

# Influence of Dispersant and Elevated Pressure on Crude Oil Degradation by a Deep Sea *Rhodococcus erythropolis* Strain

S. Hackbusch<sup>1</sup>, N. Noirungsee<sup>1</sup>, J. Viamonte<sup>1</sup>, X. Sun<sup>2</sup>, J. Kostka<sup>2</sup>, P. Bubenheim<sup>1</sup>, R. Müller<sup>1</sup>, A. Liese<sup>1</sup>

TUHH

<sup>1</sup>Hamburg University of Technology, Institute of Technical Biocatalysis, Hamburg, Germany

<sup>2</sup>Georgia Institute of Technology, School of Biology and Earth & Atmospheric Sciences, Atlanta, USA

IMAGE-II

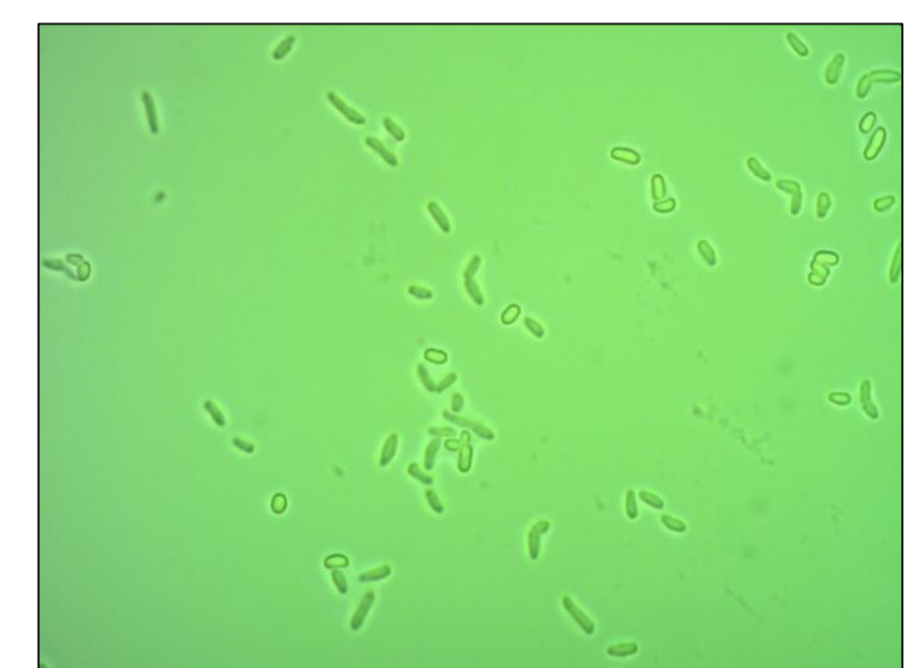
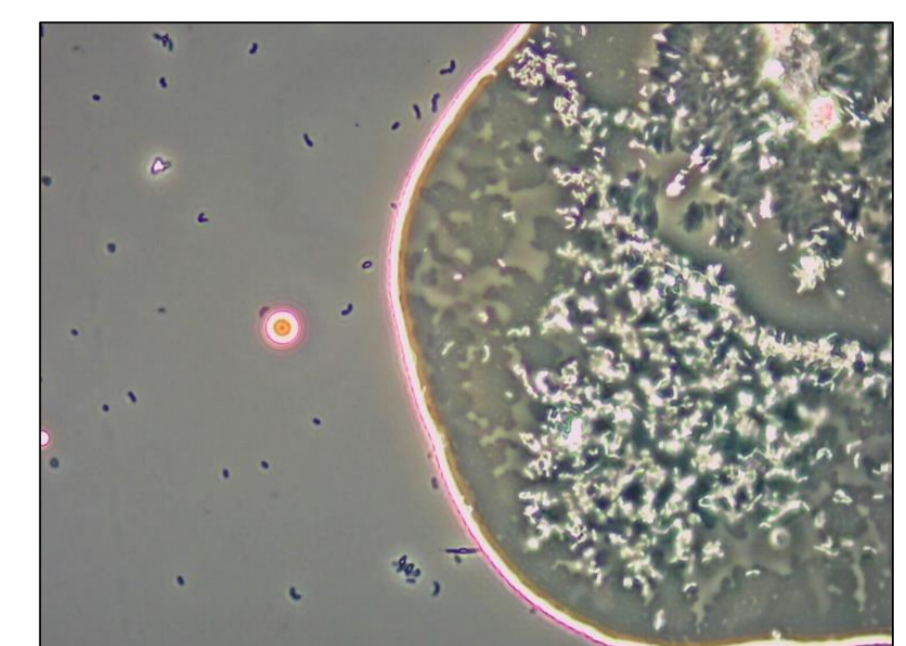
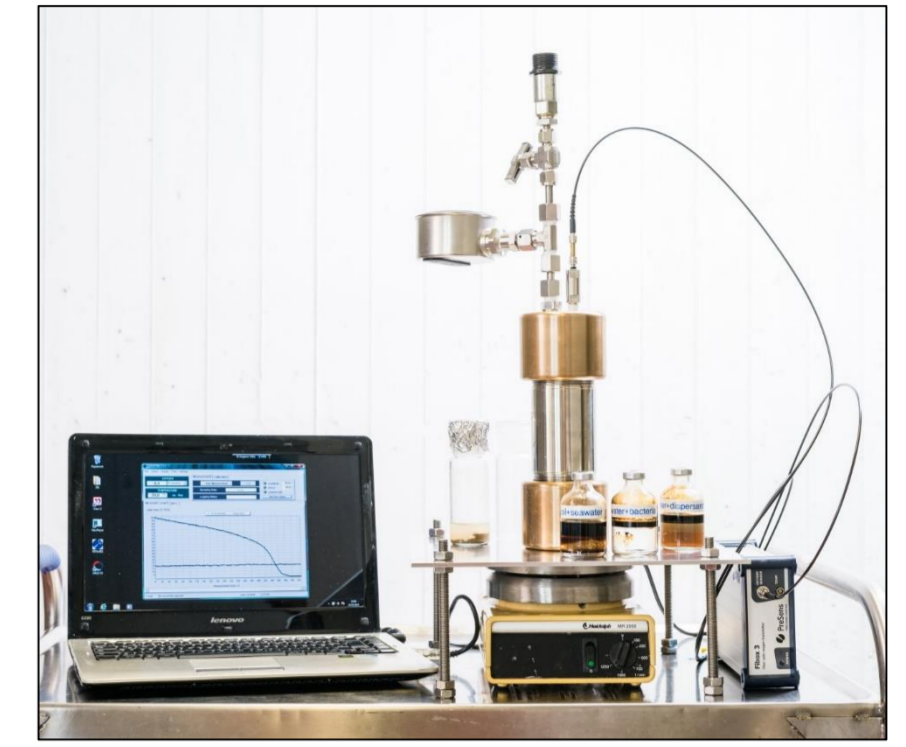
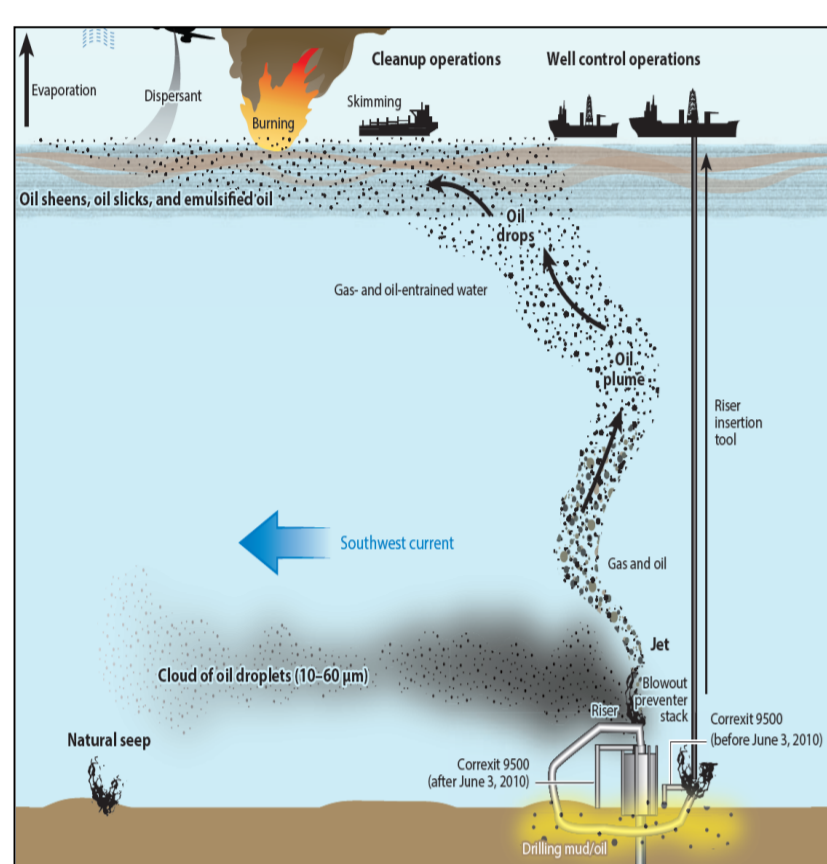
## Introduction

