Center for Energy Resources Engineering
CERE

Enzymes:
Mechanisms of action and related problems

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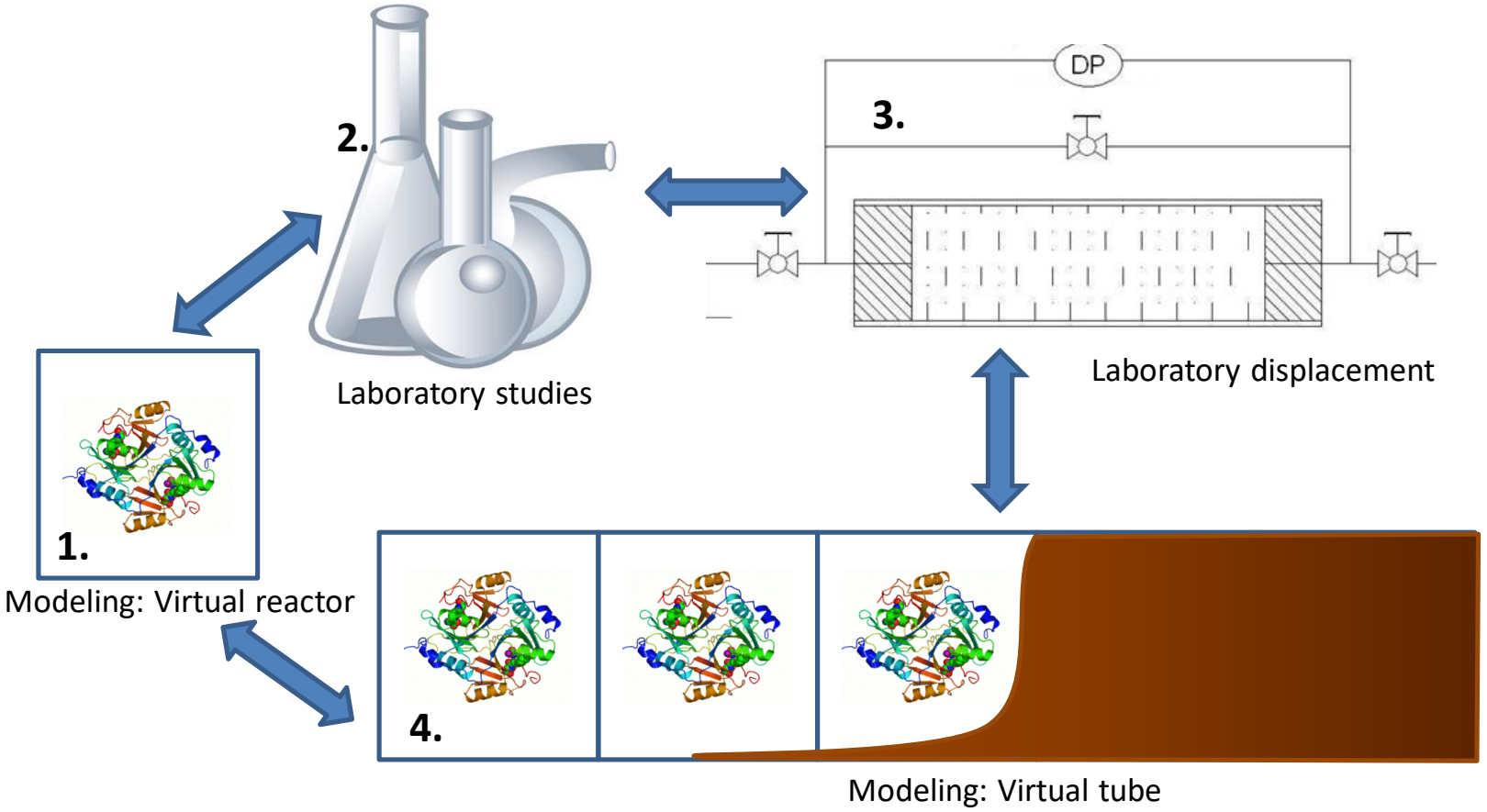
Overview of BioRec

