

Enhanced Oil Recovery with Application of Enzymes

Alsu Khusainova

PhD Thesis

Center for Energy Resources Engineering
Department of Chemical and Biochemical Engineering
Technical University of Denmark
Kongens Lyngby, 2800
Denmark

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Preface

This thesis is submitted as a partial fulfillment of the requirement for the PhD degree at Technical University of Denmark (DTU). The work has been carried out at the Department of Chemical and Biochemical Engineering, Center for Energy Resources Engineering (CERE) from December 2011 to February 2016 under the supervision of Alexander Shapiro and John Woodley. The project was funded by Innovation Fund Denmark, Novozymes AS, Mærsk Oil and Gas AS and DONG AS within the scope of the BioRec project.

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Alsu Khusainova
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Kongens Lyngby, Denmark

This dissertation is dedicated to my parents for their passion for knowledge and education that they transferred to me...

Summary

Enzymes have recently been reported as effective enhanced oil recovery (EOR) agents. Both laboratory and field tests demonstrated significant increase in the ultimate oil production. Up to 16% of additional oil was produced in the laboratory conditions and up to 269 barrels of additional oil per day were recovered in the field applications. The following mechanisms were claimed to be responsible for the enhancement of the oil production due to enzymes: wettability improvement of the rock surface; formation of the emulsions; reduction of oil viscosity; and removal of high molecular weight paraffins. However, the positive effect of enzymes on oil recovery is not that obvious. In most of the studies commercial enzyme products composed of enzymes, surfactants and stabilisers were used. Application of such samples makes it difficult to assign a positive EOR effect to a certain compound, as several components of commercial mixture might possess surface-active properties. Hence, the main goals of the present study were to establish whether enzymes alone can improve oil production and to identify mechanisms that might underlie enzymatic EOR (EEOR), especially, under conditions of the North Sea petroleum reservoirs.

At the first stage of the work enzyme samples that might have potential for EOR applications were selected. Wettability tests such as measurements of contact angles and determination of adhesion behaviour were applied as screening tools. The group of lipases/esterases demonstrated strong ability to detach oil from the calcite surface and was identified as the most promising group for further investigations. Wettability improvement due to protein adsorption onto the mineral was proposed as the main mechanism for EEOR. It was also proved that the enzyme molecules themselves caused change of the wetting state of calcite, while presence of stabilising ingredients did not interfere the results.

Implementation of such a mechanism of enzymatic action under reservoir conditions might be limited by retention of the protein molecules in the porous medium. In order to verify this hypothesis, adsorption behaviour of enzymes/proteins on the reservoir rocks was studied by application of the static adhesion tests and adsorption experiments on powders, as well as of dynamic flow-through experiments. It was established that enzymes are indeed significantly lost during the transport in the porous media due to the irreversible adsorption. The adsorption capacity of carbonate material was found to be much higher compared to sandstone. Various methods (for example, change of ionic strength and pH of the enzyme solution and displacing fluid) were applied in order to desorb attached protein molecules, but no desorption was observed.

Another possible mechanism that might underlie EEOR is formation of enzyme-stabilised emulsions. Similar to the wettability screening, lipases/esterases demonstrated the best surface-active properties: they formed the most stable emulsions with rather small drops. Light fractions of the crude oil participated mostly in formation of the protein-stabilised emulsions. Incubation of the oil-[enzyme + sea water] systems was found to be important in order to obtain high stability of emulsions. Combined application of enzymes and solid particles was an alternative way to increase emulsion stability.

Summary

Other crude oil-brine interaction tests revealed additional problems that can rise during the application of enzymatic EOR. Interaction of the enzyme solution with the crude oil can induce gelation/emulsification of the propylene glycol (the main component of the enzyme product stabilisers). Moreover, when purified enzyme containing almost no stabilisers was used, a highly viscous oil-in-water emulsion was formed.

Finally, assessment of enzymes as EOR agents under conditions similar to the conditions of the petroleum reservoirs was carried out in core flooding experiments. Two types of enzymes (lipase and amylase) were selected based on the results from the wettability and emulsion studies. They were only tested in tertiary mode, employing various injection schemes. Application of enzymes in sandstone core samples resulted in increase of the ultimate oil production by 0.23-1.69% relative to original oil in place, while no additional oil due to enzymes was produced from chalk. Wettability change was confirmed to be the main EOR mechanism, while emulsification plays less significant role.

Overall, enzymes have possessed low potential for EOR applications at least in sandstone and chalk reservoirs containing light crude oils. An alternative technique that will shift adsorption balance towards reversible adsorption should be established in order to make enzymatic EOR an effective and economically feasible oil recovery method.

Dansk Resumé

Enzymer er for nylig blevet rapporteret, som effektive stoffer for forbedret olieindvinding (EOR). Både laboratorie undersøgelser og felttest viste en markant stigning af olieproduktion. Op til ekstra 16 % af olien blev produceret i laboratorie eksperimenter og op til ekstra 269 tønder olie per dag blev fremstillet under feltforsøg. Det var foreslået, at følgende mekanismer har medvirket til øget olieproduktionen på grund af enzymer: forbedringer af bjergartsoverfladens befugtningsevne; dannelse af emulsioner; reduktion af olieviskositet; og fjernelse (kemisk nedbrydning) af højmolekylære paraffiner. Umiddelbart kan gevinsten ved at bruge enzymer til olieindvinding være svær at få øje på. I de fleste af de undersøgelser, har man anvendt kommercielle enzymprodukter bestående af enzymer, overfladeaktive stoffer og stabilisatorer. Anvendelse af sådanne produkter gør det besværligt at tildele én positiv EOR effekt til én bestemt komponent, da en eller flere komponenter kan have overfladeaktive egenskaber. Hovedformålet for denne undersøgelse var derfor at fastslå, om enzymer alene kan forbedre olieproduktionen og identificere de mekanismer, der kan ligge til grund for enzymatiske EOR (EEOR), især under de forhold der findes i reservoirer i Nordsøen.

I den første del af projektet, blev enzymer, der kunne have et potentiale for forbedret olieindvinding, valgt. Befugtningsevnen var bestemt via screening af kontaktvinkler og adhæsion. Lipaser/esteraser viste en stærk evne til at frigøre olie fra calcit overflader og blev identificeret som mest interessante gruppe for yderligere undersøgelser. Forbedringer af befugtningsevne, som følge af protein adsorption på mineralet, blev foreslået som den vigtigste mekanisme i EEOR. Det blev også fundet, at selve enzymmolekylet forårsagede ændringer af befugtningstilstanden af calcit, mens stabiliserende ingredienser ikke havde indflydelse på resultaterne.

Det er muligt, at tilstedeværelse af sådan mekanisme i oliereservoir kan være begrænset pga. retentionen af proteiner i porøst medium. For at verificere denne hypotese blev adsorption af enzymer/proteiner på reservoirsten undersøgt ved hjælp af de statiske adhæsion test og adsorption på pulvere, samt dynamiske gennemstrømningseksperimenter. Det blev konstateret, at en signifikant del af enzymer tapes under transporten gennem det porøse medium på grund af den irreversible adsorption. Det blev fundet at adsorptionsevnen på karbonat materialer er meget højere i forhold til sandsten. Forskellige metoder (fx ændring af ionstyrke og pH af enzymopløsningen og forskydningsfluid) blev anvendt i forsøget på at desorbere proteinmolekylerne, men desorptionen lykkedes ikke.

En anden muligt mekanisme som kan forklare EEOR er dannelse af enzym-stabiliserede emulsioner. I overensstemmelse med screeningen af befugtningsevnen, udviste lipaser/esteraser de bedste overfladeaktive egenskaber: De dannede de mest stabile emulsioner med meget små dråber. De lette fraktioner af råolien dannede primært protein-stabiliserede emulsioner. Inkubering af olie-[enzym + havvand] systemet viste sig at være vigtig for at opnå høj stabilitet af emulsioner. Anvendelse af en kombination af enzymer og faste partikler var en alternativ måde at øge emulsionsstabiliteten på.

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Flere studier af råolie-saltvand interaktioner afslørede yderligere problemer, som kunne opstå ved anvendelse af enzymatisk EOR. Interaktion af enzymopløsningen med råolien kan medføre geldannelse/emulgering af propylenglycol (den vigtigste komponent af stabilisatorerne i enzymproduktet). Derudover, førte anvendelse af det oprensede enzym med et minimalt indhold af stabilisatorer til dannelse af høj-viskøse olie-i-vand emulsioner.