### The Background

- After the Deepwater Horizon oil spill (1500 m deep) in 2010, up to 61 % of the hydrocarbons spilled into the Gulf of Mexico (GoM) were presumably consumed by microorganisms<sup>1</sup>.
- The chemical composition of a persistent plume between 900-1300 m depth was characterized by high concentrations of polycyclic aromatic hydrocarbons (PAHs), medium length alkanes, methane and other gases.
- Bacterial community diversity within this plume dropped drastically down to a single dominant species<sup>1,2,3</sup>.

### Our Approach

- High pressure reactors (N<sub>2</sub>; < 400 bar) were used to simulate hydrostatic pressures in the deep sea.
- Most lab-based research investigating microbial hydrocarbon degradation under deep sea conditions neglected the effect of elevated pressure on microbes so far<sup>4</sup>.
- A *Rhodococcus* GoM model strain PC20 was isolated from sediment (1200 m depth) near the spill site with crude oil as sole carbon source.
- A *Cobetia* strain was isolated from ex-situ incubated GoM deep sea sediment.



## Growth and Activity of *Rhodococcus* PC20

*Rhodococcus* strain PC20 is able to degrade and grow on single oil components (e.g. hexadecane; results not shown) as well as complex crude oil mixtures. Our experiments showed that elevated pressure had little effect on the growth of *Rhodococcus*. The addition of dispersant resulted in reduced growth, while pressure and dispersant might have a synergistic effect with even lower CFU detected (at 72h; Fig. 3).