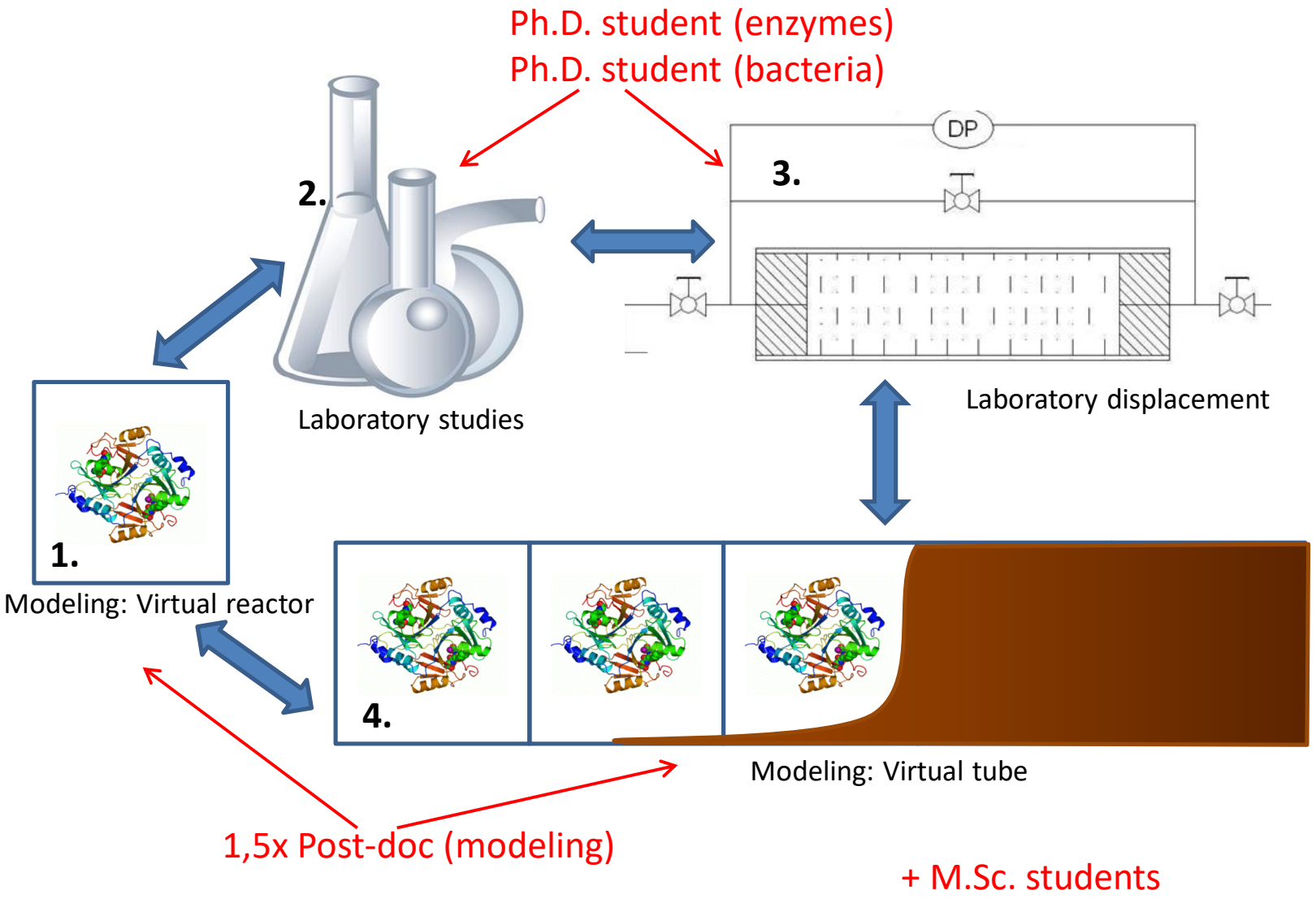
General

- Duration: *2010 - 2015*
- Industrial collaborators: *Mærsk, DONG, NovoZymes,*
- *Academic collaborators: DTI, RUC, DTU*
- Objective:
  - *To develop biotechnological knowledge and technology that will increase the amount of recoverable oil in Danish oil fields in the North Sea*
  - *(WP1) To investigate possible recovery mechanisms for Bio-EOR (enzymes and microorganisms) in the Danish North Sea sector.*
- Budget 33 000 000 DKr

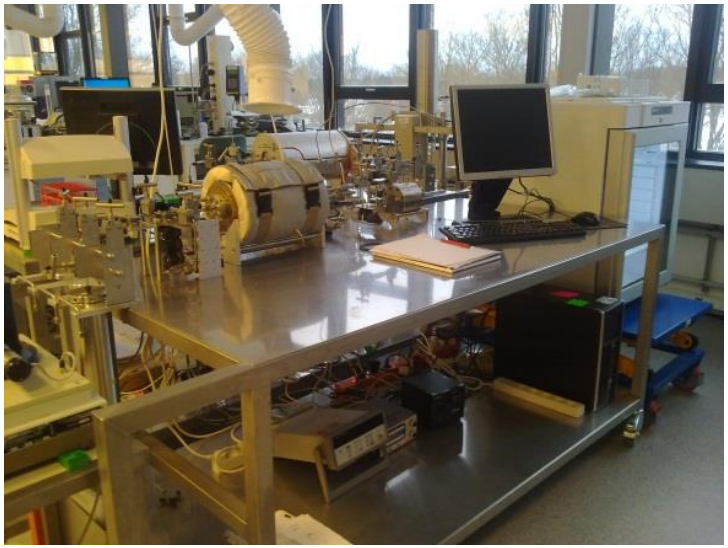
Organization of the project (WP1)