Endelig blev enzymer testet under betingelser svarende til olie reservoirer, ved hjælp af kerne fortrængningsforsøg (*core flooding experiments*). To typer af enzymer (lipase og amylase) blev udvalgt på baggrund af resultater fra studierne af befugtningsevnen og emulsioner. De blev kun testet i tertiær ”mode”, og der blev brugt forskellige injektionsplaner. Anvendelse af enzymer i sandsten kernepøver resulterede i en forøgelse af olieproduktionen med 0,23-1,69% i forhold til den oprindelige oliemængde i kernen (*original oil in place - OOIP*), mens ingen ekstra olie blev produceret i kridtprøverne. Det blev bekræftet at ændringen af befugtningsevnen var den primære EOR mekanisme, mens emulgering spillede en mindre rolle.

Samlet set konkluderes, at enzymer har et lavt potentiale for forbedret olieindvinding, når der er tale om sandstens- og kridtreservoirer, som indeholder lette råolier. En alternativ teknik, der vil ændre adsorptionsbalancen mod reversibel adsorption, vil skulle udvikles førend enzymatisk EOR kan anses at være en effektiv og økonomisk rentabel olieudvinding metode.

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Chapter 1. Introduction

*Burning petroleum as a fuel “would be akin to firing up a kitchen stove with bank notes”
Mendeleev D.I. (1834-1907)*

Although the economy of the modern society heavily relies on hydrocarbons and its derivatives, the efficiency of oil extraction is still quite low. Approximately 1/3 of the existing oil reserves is being recovered, mainly by utilisation of primary and secondary methods of recovery (Sandrea and Sandrea, 2007). There is plenty of room to improve the production, even if 100% recovery might be physically impossible.

According to ExxonMobil (Cohen, 2011), in USA, approximately half of the oil is processed to produce gasoline. Another third of the produced oil is used for the production of diesel and jet fuels. However, in the recent years, the share of oil in the energy sector is slowly, but surely decreasing, due to the development of the alternative energy sources and constant improvement of technologies for harvesting non-fossil derived energy (Pickens, 2015). Nevertheless, even if energy sector becomes 100% hydrocarbon-free in the future, the demand in oil will still be significant. The reason for this is that oil serves as a raw material for production of, for example, polymers, detergents, solvents, *etc.* Therefore, it is hard to imagine an oil-free economy in the foreseeable future, which underlines the necessity to apply more efficient, sustainable and economically viable oil recovery methods. Even though the oil prices are volatile, there are at least two major reasons for development of such methods:

1. The economical reason: to expand the lifetime of already developed oil reservoirs, thus decreasing the prime cost of the extraction.
2. Sustainability: extended reservoir lifetime implies that oil, which is a limited natural resource, will be available for a longer time.

In this work we have concentrated on one of the possible methods for enhancing the oil recovery, namely, tertiary recovery with the aid of enzymes.

1.1. Enzymes for Enhanced Oil Recovery: Why can it be Interesting?

Enzymes are rather well known agents in the field of petroleum engineering (Harris and McKay, 1998). They have been successfully used for the following purposes: enzyme pre-treatment of biopolymers, gel breaking, desulphurization, and enzyme-based production of an acid. However, the area of enhanced oil recovery (EOR) is quite new for the enzyme application. Laboratory studies and field cases have reported enzymes to be quite effective bioagents that can significantly improve oil production (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Nasiri *et al.*, 2009; Ott *et al.*, 2011). The recovery factor was increased by up to 16% in the laboratory displacement studies and up to 269 barrels of oil per day (BOPD) more were produced in the field applications (Feng *et al.*, 2007; Moon, 2008; Nasiri *et al.*, 2009; He and Zhonghong, 2011). Summary of the field applications of enzymatic enhanced oil recovery (EEOR) is given in Table 1.

Chapter 1. Introduction

Table 1. Summary of enzymatic enhanced oil recovery field applications (? – unknown).

Oil Field	Used enzyme	Additional Components added to the enzyme	Concentration of the Enzyme Product	Amount of the Enzyme Product Injected	Type of Injection	Increase in Oil Production	Incremental Oil Produced	Reference
Dagang, China	Enzyme derived from the bacteria that can detach oil from sand	Surfactant (to reduce the cost of the enzyme product and ensure synergetic effect)	8% of the modified enzyme	125.8 bbl	Huff and Puff	From 29.2 BOPD to 77.6 BOPD	2409 bbl (for 60 days)	Feng <i>et al.</i> , 2007
Bailse, China	Enzyme derived from the bacteria that can detach oil from sand	Surfactant	8% of the modified enzyme	?	Huff and Puff	From 4.4 BOPD to 12.4 BOPD	496.4 bbl	Feng <i>et al.</i> , 2007
Dagang, China	Enzyme derived from the bacteria that can detach oil from sand	Surfactant	First plug – 4% of the modified enzyme, second plug – 6%.	73 bbl	Flooding	From 14.6 BOPD to 36.5 BOPD	7902 bbl	Feng <i>et al.</i> , 2007
Mann, Myanmar	Apollo GreenZyme®	Stabiliser (sodium diacetate)	2% in KCl solution (well 395) Recycled enzyme from well 395 (well 101)	?	Flooding	From 14 BOPD to 18 BOPD (well 395) From 10 BOPD to 16-17 BOPD (well 101)	530 bbl (well 395) 1636 bbl (well 101)	Ott <i>et al.</i> , 2011

Chapter 1. Introduction

Table 1 (continuation). Summary of enzymatic enhanced oil recovery field applications (? – unknown).

Oil Field	Used enzyme	Additional Components added to the enzyme	Concentration of the Enzyme Product	Amount of the Enzyme Product Injected	Type of Injection	Increase in Oil Production	Incremental Oil Produced	Reference
Daqing Chaoyang gou, China	Biology enzyme	?	2%	$1.265 \cdot 10^4 \text{ m}^3$ (0.6%PV)	Flooding	From 27.9 t to 30.7 t	2208 t	He and Zhonghong, 2011
Pekabyry, Indonesia	Apollo GreenZyme®	Stabiliser (sodium diacetate)	?	?	Flooding	From 40-68 BOPD to 89-121 BOPD (in different wells)	?	Apollo GreenZyme® official website
La Salina, Venezuela	Apollo GreenZyme®	Stabiliser (sodium diacetate)	?	?	Flooding	From 18-158 BOPD to 40-269 BOPD	?	Apollo GreenZyme® official website

Researchers distinguish four different mechanisms that cause increase of oil production after application of enzymes: improvement of the rock surface wettability towards more water-wet state (Nasiri *et al.*, 2009); emulsification due to decrease of the interfacial tension (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Nasiri *et al.*, 2009); oil viscosity reduction (He and Zhonghong, 2011; Moon, 2008); removal of high molecular weight paraffins (Moon, 2008). Based on field trials, it has been suggested that main mechanisms underlying EEOR are unplugging of low permeable layers from clogged organic material, as well as modification of oil and rock surface properties.

Besides these promising results, there are other advantages for application of EEOR. Enzymes are environmentally friendly compounds. When oil is recovered it is possible to restore and re-use enzymes (Apollo GreenZyme® official website; Feng *et al.*, 2007).

Even though results on application of EEOR are quite optimistic, application of commercial products or mixtures of enzymes and surfactants in most of the investigations and field studies makes it difficult to determine, what component of the mixture and to what extent affects amount of the recovered oil. Nasiri (2009) reported some results on experiments, in which a specific class of enzymes, lipases/esterases, was employed. To the best knowledge of the author this was the first case where pure enzyme products were studied as EOR agents. Still, efficiency of EEOR as well as working mechanisms require additional verification with regard to the ingredient that causes enhancement of oil recovery.

1.2. Enzyme Products Designed for EOR Applications Available on the Market

The survey showed that there are five commercially available enzyme products on the market:

1. StimuZyme™, manufactured by BreakThrough Ventures, LLC. Website: www.btventuresllc.com/index.html. The product is claimed to change the wetting state of the reservoir rock from oil- or intermediate-wet to water-wet state, thus enabling the oil mobilisation. StimuZyme™ was successfully applied in China for years, with the increase of recovery factor of 25%. According to the data from the company website, the effect was also observed after the injection of the enzyme solution during quite long period.
2. Petrozyme®, manufactured by Petrologic LLC. Website: www.petrologicusa.com. The product is claimed to be based on enzymes where the oil digestion properties are neutralized, but the ability to attach to hydrocarbons and to release them is kept. The enzymes are deemed to seek and release hydrocarbons from oil globules, without being consumed in the releasing mechanism. The large oil globules are therefore reduced in size and covered by an enzyme layer, reducing the interfacial tension between the oil globule and aqueous phase. The technology is used in the Far East, US and South America (enhancement data not given).
3. GreenZyme® is manufactured by Apollo Separation Technologies Inc. Website: www.apollogreenzyme.com. The enzyme product is believed to bind to the surface of the oil-particle complex in the reservoir rock, triggering breakdown of the complex. Then the enzyme

covers the surface of the released particle. Thereby the particle is prevented from re-agglomeration with the oil, which enables the oil to be carried away with the flow. The product is claimed to be the first bio-enzyme used in EEOR, which is nowadays utilised for oil recovery projects all over the world.