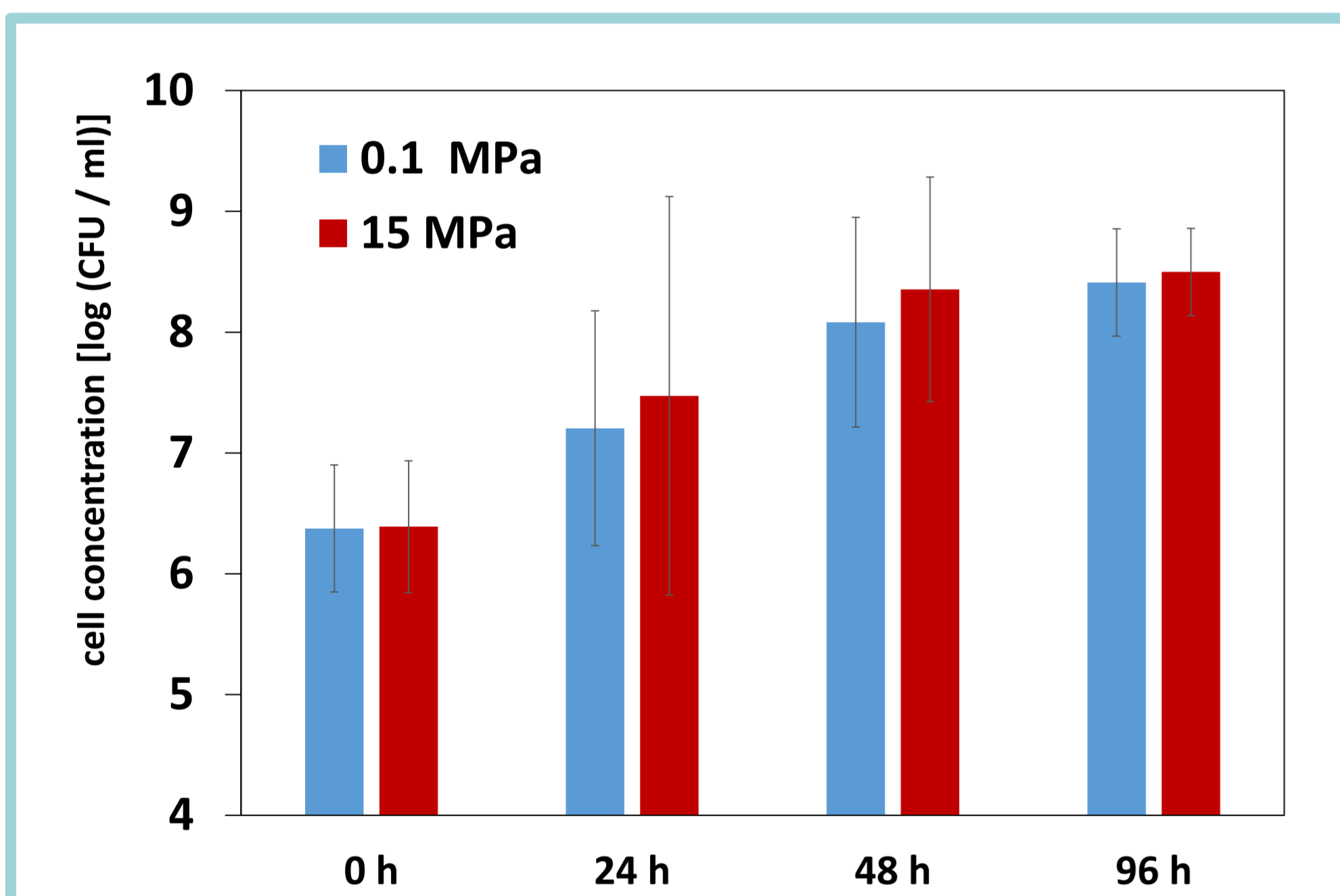


Fig. 1: Growth of PC20 at 0.1 and 15.0 Mpa. n = 3. 20 ml MM2 + 3% NaCl + vitamin / trace element solution, 1 % v/v LLS crude oil, 200 rpm, RT.