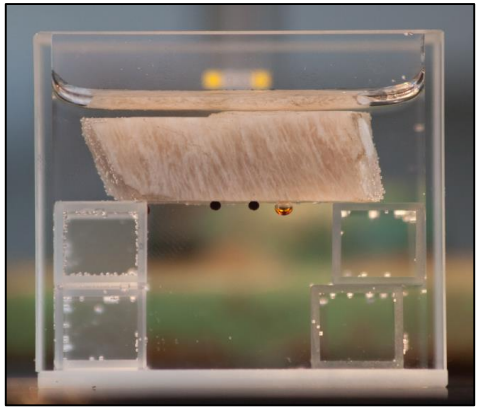


Organization of the project (WP1)



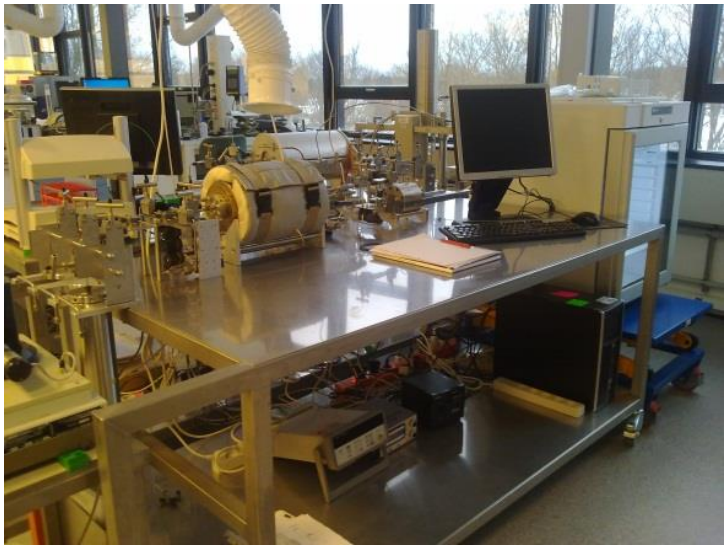
Experimental

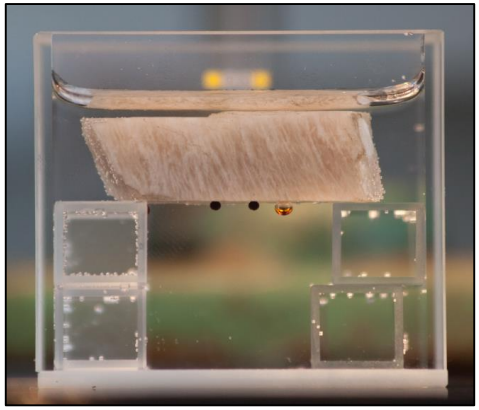




- Wettability
  - *Mineral surfaces*
  - *Enzyme solutions*
  - *Contact angles/adhesion*
- Adsorption (statics and dynamics)
  - *Mineral powder/surfaces*
  - *Desorption times*
- Enzyme/bacteria penetration
  - *Penetration depths*
  - *Spore-forming vs non-spore forming*
  - *Retention of enzymes*
- Bacteria growth and sporulation
  - *Optimal nutrition*
  - *Effects of acidity, temperature…*
- Flooding
  - *Homogeneous vs heterogeneous rock*
  - *Secondary vs tertiary injection*