4. WF-E OilStim Catalyst, manufactured by Wellfix. Website: www.wellfixtechnology.com . The suggested working mechanism is that enzymatic treatment improves oil recovery by catalysing breakdown of larger molecules in oil into smaller molecules, which improve flow characteristics of oil, including heavy oil. According to the data from the website, the product was successfully tested in China, Indonesia, Venezuela and Texas.

5. Z Enzyme. Website: www.zenzyme.webs.com . It is claimed that the viscosity of the oil is reduced, and thus its mobility is enhanced. It is, however, not specified which mechanism is responsible for the reduction of the viscosity. The data regarding field studies was not found.

It can be concluded that the main working principle of the enzymes is to change the state of the system from oil-wet to water wet, i.e. to “free” the oil globules into the porous structure of the reservoir rock by reducing the adhesion of the oil to the rock. Furthermore, the enzyme is claimed to attach to the oil globules, breaking them down into smaller oil drops. As a result, the diffusion of the smaller droplets out of the porous medium (in the direction of the flow) is made easier.

The data given in the open sources on these commercially available enzyme products is, however, very limited. The available information does not allow drawing an unambiguous conclusion that the enzyme itself, but not another component (or components) of the enzyme product is responsible for the enhancement of the oil recovery. Only for the WF-E OilStim Catalyst it is mentioned, that the recovery tests were performed both with and without enzyme treatment, and in the latter case the positive effect was achieved. Generally, the mechanisms of enzymatic action, as well as conditions for application of the EEOR and possible challenges are not well studied and systematised. Therefore, the present study was initiated.

1.3. Research Objectives

The study was carried out in order to assess potential of enzymes as EOR agents. The following questions have been answered during the investigation:

- Can enzymes enhance oil recovery?
- Which group(s) of enzymes has (have) the highest potential for EOR applications?
- Which mechanisms underlie EEOR?
- What problems might arise during application of EEOR?
- What conditions and injection schemes will be optimal for implementation of EEOR technique?

In order to fulfil these objectives, several experimental procedures have been developed and followed during the project. In particular, an advanced core flooding set-up was built for penetration and recovery studies.

1.4. Thesis Outline

This thesis is divided into eight chapters. In the first two chapters general overview of the research topic is given. The rest of the thesis deals with the accomplished experimental work. Brief description of the chapters is presented below.

Chapter 1 introduces the reader to the subject of EEOR and explains the motivation behind the project. Research goals as well as organisation of the thesis are also described in *Chapter 1*.

Chapter 2 presents a discussion on potential role of enzymes in EOR applications. Protein behaviour at oil-water and water-solid interfaces is described.

Chapter 3 is the first stage of the entire experimental investigation that was conducted to identify the group of enzymes with the highest potential for EOR applications. Initial selection of the samples for the screening was accomplished under the expertise of Novozymes A/S. Adhesion behaviour tests in conjunction with contact angle measurements were used as screening tools. The group of lipases/esterases demonstrated ability to fully detach oil from the calcite surface and was chosen as the most promising enzyme group. Effect of the pure enzyme was confirmed by experiments with purified enzyme samples. EOR mechanisms that might be realised after application of enzymes are proposed in the chapter.

Chapter 4 describes investigation of enzyme/protein adsorption at water-solid interface. Various substrates such as minerals, powders, rock samples were used to imitate sandstone and chalk reservoir materials. Two types of static (adhesion test on minerals and adsorption on powders) and one type of dynamic (flow-through test) experiments were carried out. Irreversible protein adsorption was found to be significant. Various methods were applied in order to desorb attached biomolecules, but no desorption was obtained. Hence, enzyme retention in porous media might become a serious obstacle on the way of application of EEOR.

Chapter 5 is emulsion and crude oil-brine interaction study. A methodology of the emulsion preparation was developed. Several groups of enzymes were screened for the highest surface-activity at oil-brine interface. The lipase/esterase-stabilised emulsions were found to be the most perspective from the EOR point of view. Combined effect of enzymes and solid particles was also investigated. The crude oil-brine interaction experiments revealed potential problems that might affect work of enzymes.

Chapter 6 presents results of the core flooding experiments. Effect of lipase and amylase on oil recovery was tested in tertiary mode in sandstone and chalk cores. Various injection schemes were tested. Application of the enzymes resulted in relatively low recovery values during the tertiary recovery stage (up to 1.69% relative to original oil in place in sandstone and no additional

oil produced in chalk). Factors affecting successful application of EOR as well as overall performance of enzymes for the purpose of oil production are discussed in the chapter.

Chapter 7 summarizes the key results of the thesis, while recommendations for future works are given in *Chapter 8*.

A detailed description of an oil displacement experiment can be found in Appendix 1. The joint author statements for published and submitted manuscripts are included in Appendix 2.

1.5. List of Research Papers and Conference Contributions

The results of the research that was carried out during the PhD project have been presented through the following research papers and conference contributions:

Research Papers:

Khusainova, A., Nielsen, S.M., Pedersen, H.H., Woodley, J.M., Shapiro, A., 2015. Study of Wettability of Calcite Surfaces Using Oil–Brine–Enzyme Systems for Enhanced Oil Recovery Applications. *Journal of Petroleum Science and Engineering*, 127, 53–64.

Khusainova, A., Shapiro, A., 2016. Study of Enzyme/Protein Adsorption for Chalk and Sandstone Reservoir Rocks. *Submitted to Journal of Petroleum Science and Engineering*.

Khusainova, A., Shapiro, A., 2016. Experimental Investigation of Enzymatic Enhanced Oil Recovery. *Submitted to Journal of Petroleum Science and Engineering*.

Conference Contributions:

Khusainova, A., Shapiro, A.A., Stenby, E.H., Woodley, J.M. Wettability improvement with enzymes: application to enhanced oil recovery under conditions of the North Sea reservoirs. The IEAEO's 33rd Annual Workshop and Symposium, August 27-30th, 2012, Regina, Canada. (Oral)

Khusainova, A., Shapiro, A.A., Stenby, E.H., Woodley, J.M. Potential of Enzymes as Enhanced Oil Recovery. 75th EAGE Conference & Exhibition incorporating SPE EUROPEC 2013, 10 – 13 June 2013 in London, United Kingdom. (Oral)

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Cohen, K., 2011. There's More in a Barrel of Oil than Just Gasoline. ExxonMobil Perspectives <http://www.exxonmobilperspectives.com/2011/06/05/theres-more-in-a-barrel-of-oil-than-just-gasoline/>

Feng, Q.X., Ma, X.P., Zhou, L.H., Shao, D.B., Wang, X.L., Qin, B.Y., 2007. EOR Pilot Tests with Modified Enzyme in China. In: Proceedings of the SPE 107128 Presented at SPE Europe/EAGE Annual Conference and Exhibition, London, United Kingdom, June 11–14.

Chapter 1. Introduction

Harris, R.E, McKay, I.D., 1998. New Applications for Enzymes in Oil and Gas Production. SPE European Petroleum Conference held in The Hague, The Netherlands, 20-22 October 1998, 467-475.

He, L., Zhonghong, Z., 2011. Biology Enzyme EOR for Low Permeability Reservoirs. In: Proceedings of the SPE 114281 Presented at the SPE Enhanced Oil Recovery Conference, Kuala Lumpur, Malaysia, July 19–21.

Moon, T., 2008. Using Enzymes to Enhance Oil Recovery. JPT online, SPE.

Nasiri, H., Spildo, K., Skauge, A., 2009. Use of Enzymes to Improve Waterflood Performance. In: Presented at the International Symposium of the Society of Core Analysis, Noordwijk, Netherlands, September 27–30.

Ott, W.K., Nyo, T., Aung, W.N., Khaing, A.T., 2011. EEOR Success in Mann Field, Myanmar. In: SPE 114231 Presented at the SPE Enhanced Oil Recovery Conference, Kuala Lumpur, Malaysia, July 19–21.