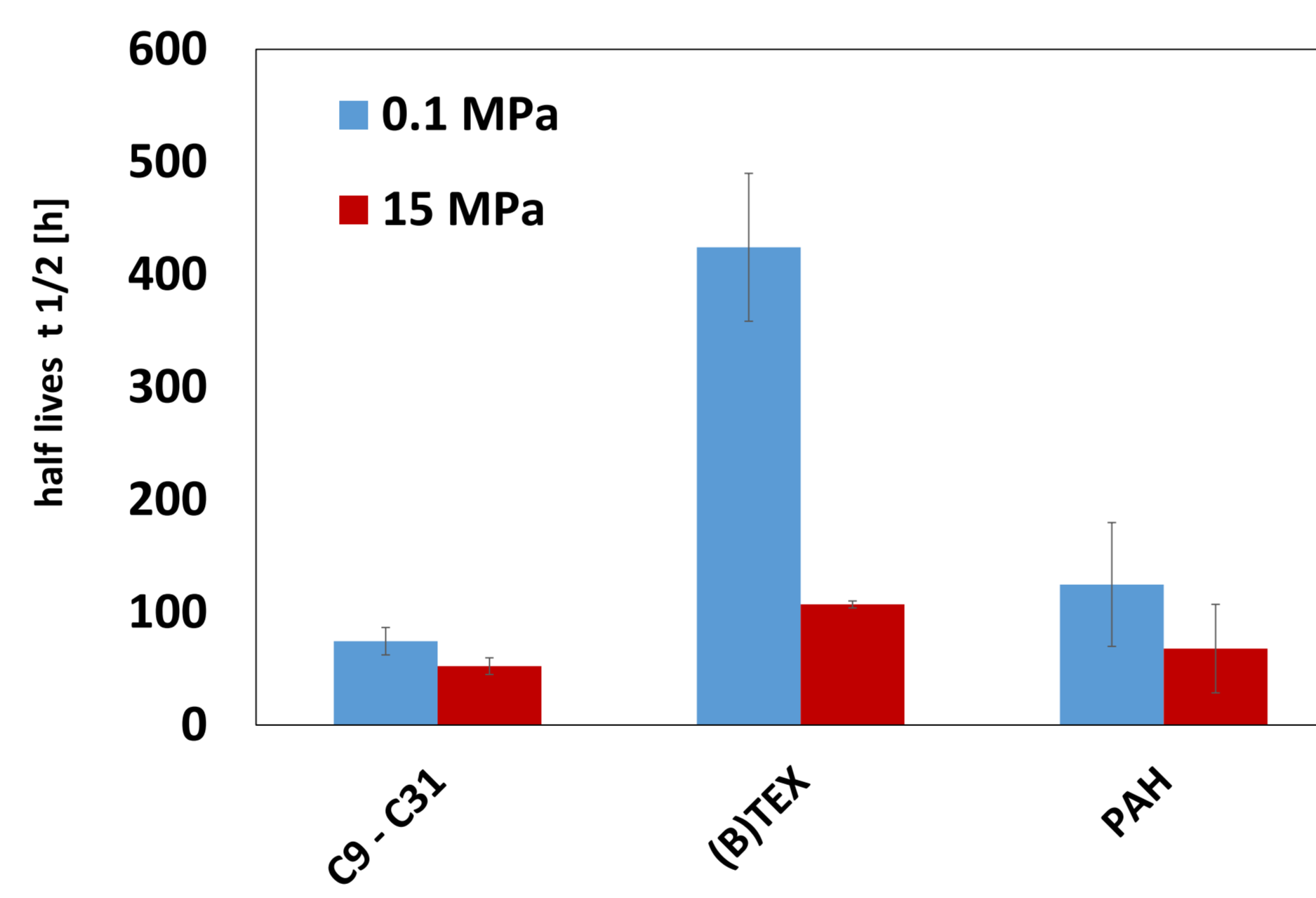


Fig 2: Half lives calculated for average compound classes: saturates (nC9-nC31), (B)TEX (toluene, ethylbenzene, m-/o-/p-xylene) and PAH (naphthalene, 2-methylnaphthalene, 1-methylnaphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, benz(a)anthracene).