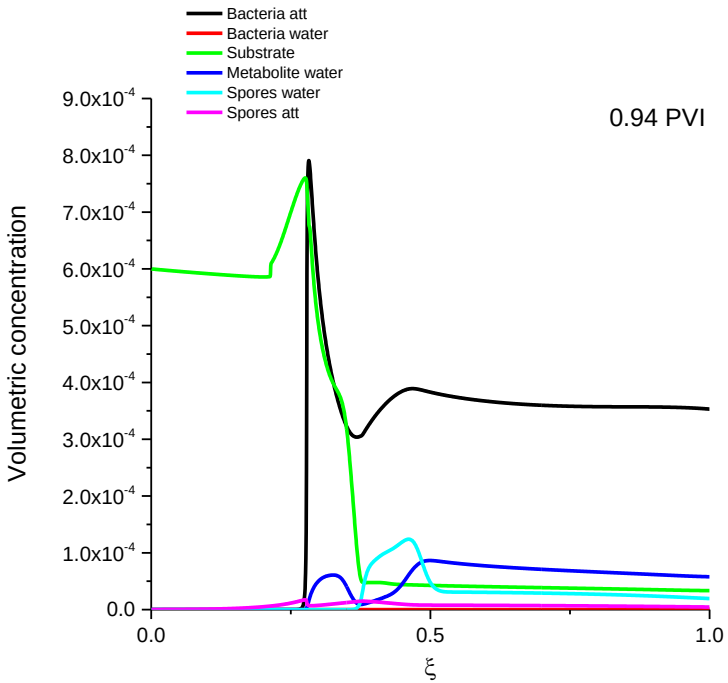
Enzymes: Experimental





- Wettability
  - *Mineral surfaces*
  - *Enzyme solutions*
  - *Contact angles/adhesion*
- Adsorption (statics and dynamics)
  - *Surfaces: mineral powder*
  - *Desorption times*
- Enzyme/bacteria penetration
  - *Penetration depths*
  - *Spore-forming vs non-spore forming*
  - *Retention of enzymes*
- Bacteria growth and sporulation
  - *Optimal nutrition*
  - *Effects of acidity, temperature…*
- Flooding
  - *Homogeneous vs heterogeneous rock*
  - *Secondary vs tertiary injection*

Modeling
(to be presented by Sidsel)



- Microbial EOR (not enzymes)
- 1D model (but with the 3D perspective)
- Different mechanisms
  - Filtration vs adsorption
  - Sporulation
  - Surfactant production
  - Plugging
  - …
- *Daily interaction with the laboratory*

Important effects for EnzEOR

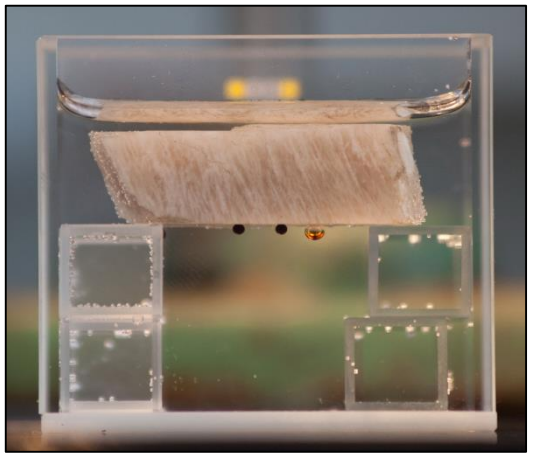
Previous studies

- Enzymes help producing more oil
- The main mechanism is supposed to be changing wettability (e.g. H. Nasiri, 2011)
- *What are the main mechanisms of enzyme behavior/action?*
  - *Interfacial tension*
  - *Adhesion*
  - *Transport*
  - *Attachment*
  - *…*

Wettability tests

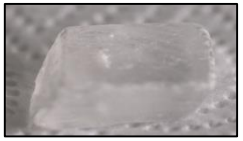
Experiments by A. Khusainova

- Room **T** and **p**
- Brine – synthetic **North Sea water**
- **15 enzyme** products: novozymes®
- 2 Commercial Mixtures



"Chalk" Oil

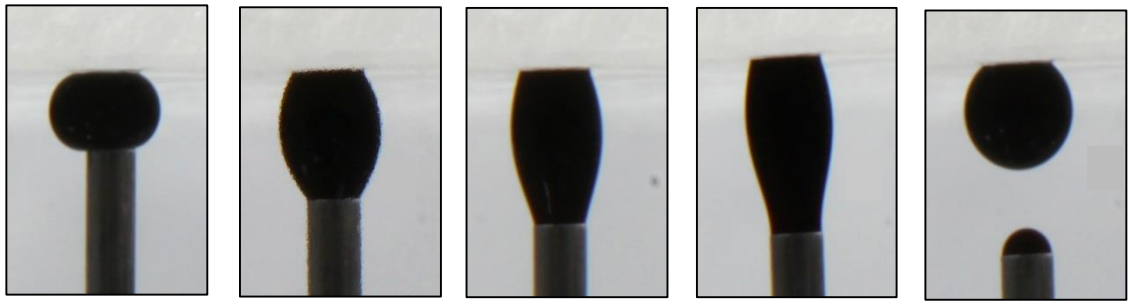
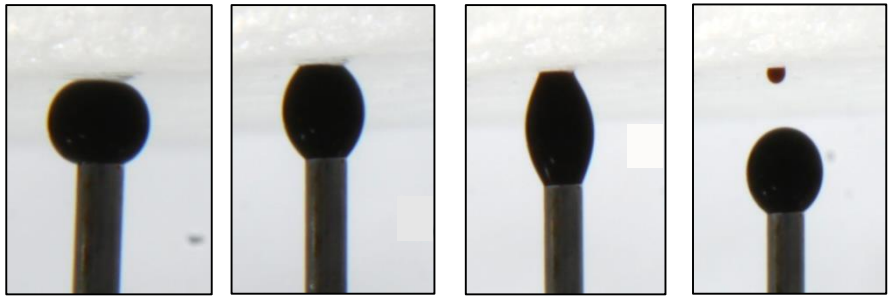
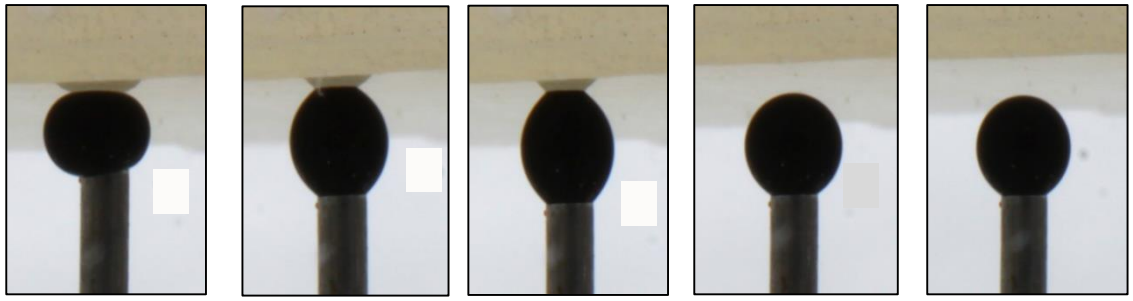
Calcite ≈ Chalk