Pickens, A. 2015. Global Renewable Energy Capacity Increased 120% since 2000. The Climate Group <http://www.theclimategroup.org/what-we-do/news-and-blogs/global-renewable-energy-capacity-increased-120-since-2000/>

Sandrea, I., Sandrea, R., 2007. Global Oil Reserves – Recovery Factors Leave Vast Target for EOR Technologies. Oil & Gas Journal.

Chapter 2. What can We Expect from Enzymes?

In the chapter potential roles of enzymes in enhanced oil recovery (EOR) applications are presented. Typical protein behaviour at oil-water interface is described. Key parameters regulating enzyme/protein adsorption/desorption at water-solid interface are reviewed.

2.1. What are Enzymes?

Enzymes are naturally occurring biological catalysts of the protein nature (Copeland, 2000). Being a globular protein, enzyme molecules comprise of amino acid residues that fold and form three-dimensional (3D) structures. The sequence of the amino acids determines the spatial configuration of the biomolecule and defines its catalytic properties. In addition to the enhancement/inhibition of chemical reactions, enzymes possess unique surface activity, which is determined by the presence of hydrophobic and hydrophilic functional groups. Out of the definition and characteristics given above, three potential roles of enzymes during the EOR process can be proposed *a priori* (Figure 1).

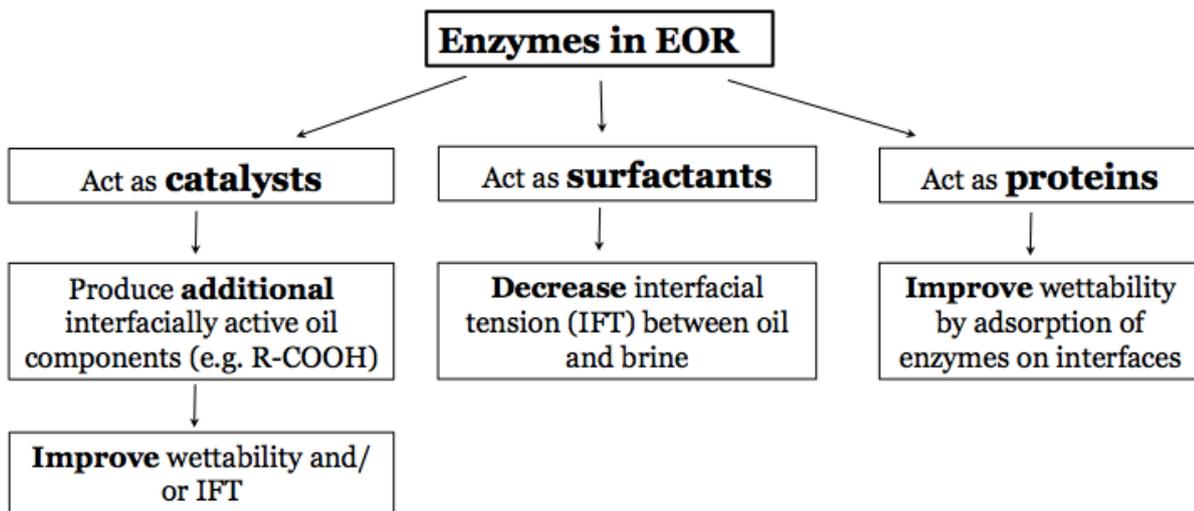


Figure 1: Potential functions of enzymes as EOR agents.

First, enzymes are biocatalysts, therefore, they can potentially use some crude oil components as a substrate. For example, one of the samples that was tested in the study is the NS44164 esterase (Chapter 3, Table 2). Its catalytic function is to enhance hydrolysis of ester bonds in lipids and other compounds. Crude oil might include esters in the form of either free compounds, e.g. a dioctylphthalate (Phillips and Breger, 1958), or as binding elements within high-molecular compounds (Kam'yanov *et al.*, 1990). Thus, hydrolysis of the ester bonds might result in the formation of carboxylic acid and alcohol. Both of the reaction products are surface-active compounds and can affect the ultimate oil recovery (Chen and Zhao, 2015; Fathi *et al.*, 2011).

Second, enzymes have both hydrophilic and hydrophobic functional groups. Hence, they can work in a way similar to surfactants: by reducing an interfacial tension (IFT) between oil and water

phases, application of enzymes can cause an emulsification (more details in Section 2.2), while decrease of IFT followed by formation of emulsions is one of the key mechanisms of EOR techniques (Dake, 1978).

Finally, enzymes demonstrate high affinity to the water-solid interface (Norde, 2008). Adsorption of the biomolecules might improve wettability of the rock surface towards oil recovery favourable conditions. More details on protein adsorption onto the water-solid interface can be found in Section 2.3.

2.2. Enzymes/Proteins and Oil-Water Interface

Adsorption of enzymes/proteins at the oil-water interface might be advantageous (e.g. emulsion and foam stabilisation in food industry) or disadvantageous (e.g. reduction of the therapeutic efficiency of drugs). From the EOR point of view, formation of the adsorbed protein layer between oil and water phases is rather beneficial as formation of such layer reduces IFT and, consequently, promotes formation of the emulsion. The EOR mechanism would be similar to that of surfactant flooding.

However, surfactant molecules and protein macromolecules perform differently at the oil-water interface (Damodaran, 2005). In general, surfactants are more efficient IFT reducers compared to proteins (Razumovsky and Damodaran, 1999). The reason for such a difference is fairly simple molecular structure of surfactants and complex structure of protein molecules. Surfactants are relatively small molecules with clearly defined hydrophilic head and hydrophobic tail. In contrast, hydrophilic and hydrophobic functional groups are spread on the surface of the protein molecule as well as some functional groups are remaining inside the globular structure. The functional groups at the surface form some hydrophilic and hydrophobic zones, but the separation is not as clear as in surfactant molecules. Nevertheless, protein molecules provide better emulsion stabilisation due to gel-like structure of the adsorbed protein layer, which can be reached at saturated monolayer and multilayer coverage modes (Dickinson, 2001). Protein fragments that do not participate in the surface interactions and become available after protein unfolding provide additional stabilising effect, which prevents coalescence of the oil drops. Steric stabilisation is also more pronounced for proteins compared to surfactants.

Protein adsorption at the oil-water interface occurs in three stages (Figure 2) (Baldursdottir *et al.*, 2010; Beverung *et al.*, 1999):

1. **Induction.** During the induction stage, IFT almost does not change. Diffusion of the protein molecules to the oil-water interface is determining parameter of the adsorption at this stage. Once protein molecules reach the surface, they begin to undergo conformational changes: macromolecules start unfolding in order to release functional groups hidden inside the globular structure. The induction stage is only characteristic for dilute protein solutions ($\leq 10\mu\text{g/ml}$) as at higher concentrations time measurement of the first two stages is limited by their fast speed.

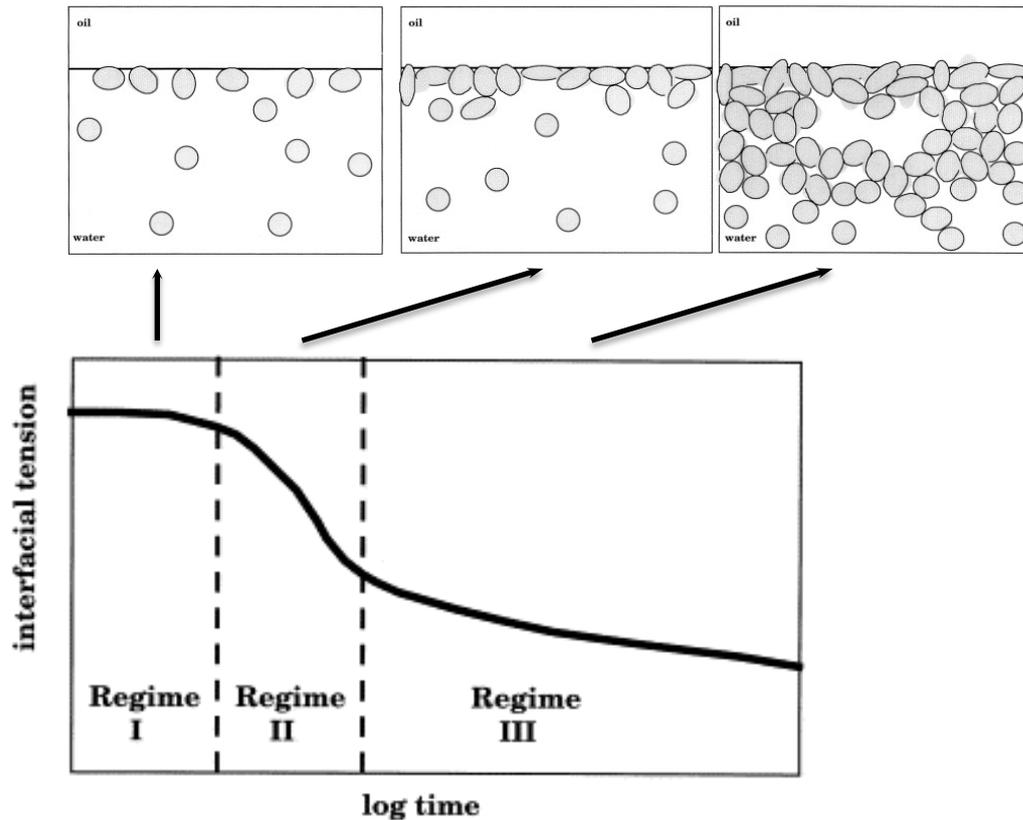


Figure 2: Three regimes of protein molecules adsorption at an oil-water interface. The circles represent original molecular structure, while the ovals represent protein molecules that underwent conformational changes (adapted from Beverung *et al.*, 1999).

