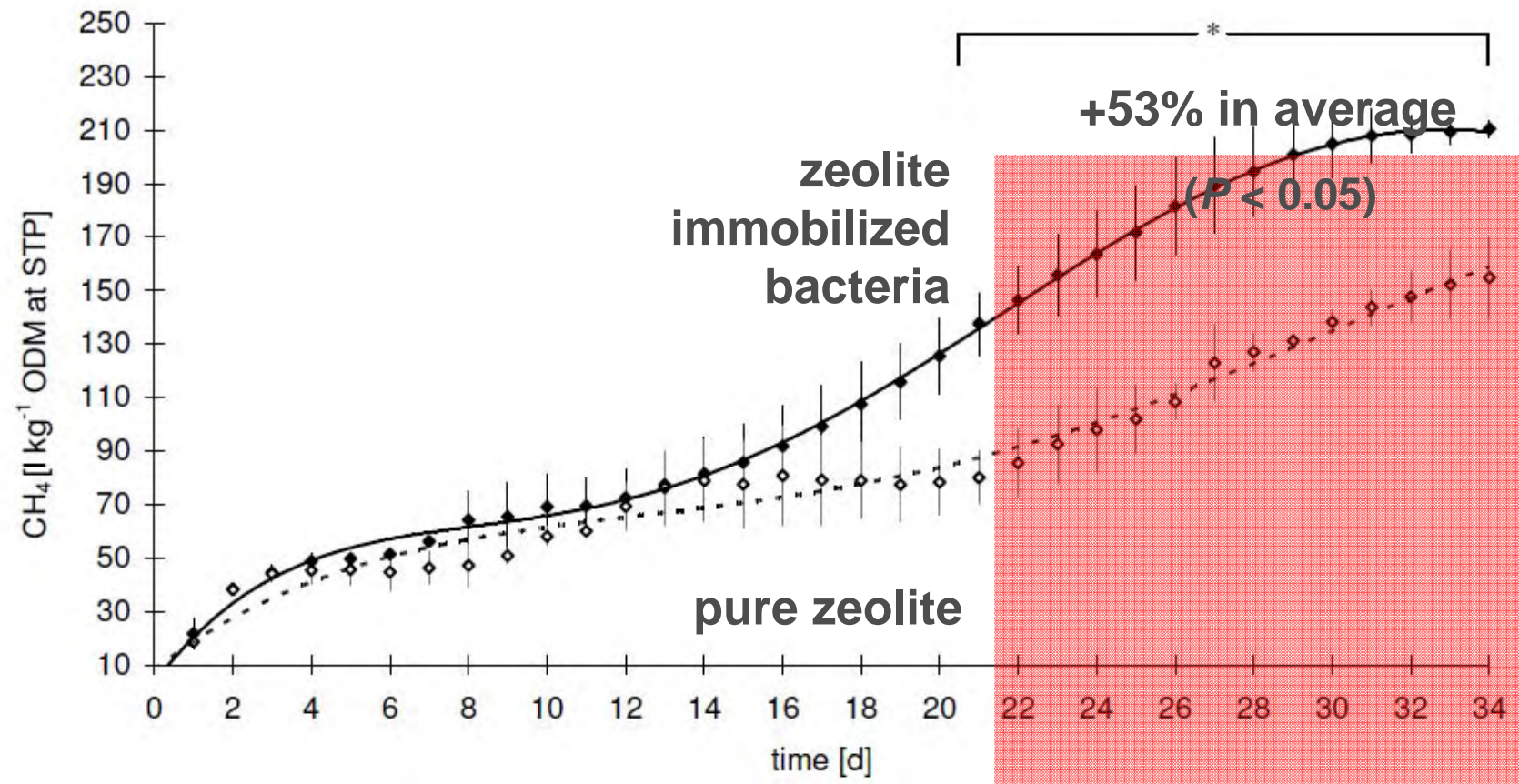
*Clostridium* sp. (*C. beijerinckii*, *C. diolis*, *C. butyricum*)