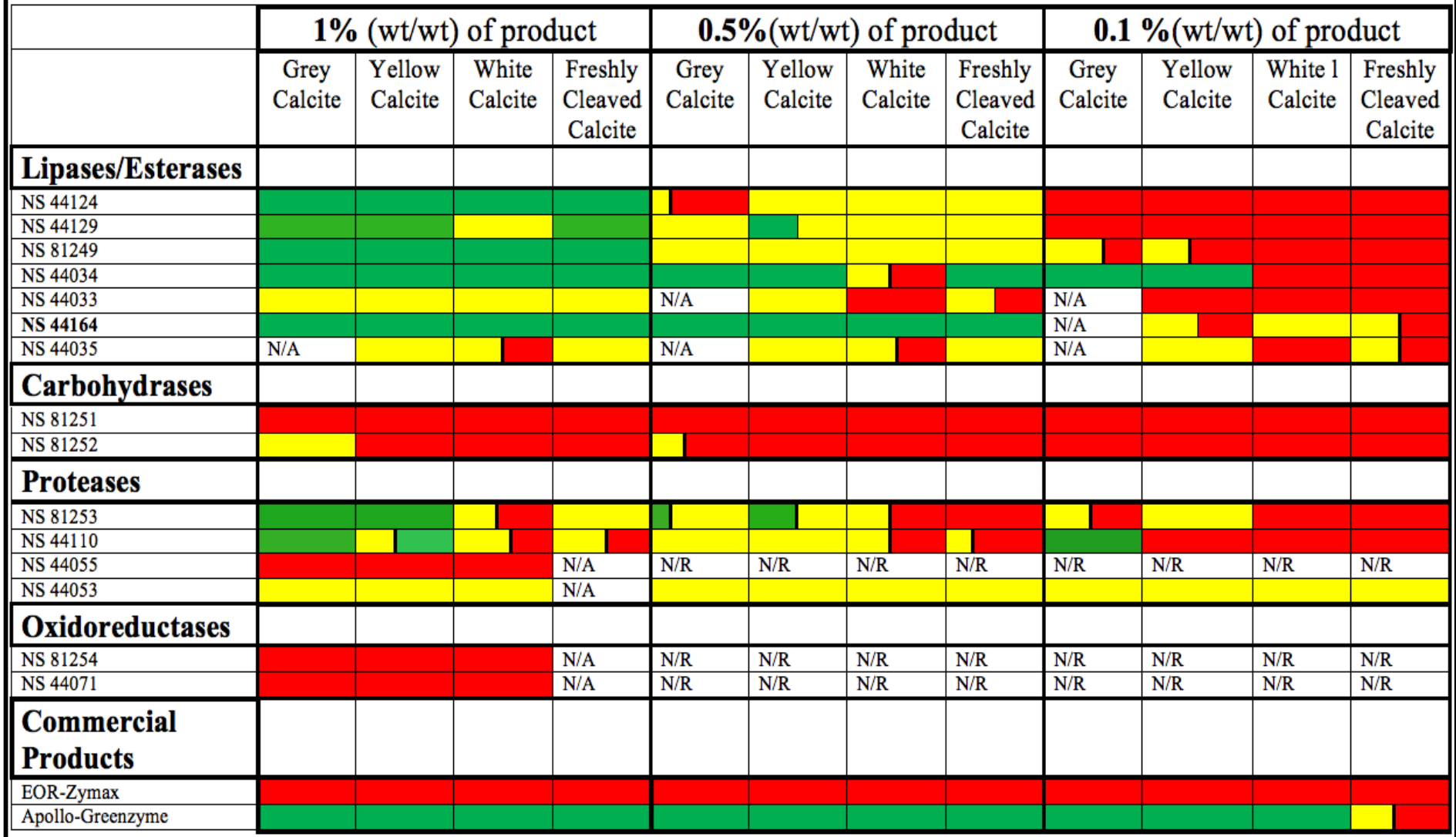
"Sandstone" Oil

Quartz ≈Sandstone & Mica ≈ Clay

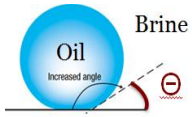

Adhesion Behavior Test: *Terms*

Adhesion	
Temporary Adhesion	
Non-adhesion	

Adhesion Map



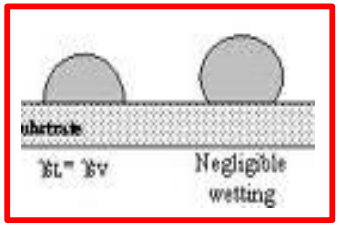
	1% (wt/wt) of product				0.5%(wt/wt) of product				0.1 %(wt/wt) of product			
	Grey Calcite	Yellow Calcite	White Calcite	Freshly Cleaved Calcite	Grey Calcite	Yellow Calcite	White Calcite	Freshly Cleaved Calcite	Grey Calcite	Yellow Calcite	White l Calcite	Freshly Cleaved Calcite
Lipases/Esterases												
NS 44124												
NS 44129												
NS 81249												
NS 44034												
NS 44033					N/A				N/A			
NS 44164									N/A			
NS 44035	N/A				N/A				N/A			
Carbohydrases												
NS 81251												
NS 81252												
Proteases												
NS 81253												
NS 44110												