2. Monolayer saturation. During the second stage the sharpest decrease of IFT is observed due to increase of the protein surface concentration. Further saturation of the adsorbed layer occurs in the two parallel ways. New protein molecules are continuously supplied by diffusion from the bulk aqueous solution. Meanwhile, proteins that are already adsorbed continue unfolding, providing new contact sites. So the molecules become attached stronger due to larger number of the available functional groups. Such conformational changes are always irreversible and no protein desorption is observed from the emulsion droplets (Bos and Vilet, 2001). Loss of 3D structure leads in turn to loss of enzymatic activity. It should be noted that for already denaturated proteins the adsorption scenario might differ from what was described above. As a result, they can possess less surface-active properties. Multilayer formation may also be initiated at the stage of monolayer saturation if proteins from the bulk solution start being attracted by the initial adsorbed layer.

3. Interfacial gelation. The IFT values demonstrate steady decline during the last stage of the protein adsorption. Molecules in the already adsorbed layers continue undergoing structural rearrangements to reach the energetically favourable state, while the monolayer continues accumulating new protein molecules. As a result, a gel-like network structure, stabilised by intramolecular conformational adjustments and intermolecular interactions, is formed.

2.3. Enzymes/Proteins and Water-Solid Surface Interface

Protein adsorption from a fluid on a solid surface has been widely investigated in various areas, such as biological, medical and technological systems. Different combinations of proteins, fluids and solid adsorbents depending on an area of the interest have been extensively investigated for years. However, generalisation of the results is still quite difficult as different research groups use different procedures and the obtained results sometimes contradict each other.

Parameters controlling adsorption and further desorption of the proteins can be divided into four groups:

1. Nature and concentration of the protein. Depending on the internal stability of proteins their adsorption can be governed by the electrostatic forces or by the protein surface-induced structural changes (Norde, 1998; Norde, 2008). Hard proteins are proteins that are characterised by high internal stability. They can keep their globular structure upon attachment, and adsorption occurs only due to electrostatic attraction between the biomolecules and the solid surface. For the soft proteins with low structural stability conformational changes play a major role during adsorption and can outweigh electrostatic forces.

The proteins at low concentrations adsorb in monolayer mode. Due to surface availability they can undergo structural modifications that result in a higher contact area (Evers *et al.*, 2008). Thus, desorption of those molecules is often irreversible due to strong binding between a monolayer and a solid sorbent (Kirchman *et al.*, 1989). During adsorption at high concentrations, the proteins form multilayers, where protein molecules are packed compactly and stay folded, therefore desorption will be much higher.

2. Solid surface. Substrates for the protein adsorption can differ in surface tension, polarity and charge. The highest amount of adsorbed proteins would be on a non-polar charged material with high surface energy. (Hlady *et al.*, 1999). Wettability of the surface also highly affects protein adsorption. Adsorption of proteins is higher on the hydrophobic surfaces, whereas desorption occurs more easily from the hydrophilic sorbent surfaces (Lai *et al.*, 2008; Sethuraman *et al.*, 2004).

3. Solution. Two characteristics of the solution and of the solvent can influence their adsorption. The value of pH determines the surface charge of the protein molecules. At pH below isoelectric point, proteins are positively charged; at isoelectric point negative and positive charges of the different protein fragments are balanced, so the molecules are neutrally charged; and at pH above the isoelectric point the proteins have a positive charge. In most of the cases maximum adsorption of the proteins occurs near the isoelectric point (McLaren, 1957; Norde, W., 1986; Skujiņš *et al.*, 1974). If the charge of the sorbent is known, by changing pH it is possible to enhance or inhibit adhering of the proteins to the surface (i.e. it is possible to control electrostatic attraction/repulsion forces).

Increase of the ionic strength of the solution has an opposite effect compared to increase of pH (Jones and O'Melia, 2000; Kirchman *et al.*, 1989). The increased salt content leads to compression of the double layer, which in turn decreases electrostatic repulsive forces between like-

charged protein molecules and solid surface and reduces electrostatic attractive forces between oppositely charged protein molecules and solid surface.

A special example of protein adsorption from high ionic strength solution is adsorption from seawater, that is, the case for application of enzymatic EOR. There are few studies of the protein adsorption from seawater (Kirchman *et al.*, 1989; Raspor, 1991; Taylor *et al.*, 1994), and they are in a good agreement between each other. Independently of the surface type, protein adsorption is higher in seawater than in low ionic solutions (Kirchman *et al.*, 1989). The process is mainly governed by hydrophobic interactions as solution counter-ions neutralize protein charge and electrostatic forces are not that important (Kirchman *et al.*, 1989; Raspor, 1991). It was found that among all the counter-ions contained in genuine seawater (pH=7.8) ions of Ca^{2+} and Mg^{2+} made the highest contribution for neutralizing BSA, whereas effect of Na^+ ions was quite negligible (Raspor, 1991). Adsorption was reversible when bulk protein concentrations were high ($>10 \mu\text{g/ml}$) (Kirchman *et al.*, 1989). 65% of protein could be recovered within 1 min. Secondary binding of the desorbed proteins was also observed (Taylor *et al.*, 1994).

4. External parameters. External parameters include temperature, pressure and porosity of the solid adsorbent. Increase of the temperature in most of the cases promotes adsorption rate and consequently amount of the adsorbed protein (Eltekova and Eltekov, 2008; Kiesel *et al.*, 2014). It should be noted that depending on conformational stability of the protein desorption of the protein molecules in protein-free buffer can also be heat-induced (Kiesel *et al.*, 2014). Hard proteins (e.g. lysozyme and RNase A) keep their mobility at the interface and it increases with increase of the temperature. Soft protein molecules (e.g. BSA) due to low internal stability start denaturing at about 60°C . Unfolding of the protein leads to higher protein-surface contact area that hinders desorption of the biomolecules.

Recent studies (Koo *et al.*, 2013; Wirkert *et al.*, 2014) revealed that adsorption of proteins is also pressure-induced process. The effect of pressure can be explained by conformational changes during which a “hard” protein becomes more “soft”. It should be emphasized that elevated pressure in the framework of the protein adsorption implies values of 2500-5000 bar. However, the pressure effect can be avoided by addition of stabilisers (e.g. glycerol) that keeps globular confirmation of the protein molecules (Koo *et al.*, 2013).

A general trend for the porosity dependence of the protein adsorption is increase of the amount of adsorbed molecules with increasing pore diameter (Eltekova and Eltekov, 2008). Investigation of the BSA adsorption on silica with pore diameter range between 3 and 160 nm showed that maximum of adsorbed amount occurred at a pore diameter of 80 nm with no further significant increase. Thus, dependence of the pore diameter is important for microporous and sometimes mesoporous media, and is not relevant to the most porous media of petroleum reservoirs. On the contrast, Kondo *et al.* (1989) reported that adsorption was faster and higher in small and large pores. Most likely, effect was due to lack of the space for small pores and due to availability of the space and easy diffusion for large pores.

Chapter 2. What can We Expect from Enzymes?

When it comes to the desorption of proteins, two generally observed features should be taken into account (Norde, 1986). First, desorption of proteins can be achieved in three different ways:

1. Desorption by diluting – dilution of the system with the solvent that was used for protein adsorption. When protein adsorbs to a solid surface, attachment of a molecule occurs via several segments, therefore desorption of these molecules can be quite difficult and often diluting the system with the solvent is not enough to detach the protein molecules;
2. Change of pH, ionic strength of the solvent or change of some external parameters (e.g. temperature);
3. Replacement of the adsorbed protein molecules by other protein that has higher affinity for the sorbent.