*Azospira oryzae*



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

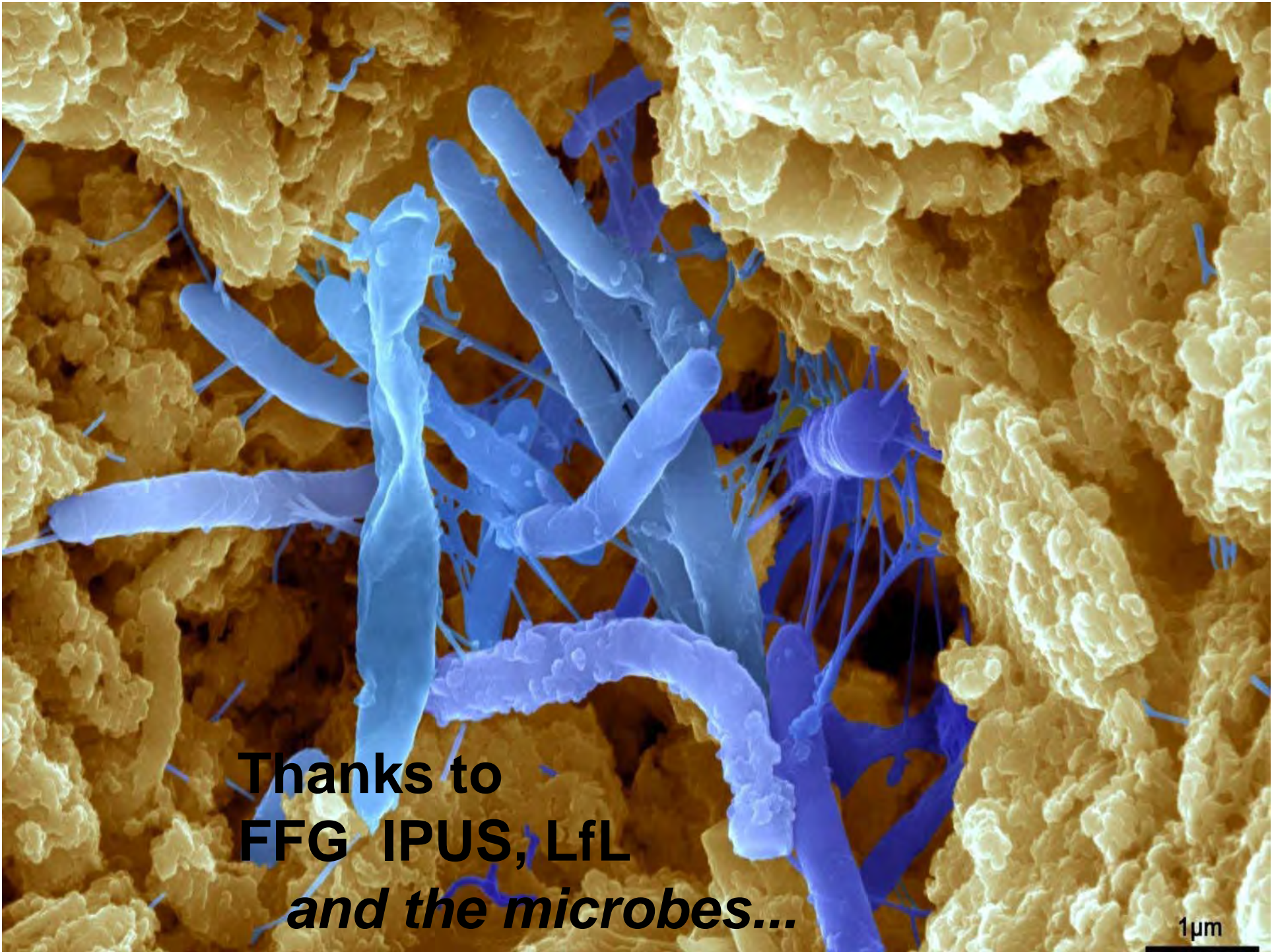
# CH<sub>4</sub>-yield in batch-experiments



•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 immobilised on zeolite



**Thanks to  
FFG IPUS, LfL  
*and the microbes...***

1 μm