NS 44055				N/A	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
NS 44053				N/A								
Oxidoreductases												
NS 81254				N/A	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
NS 44071				N/A	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
Commercial Products												
EOR-Zymax												
Apollo-Greenzyme												

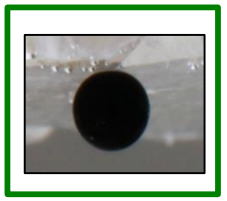


Water *– Wet State*
Θ =0-70
Andersen, 1986

Contact Angles

Calcite in SW Θ = 38 ± 7°

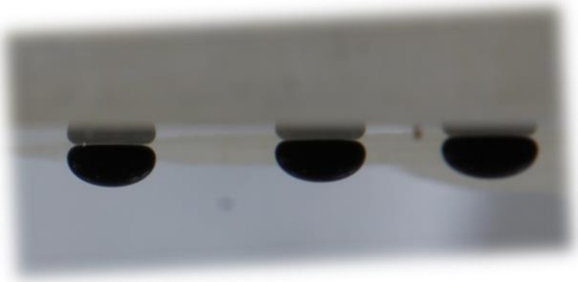




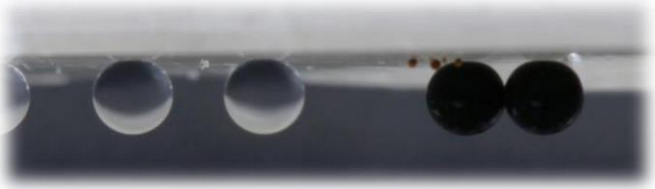
Lipases/Esterases	**0°** (100 % reduction)
Carbohydrases	No significant changes
Proteases	Mixed Result
Oxidoreductases	No significant changes
EOR-Zymax	No significant changes
Apollo-Greenzyme	25 % decrease

Enzyme vs surfactant

Surfactant
(*Sodium dodecyl sulfate*)

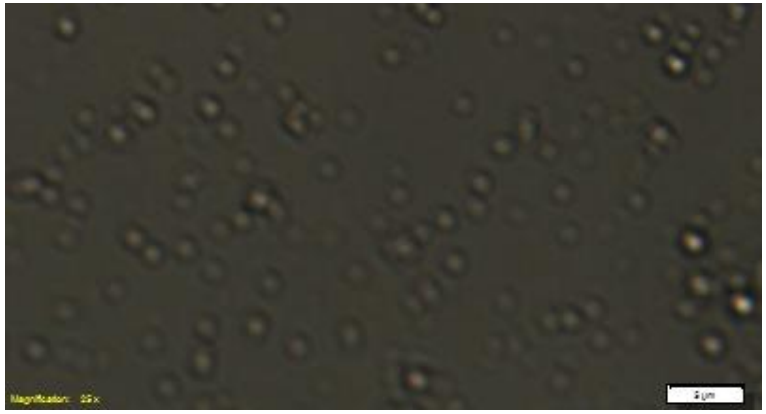


Enzyme (*lipase*)