Second, adsorption of the proteins is often irreversible. Either protein strongly adsorbs to the surface and cannot be detached, or the desorbed molecules undergo some structural changes (such as unfolding, for example) during the attachment and cannot return to the original structure.

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Chapter 3. Study of Wettability of Calcite Surfaces using Oil-Brine-Enzyme Systems for Enhanced Oil Recovery Applications

The chapter was published in Journal of Petroleum Science and Engineering:

Alsu Khusainova, Sidsel Marie Nielsen, Hanne Høst Pedersen, John M. Woodley and Alexander Shapiro, 2015. Study of Wettability of Calcite Surfaces Using Oil-Brine-Enzyme Systems for Enhanced Oil Recovery Applications. Journal of Petroleum Science and Engineering, 127, 53–64.

Abstract

Enzymes have recently been considered as possible agents for enhanced oil recovery (EOR) acting at the liquid–solid interface. One way to assess this is via measuring the wettability of calcite surfaces, important for EOR methods in carbonaceous reservoirs. In the present work, we have experimentally investigated the effect of enzymes on the wettability of calcite mineral surfaces with oil–brine systems. The action of various enzymes, including esterases/lipases, carbohydrases, proteases and oxidoreductases (along with two commercial mixtures) was studied by contact angle measurements and adhesion behaviour tests. Comparative studies with a surfactant, protein, purified enzyme, enzyme stabiliser using *n*-decane (as a model for the oil) have also been carried out in order to verify experimental results. The enzymes that have the highest effect on the wettability have been identified. Those enzymes, which were found the most promising from a practical perspective, have shown the ability to fully detach oil from the surface, even at very low enzyme concentrations. For example, esterases/lipases were found to strongly affect the wettability and to remove adhesion at concentrations as low as 0.1% of the enzyme product (corresponding to 0.002–0.005% protein). Likewise, proteases could also improve wettability, although the effect was not consistent and was dependent on impurities. Other enzymes had no effect on the wettability of calcite at the concentration studied. The main mechanism of enzymatic action has been found to be replacement of oil at the solid surface by the enzyme. Other mechanisms (modification of the surface tension or catalytic modification of hydrocarbons resulting in reducing the oil viscosity) have shown to be much less pronounced from the measurements reported here.

Keywords: Enhanced Oil Recovery; Enzymes; Wettability; Adhesion; Carbonaceous Reservoirs

1. Introduction

Today, application of enhanced oil recovery (EOR) to carbonaceous reservoirs is becoming increasingly important, given the growing oil demand. Indeed, the recovery of oil from such reservoirs is usually considerably lower than that from sandstone reservoirs. Recently reported methods for EOR are mostly based on the application of biological agents such as enzymes (Feng *et al.*, 2007; Nasiri *et al.*, 2009; He and Zhonghong, 2011; Ott *et al.*, 2011). Enzymes may be particularly advantageous as EOR agents, since they are biologically produced, environmentally friendly, surface-active substances, which usually act at extremely low concentrations. Several initial field trials in China, Indonesia, Venezuela and USA have demonstrated quite promising results (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Ott *et al.*, 2011). Meanwhile, the

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mechanism of enzyme action and their efficiency have not been thoroughly investigated, especially, with respect to carbonaceous reservoirs. Consequently, there is currently no method for the selection of suitable enzymes and co-solvents, or their concentrations to apply to EOR.

Based on laboratory experiments, three potential mechanisms have been proposed to explain the positive effect of enzymes on oil extraction from the reservoir rocks (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Nasiri *et al.*, 2009; Ott *et al.*, 2011): (1) breaking the connections between oil and internal porous surface; (2) decreasing the interfacial tension (IFT) and creation of emulsions; and (3) decreasing the oil viscosity.

In all cases the mechanistic explanations result in an increase of oil mobility and, as a result, increased oil production.

The primary mechanism responsible for the successful action of enzymes is claimed to be their activity on the rock surface, breaking the oil–rock bonds (Feng *et al.*, 2007). Some authors (Moon, 2008; Ott *et al.*, 2011) have also reported a change of oil properties due to application of enzymes. For example, breakage of carbon bonds and a decrease of wax content with a consequent decrease of oil viscosity were previously reported for Apollo GreenZyme™ commercial product (Moon, 2008).

Most of the published scientific reports have used enzymes in the form of commercial mixtures. In such mixtures, enzymes are usually present in combination with stabilisers and surfactants (see for example, Apollo GreenZyme™ Material Safety Data Sheet; Feng *et al.*, 2007). This makes it difficult to assign observed effects to a particular component of the mixture, meaning that experimental work with these commercial products may lead to misinterpretations. Further research is needed in order to identify the working mechanisms of pure enzymes and the relevant concentrations that can be applied in the field.

In general, data on specific classes of enzymes that might be effective for EOR application is very restricted. Indeed, to the best of our knowledge, only lipases have been applied as pure enzymes in previous reports (Nasiri, 2011).

In this study we have carried out a systematic screening of the four most promising groups of enzymes (esterases/lipases, carbohydrases, proteases and oxidoreductases) with respect to their ability to alter the wettability of the calcite surface, characteristic of the chalk reservoir rock and, ultimately, to detach oil from the surface.

Among different techniques, adhesion tests of oil drops on mineral surfaces, in the presence of known enzyme solutions, are the most suitable for wettability screening as they keep the balance between accuracy, timing and simplicity which is very important in the case of a large number of samples. Measurement of the contact angles in conjunction with adhesion tests gives an even better indication of wettability (Buckley and Morrow, 1990). This method was used in the present work. In order to distinguish the specific effect of the enzymes, comparative studies were conducted with a surfactant, a protein and an oil model (mimic). The obtained results should enable direct assessment of the enzyme as a working biological component and correlation of the enzyme class

with respect to its potential for EOR. Adsorption of enzymes at interfaces and/or formation of surface-active compounds were proposed to be key mechanisms underlying changes introduced into a crude oil–brine–calcite system.

The experimental program is proposed as the first step in the study of the applicability of enzymes for enhanced oil recovery. Further studies will be necessary, including dynamic adsorption experiments, flow-through experiments, flooding tests and pilot reservoir tests. However, the present study is independent of the subsequent steps and provides a thorough description of the wettability alteration mechanisms as well as reasonable screening criteria for enzyme selection and working concentrations of enzymes.

The paper is organized as follows. First, we give an overview of materials and methods applied (Section 2). Section 3 describes results of the assessment of wettability of crude oil–sea water and enzyme–calcite systems. The reference experiments and comparative studies for similar systems are discussed in Section 4. In Section 5, we discuss significance of our findings for enzymatic EOR. Finally, the key results of the work are summarized in Section 6.

2. Materials and methods

2.1. Materials

2.1.1. Fluids

All the tests were performed using light dead oil recovered from a chalk reservoir in the Danish sector of the North Sea. None of the enzymes utilised in this study interact with small hydrocarbon molecules, so that the difference between the live and dead oils was unimportant for the purpose of the experiment. In the reference experiment *n*-decane (Sigma-Aldrich, purity $\geq 99\%$) was used as the model oil phase.

Table 1: Composition of synthetic North Sea water used for adhesion behaviour and contact angle tests.

Salt	Concentration, g/l
NaCl	18.01
NaHCO ₃	0.17
KCl	0.74
MgCl ₂ .6H ₂ O	9.15
CaCl ₂ .2H ₂ O	1.91
Na ₂ SO ₄	3.41
Total Dissolved Salts	33.39

The aqueous phase was synthetic North Sea water (pH=7.78; composition as given in Table 1). Chemicals for brine preparation were purchased from Fluka (purity $\geq 99.5\%$) and were not subjected to further purification.

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2.1.2. Enzyme, protein and surfactant samples

Fifteen enzyme products kindly provided by Novozymes A/S, and two enzyme-based commercial mixtures (Apollo GreenZyme™ and EOR-ZYMAX™) were investigated in the study (Table 2). Each of the Novozymes enzyme products belonged to one of four classes (esterases/lipases, carbohydrases, proteases, oxidoreductases). Three solutions (0.1%, 0.5%, and 1% (weight/weight)) were prepared for each enzyme sample by dilution of the enzyme products in the sea water (SW). The actual content of protein is much lower, typically in the range of 2–5% of the enzyme products. This is further discussed in Section 4.1.

Table 2: Enzyme samples used in the study.