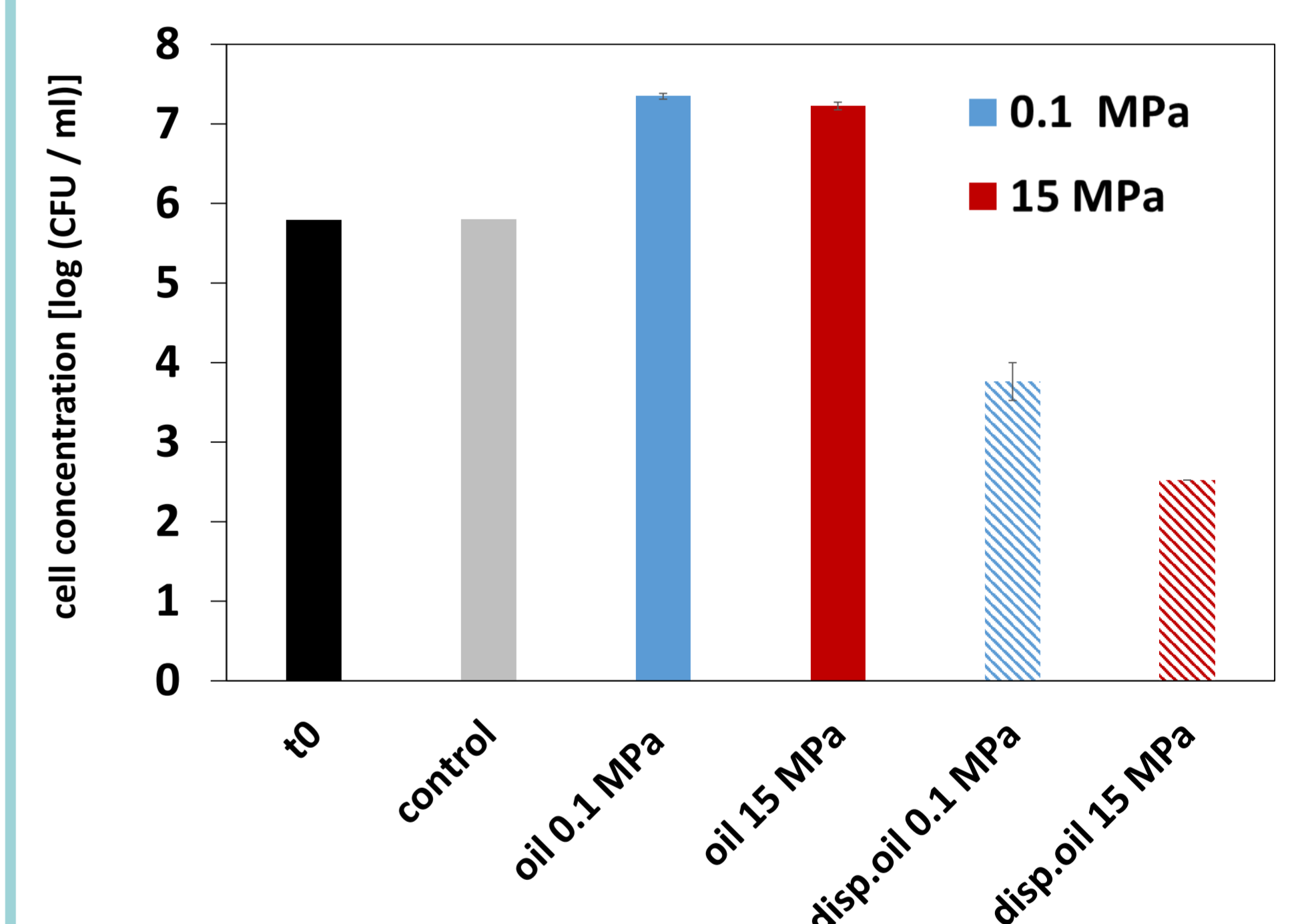


Fig. 3: Incubation of PC20 with crude oil and with/without dispersant (1:100) after 72h 20 ml MM2 medium (+3% NaCl, + 1x vitamin mix (DSMZ141), + 1x trace elements (DSMZ141)); 200 µl crude oil; 20 µl Corexit 9500A; RT; 200 rpm n = 2.

## Isolation of *Cobetia* C6 from deep sea sediment

Experiments with GoM deep sea sediment for 7 days at 10 MPa and 4°C with different substrates (oil/dispersant) and pressure suggests a pressure sensitivity of *Cobetia*, judging by their relative abundances. *Cobetia* C6 was previously isolated from the sediments, and partial 16S rRNA gene has high sequence identity to *Cobetia* variants detected from community analysis (Fig 4). *Cobetia* strain C6 is able to grow on crude oil (data not shown) and hexadecane at 4°C and 0.1 MPa.