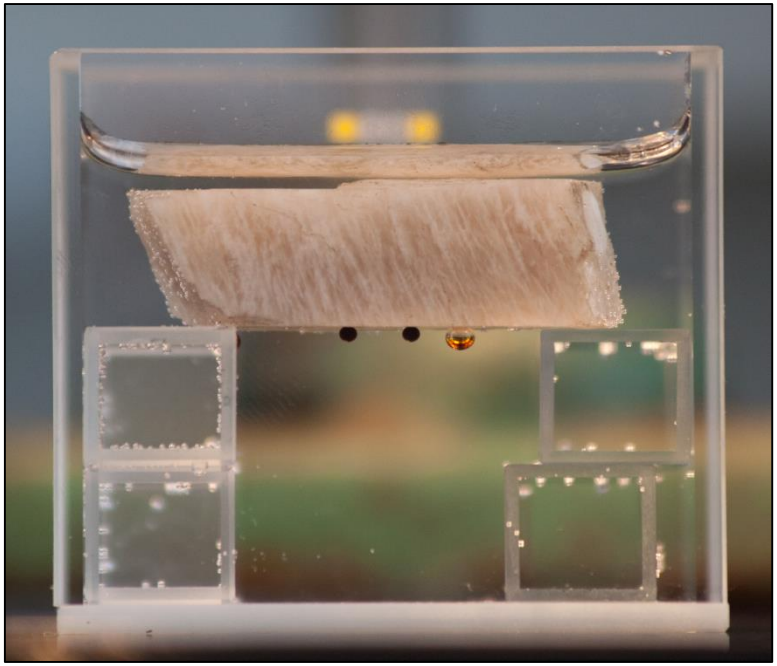
- Enzyme acts on the solid interface, while surfactant affects oil-water surface tension
- Adsorption of enzyme on solid is necessary mechanism (unlike surfactant)
- Although enzyme only slightly affects the surface tension, it may promote emulsification

Stable emulsions with submicrometer drop sizes may be formed



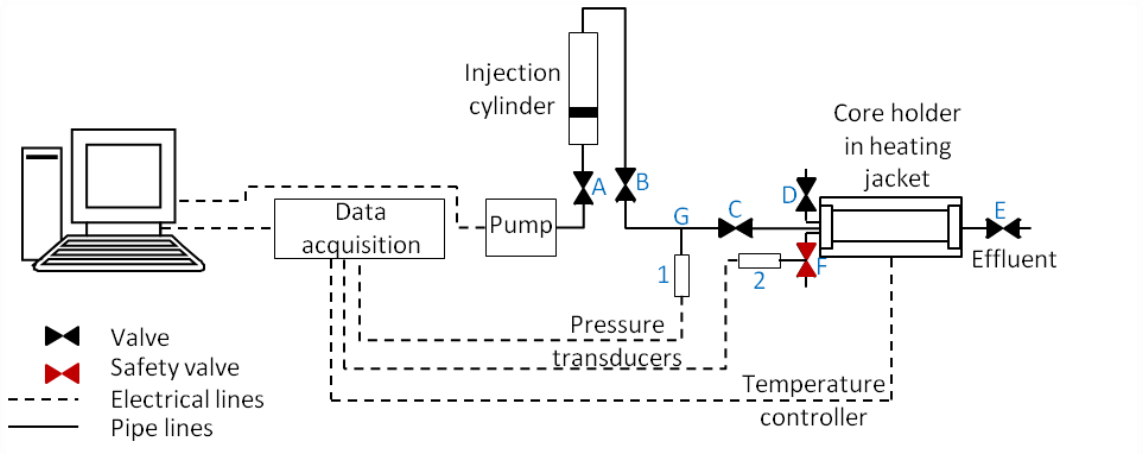
Dynamic desorption tests

- A mineral soaked for 30 min in an enzyme or enzyme product seawater solution
- Removed and put into SW
- The oil drops put in contact after 0, 30min, 60min,…,48 days
- Adhesion behavior monitored

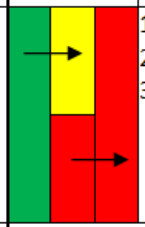
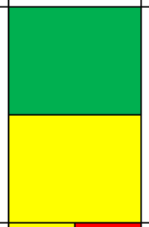
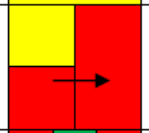
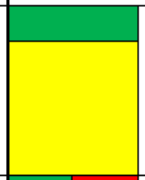
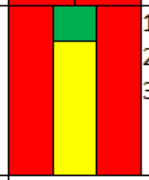


Penetration tests

- Outcrop chalk core saturated by seawater
- Injection of 1 PV enzyme/enzyme product solution
- Injection of several PV of seawater
- Measuring of enzyme in the effluent (Bradford assay)