Sample	Enzyme type	Enzymatic action
Esterases/Lipases		
NS 44034	Lipase EC 3.1.1.3	Hydrolysis of ester bonds in a lipid (activity: 100 KLU/g)
NS 81249	Lipase EC 3.1.1.3	Hydrolysis of ester s in a lipid (activity: 50 KLU/g)
NS 44124	Lipase EC 3.1.1.3	Hydrolysis of ester bonds in a lipid (activity: 100 KLU/g)
NS 44033	Lipase EC 3.1.1.3	Hydrolysis of ester bonds in a lipid (activity: 6 KLU/g)
NS 44035	Lipase EC 3.1.1.3	Hydrolysis of ester bonds in a lipid (activity: 20 KLU/g)
NS 44164	Esterase/lipase EC 3.1.1.3	Hydrolysis of ester bonds in lipids and other compounds (activity: 15 KLU/g)
NS 44129	Phospholipase EC 3.1.1.32	Hydrolysis of ester bonds in phospholipids (activity: 10 KLU/g)
Carbohydrases		
NS 81251	Amylase EC 3.2.1.1	Hydrolysis of starch (activity: 120 KNU/g)
NS 81252	Cellulase EC 3.2.1.4	Hydrolysis of cellulose (activity: 1000 ECU/g)
Proteases		
NS 81253	Subtilisin protease EC 3.4.21.62	Hydrolysis of proteins (activity: 2.5 AU/g)
NS 44110	Subtilisin protease EC 3.4.21.62	Hydrolysis of proteins (activity: 8 KNPU/g)
Multicomponent products		
NS 44053	Cellulases EC 3.2.1.4/Hemicellulases EC 3.2.1.6/EC 3.2.1.8/Amylase EC 3.2.1.1	Hydrolysis of cellulose/hemicellulose/ starch. (standardised activity: 45 FBG/g but it contains many different enzymes)
NS 44055	Pectinases EC 3.2.1.15, EC 4.2.2.10, EC 4.2.2.2, EC 3.1.1.11 Hemicellulases/ EC 3.2.1.6, EC 3.2.1.8/ Cellulases EC 3.2.1.4/ Proteases	Hydrolysis of carbohydrates/ pectins/ proteins etc. standardised activity: 100 FBG/g but it contains many different enzymes
Oxidoreductases		
NS 81254	Laccase EC 1.10.3.2	Redox reactions on phenolic or aniline/amine structures. Laccase requires oxygen as an electron acceptor (activity : 1000 LAMU/g)
NS 44071	Peroxidase EC 1.11.1.7	Redox reactions on phenolic and other structures. Peroxidases require H ₂ O ₂ as an electron acceptor (activity : 10000 POXU/g)
Commercial mixtures containing enzymes		
Apollo GreenZyme™	Undisclosed	–
EOR-ZYMAX™	Undisclosed	–

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Two oxidoreductases were applied (peroxidase and laccase) that required the presence of hydrogen peroxide (1–3 mM) and oxygen, respectively. Hydrogen peroxide (Sigma-Aldrich) was added during preparation of the peroxidase solution, while no additional amount of oxygen was supplied during application of laccase, since the amount of dissolved oxygen was considered to be sufficient.

Bovine serum albumin protein (BSA, 98% purity) and sodium dodecyl sulphate surfactant (SDS, 99% purity) were purchased from Sigma-Aldrich. Concentrations of BSA (0.001%, 0.005%, 0.01%, 0.05%, 0.1% and 1% w/w) and SDS (0.003%, 0.05%, 0.5% w/w) were chosen so that they were correlated with the amount of enzyme used in experiments. The BSA and SDS solutions in synthetic brine were prepared in an identical way to the enzyme solutions.

Other chemicals used were propylene glycol (Sigma-Aldrich, purity $\geq 99.5\%$), and a purified version of the enzyme (lipase) sample NS 44034 (Novozymes A/S) (without stabilisers).

2.1.3. Calcite minerals

In laboratory experiments it is usual practice to use various minerals to mimic specific reservoir rocks. Calcite minerals were used in this work to represent a chalk reservoir. Three calcite crystals (white, yellow and grey) with crystal faces were kindly provided by the Geological Museum of Copenhagen, Denmark. A further calcite sample with the surface created after cleavage of a larger mineral was kindly supplied by Center for Arctic Technology, Technical University of Denmark (Lyngby, Denmark). One of the crystal face samples was transparent. One of the samples with the crystal face and freshly cleaved samples were transparent and opaque calcites with no additives, correspondingly. Two other samples were yellow and grey minerals, where the colour was due to the presence of colour-changing additives. Application of these particular samples allowed assessment of the effect of different additives and effect of origin of the mineral surface.

In order to approach realistic roughness of the natural surfaces (such as pore walls), the calcite surfaces were not subjected to any treatment (e.g., polishing), although they were thoroughly cleaned, as described below. The surface roughness may significantly affect wettability, which would be expected to lead to different drop shapes and scattering of the apparent contact angles, even for a single drop. In order to acquire an axisymmetric shape, the drop size should be sufficiently large compared to the scale of roughness (Marmur, 2006). This requirement was met in all our experiments. However, if the drop size becomes large, gravity affects the value of contact angle (Vafaei and Podowski, 2005; Shojai Kaveh *et al.*, 2014). In order to check what type of forces, surface or gravity dominates in our experiments, the Bond number reflecting relative contribution of these forces was calculated:

$$Bo = \frac{(\rho_1 - \rho_2) * g * L^2}{\gamma},$$

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where Bo is Bond number; ρ_1 , the density of aqueous phase (kg/m^3); ρ_2 , the density of oil phase (kg/m^3); g , the acceleration due to gravity (m/s^2); L , the characteristic length of the drop (m); and γ , the interfacial tension (N/m).

For an average oil drop, the Bond number equals 0.3, which means that surface forces determine the drop shape (Shojai Kaveh *et al.*, 2014). Hence, the oil drops are neither too small (significantly larger than surface roughness) nor too large (the surface forces prevail over gravity).

Prior to introduction of calcite minerals into the experiments they were thoroughly washed with acetone in the ultrasonic bath, followed by cleaning with ethanol. After each experiment the mineral samples were cleaned in three steps. First, water was used to wash the bulk enzyme solution from the surface (in order to avoid potential denaturation/solidification of enzymes/proteins and subsequent clogging of the voids on the mineral surface due to following application of the solvent). Secondly, the surface was washed with the toluene, in order to remove all the crude oil components. Finally, the surface was rinsed with ethanol, to eliminate remains of the enzymes. Testing adhesion behaviour and contact angle in crude oil – SW – calcite system after experiments with enzyme samples proved the efficiency of this cleaning procedure.

2.2. Methods

The goal of this study is to investigate the effect of enzymes on crude oil/brine attachment to the surfaces of minerals representing the porous rocks of petroleum reservoirs. It is important to measure and to evaluate the quantitative characteristics of this attachment. To date, two such characteristics have been considered in the scientific literature: contact angle (Anderson, 1986) and adhesion behaviour (Buckley and Morrow, 1990). These characteristics may be studied together, in similar tests. A common opinion in the scientific literature has been that the results of the two tests are somehow correlated, and, for example, a decrease of the contact angle indicates also less adhesive behaviour (Buckley and Morrow, 1990; Nasiri, 2011).

As discussed below, our results indicate that these two measurements are not fully correlated. Moreover, they have a different meaning with respect to applicability of enzymes for EOR. Therefore, it was important for us to carry out both tests simultaneously and to analyse them in greater detail. Below we describe an experimental approach and procedure making this possible.

2.2.1. Adhesion test

Adhesion tests were carried out according the procedure developed by Buckley and Morrow (1990). All the experiments were accomplished under ambient conditions. To the best of our knowledge, the enzymes are relatively insensitive to pressure, while temperature will change their activity. Indeed, the selected enzyme samples might become more active at the elevated temperatures characteristic of petroleum reservoirs. Nevertheless, it is not expected that this will alter their behaviour (Cobianco *et al.*, 2007; Turner and Vulfson, 2000). Hence, the simple (ambient temperature) tests carried out here are to a large extent expected to be representative of the behaviour of enzymes under reservoir conditions.

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Calcite was immersed into the brine/enzyme solution in a glass container (5x5x5 cm or 6x6x6 cm dependent on the size of the mineral). The container was placed on an anti-vibration platform, accurately levelled prior to use. A drop of oil (1.5-2 μ l) was carefully deposited on to the lower crystal face using a syringe with an inverted needle (Figure 1). The oil drop was allowed to contact with the mineral in the presence of brine for 2 minutes without detachment from the needle. Afterwards the needle was moved down in order to either remove the drop from the mineral, or to leave it on the surface. At this stage three types of behaviour were observed (Figure 2):

1. Adhesion behaviour: Oil sticks to the mineral surface, the link between the needle and oil breaks and oil drop is left on the surface;
2. Non-adhesion behaviour: The oil drop does not attach to the crystal and stays on the needle leaving the mineral surface clean;
3. Temporary adhesion: Oil initially sticks to the calcite surface; while the needle is lowered, the drop detaches from the surface and stays on the needle leaving a small oil spot on the mineral.