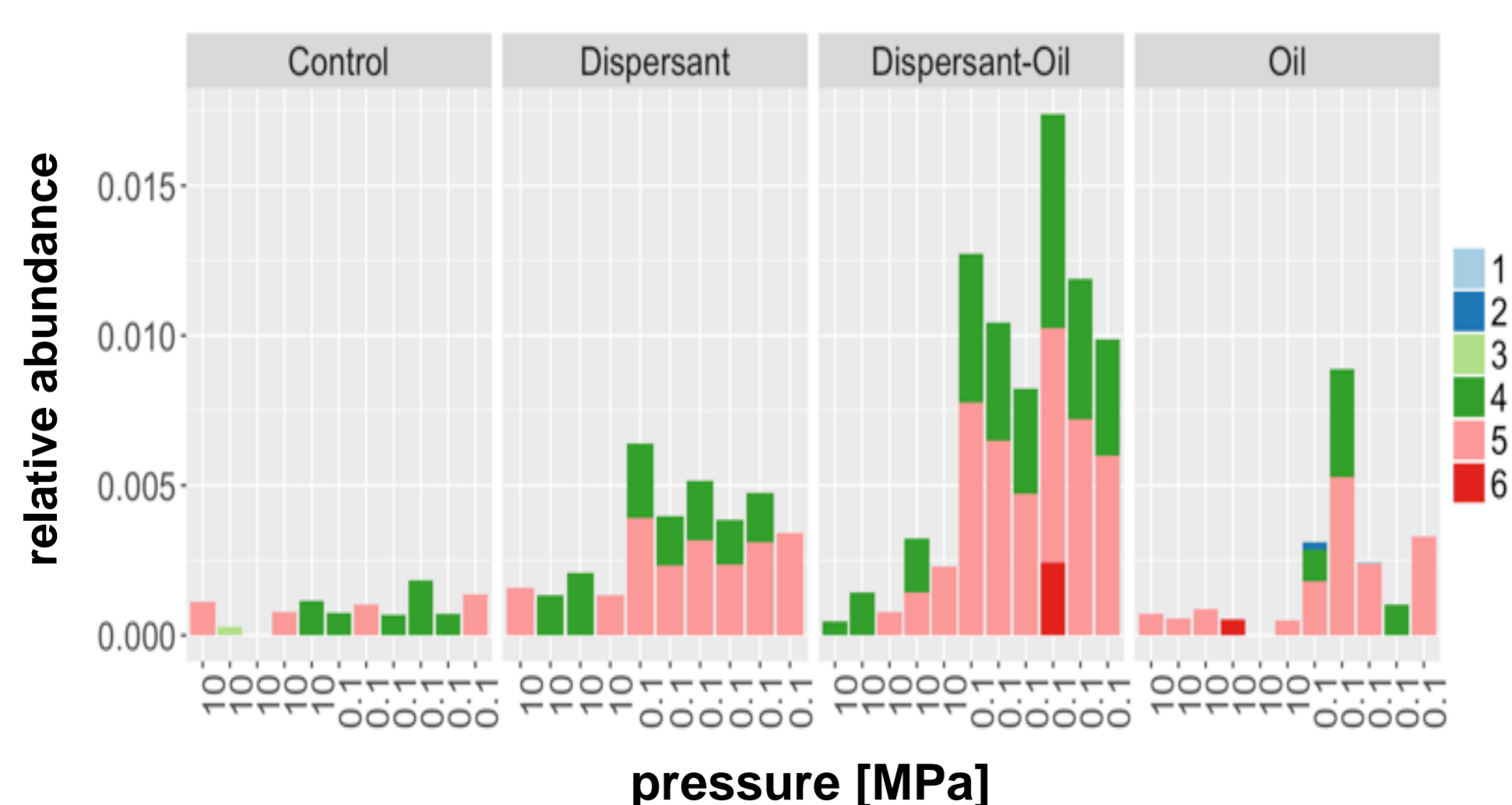


Fig. 4: Relative abundance of *Cobetia* variants found with 16S analysis after incubation for 7 days 4°C at 0.1 and 10 MPa.

Isolate #	% Identity of partial 16S rRNA sequences of Isolates to Variants					
	Variant 1	Variant 2	Variant 3	Variant 4	Variant 5	Variant 6
Isolate 6	89	100	99	100	100	99

Tab 1: Comparison of partial 16S rRNA sequences (~300 bp) of Isolate C6 with variant sequences.

Isolate #	First hit	Query cover	Submitted
6	<i>Cobetia</i> sp. 7523M	100%	2016

Tab 2: NCBI Blast result of Isolate C6 partial 16S rRNA sequence.