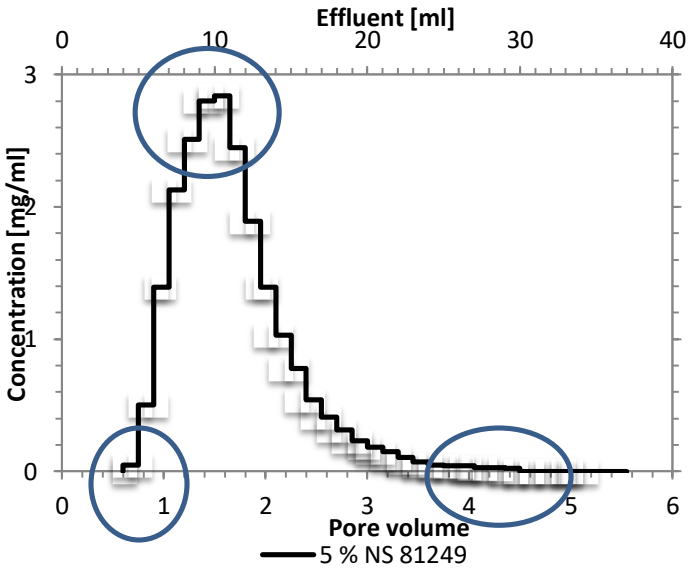
Penetration vs desorption: summary of the results

	Adhesion Test				Penetration	
	Quartz		Calcite		Sandstone	Chalk
	State	Time	State	Time		
BSA 1%		1. 0- 40 min 2. 45-70 min 3. 75 min +		During 42 days n/a-t/a	Anette: Penetrate, Overall recovery of BSA – 82-87%	Alsu: No penetration of BSA
BSA 0.5%	N/A			T/a-a was first 122 min, after 132 min – only a	N/A	N/A
NS81249-Prod		48 days		1. 0-30 min 2. 45-145 min 3. 155 min +	N/A	Birgit: Penetrate, Overall recovery 35%
NS81249-Purified		1. 0-83 min 2. 92-200 min	N/A		Anette: Penetrate, overall recovery – 87%	Alsu: Penetrate in principal
NS44164-Prod		39 days		1. 0-20 min 2. 25-90 min 3. 100 min+	N/A	Birgit: No penetration
NS44164-Purified		1. 0-202 min 2. 215-276 min	N/A		N/A	N/A
Who has done	Anette				Alsu/Anette/Birgit	

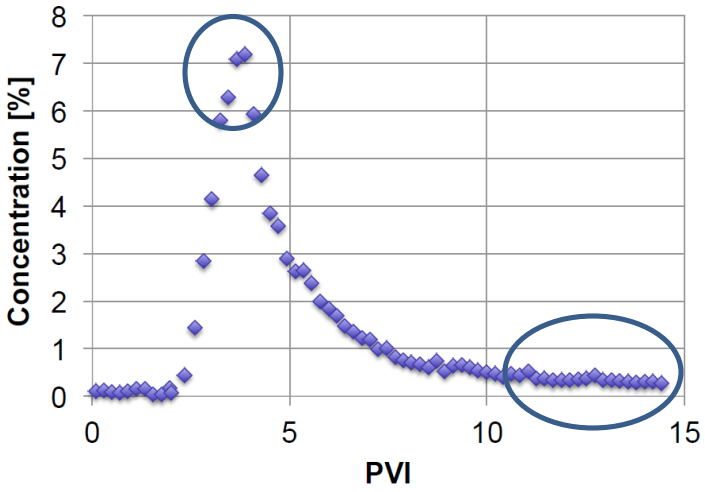
Penetration vs desorption tests

- Similar results for the BSA protein (no penetration = no desorption)
- Different results for enzyme products
  - Sometimes the results of the two tests are correlated, sometimes they are not;
  - Sandstone: no desorption, but the enzyme penetrates;
  - Chalk: reasonably fast desorption (ca. 100 minutes) but no penetration
- Behavior of pure enzymes is still to be studied

“Anomalous diffusion”?



(A. Lunde)



(B. Haastrup)

- Sometimes enzyme arrives to the outlet earlier than after 1 pvi (in sandstone)
- Highly asymmetric production profiles (unlike those predicted by the diffusion/dispersion models)
- Long “production tails”

Conclusions

- The main mechanism of enzymatic action is adsorption on the solid-liquid interface
- Lipases/esterases seem to be the most perspective group of enzymes
- Adsorption may be irreversible, or desorption may be very slow. This creates a challenge of enzyme loss inside the rock
- Penetration tests show that enzymes are sometimes “lost” inside the rock
- Production data show the signs of anomalous transport behavior (anomalous diffusion) of the enzymes