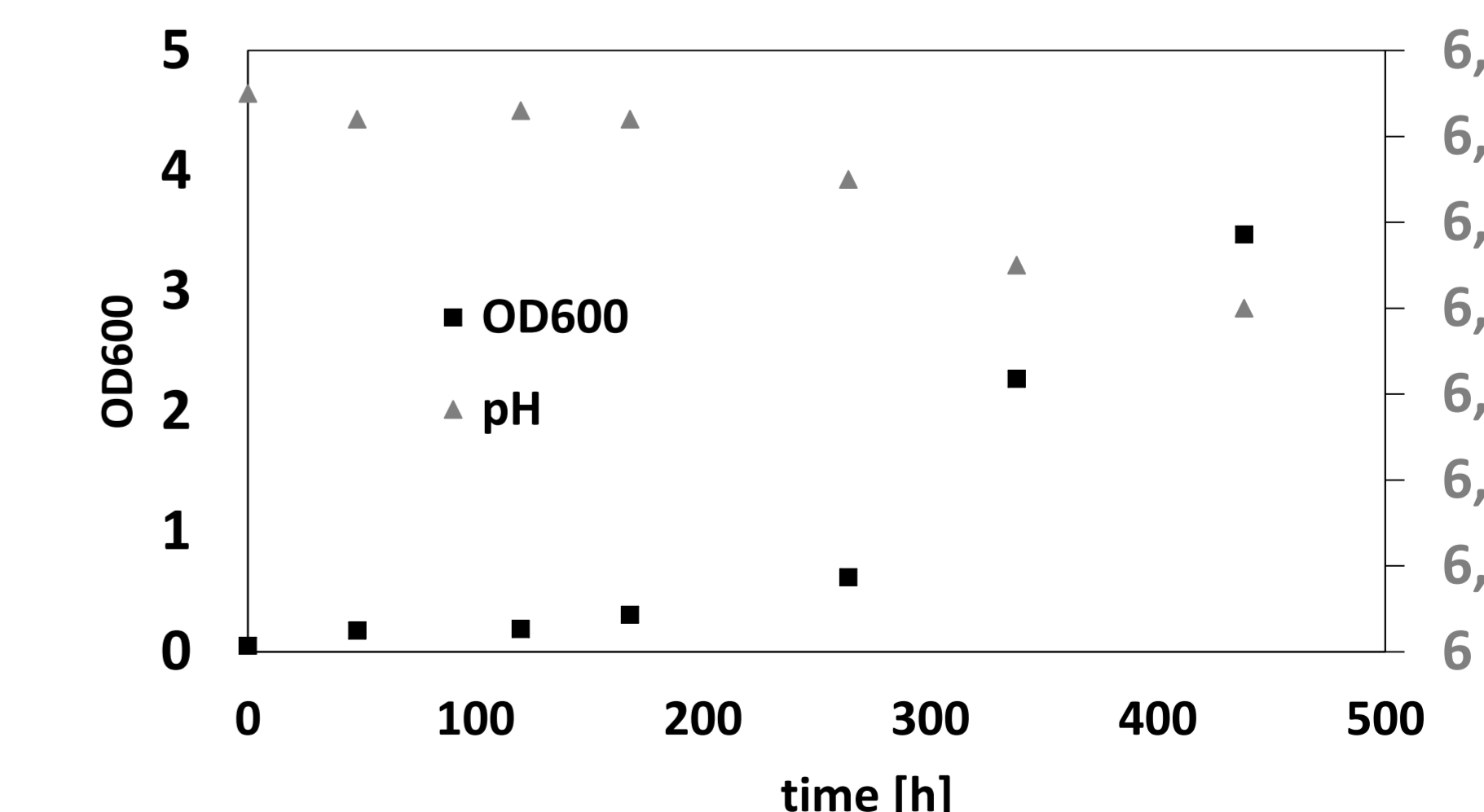


Fig. 5: Preliminary experiment of *Cobetia* isolate C6 at ambient pressure and 4°C with hexadecane (C16) as substrate.

Contact:

Steffen Hackbusch  
Institute of Technical Biocatalysis  
Hamburg University of Technology  
Tel. +49-(0)40-42878-4171  
Fax. +49-(0)40-42878-2127  
E-mail: steffen.hackbusch@tuhh.de



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