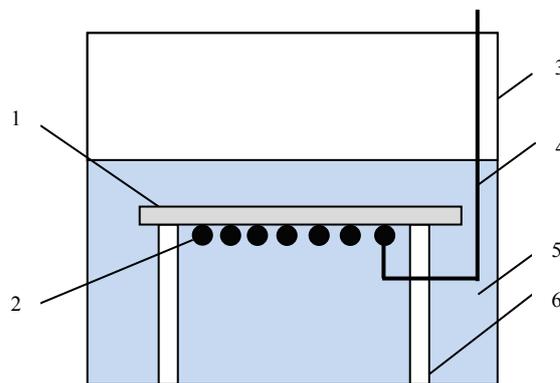


Figure 1: Scheme of the experimental setup. 1 – calcite mineral, 2 – oil drop, 3 – glass container, 4 – inverted needle, 5 – brine/enzyme solution, 6 – glass stand.

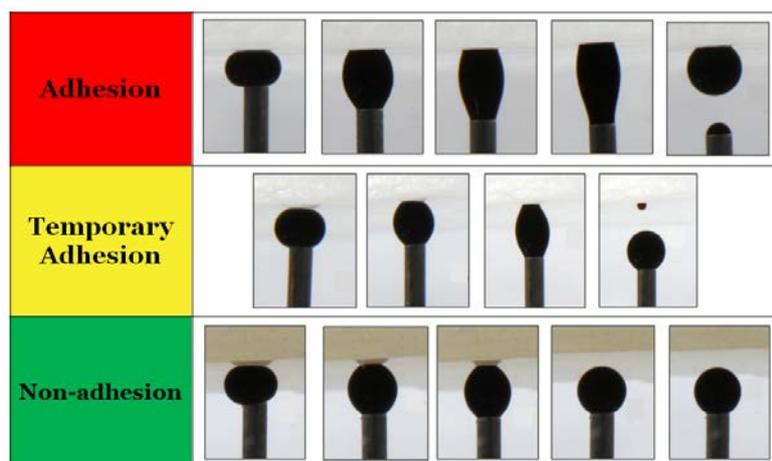


Figure 2: Adhesion behaviour types.

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Each crude oil – brine – calcite system was tested at least twice. Adhesion behaviour of a certain system was determined on the basis of 12 to 24 drops. The response of adhesion behaviour after addition of enzyme was considered to be uniform or homogeneous if more than 90% of the drops showed similar results. Otherwise the results were considered to be inconclusive.

2.2.2. Contact angle measurements

Measurements of contact angles were based on image analysis (Roero, 2004; Shojai Kaveh et al., 2014; Yang *et al.*, 2008). The procedure consisted of three steps: 1. Placing a liquid drop on a solid surface; 2. Recording the drop shape (image acquisition); 3. Image processing and analysis (determination of the final contact angle).

The oil drops were placed on a mineral surface in the same way as for the adhesion test, as described in section 2.2.1 and Figure 1. After the deposition, a drop was allowed to settle for about an hour (60 minutes was found to be the optimal interval to stabilise the drop, while achieving a reliable contact angle). An image of the drop was recorded with a Canon EOS 50D camera equipped with a Canon EF 100mm F2.8 L IS USM Macro lens in order to get high-quality images. An external flash unit was used to obtain high light-dark contrast, which also allowed accurate determination of the drop shapes, particularly of the oil-brine-mineral contact point. Settings on the camera were as follows: ISO speed 100, shutter speed 1/400 and aperture 18 – 22.

The contact lines between the two liquid phases and also between the liquids and the solid were established by applying edge detection techniques. Dependent on the quality of the images, the drop boundaries and the triple contact point of the phases were determined by image processing in the ImageJ software or, in more ambiguous cases, by a Matlab script that applied the Canny edge detector. Image analysis was performed using the drop analysis plugin of the ImageJ software. Low-Bond Axisymmetric Drop Shape Analysis (LBADSA), which is based on fitting Young-Laplace equation, was selected to determine the contact angle (Stalder *et al.*, 2010).

All the experiments were checked for reproducibility. Conditions applied in this study corresponded to water receding conditions when water is displaced by oil from the solid surface. Each value of the contact angle was determined from an average of 12 to 24 oil drops.

2.2.3. Validity of the adhesion tests and contact angle measurements

It should be verified whether the observed adhesion behaviour and contact angle values are affected by the way the experiments were carried out. Two sources of uncertainty should be checked: the effect of buoyancy on the shape of the oil drop surrounded by brine, and the effect of “pushing” during the placement of a drop on the surface. The last effect is very difficult to control, since, in order to reach equilibrium during the adhesion behaviour test an oil drop should be allowed to interact with the mineral for two minutes (this time interval was found to be sufficient for oil – brine – mineral interaction and reasonable in terms of the experimental timing (Buckley and Morrow, 1990)). During the equilibration period, the oil drop should not be detached from the needle and should be slightly ‘pushed’ towards the mineral.

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For verification of the effect of ‘pushing’, an oil drop of a defined volume was created at the tip of the needle and then the needle was slightly shaken, so that the drop floated up. The contact angle was measured after 12, 30 and 60 minutes. The result was a contact angle of $37^{\circ} \pm 7^{\circ}$, which is the same value as for slight pressing. Similar results were also obtained when applying several enzyme samples. Therefore, it may be concluded that “pushing” does not affect the formation of a certain drop shape.

During placement of the drops underneath the mineral, buoyancy might also impact the adhesive forces and affect the drop shape formation. In one of the experiments minerals were turned upside down, and the oil drops were placed on the top of the mineral. Despite the fact that oil phase is less dense than the surrounding aqueous phase, the oil drops got attached to the mineral surface due to strong adhesive forces. No changes in adhesion behaviour were observed. However, the average value of the contact angle increased from 38° up to 43° in case of SW applied as an aqueous phase.

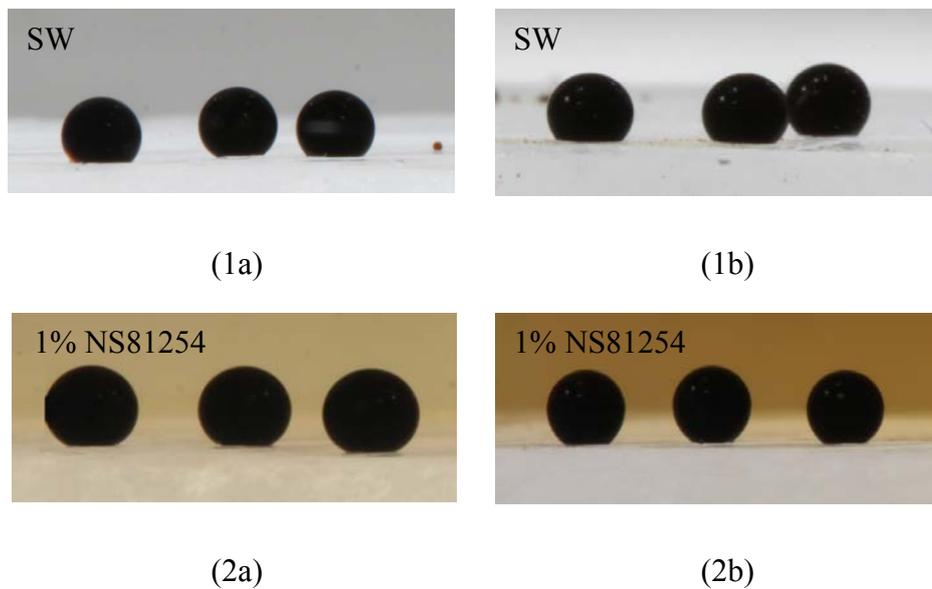


Figure 3: Effect of buoyancy on shape of the oil drops: 1a and 2a – shapes of the drops placed on the bottom surface of the mineral in presence of SW and 1% NS81254 sample, respectively; 1b and 2b – shapes of the drops placed on the top surface of the mineral in presence of SW and 1% NS81254 sample, respectively. Pictures in Figures 1a and 2a are turned upside down for easier comparison with Figures 1b and 2b.

The same experiment was carried out with the 1% NS 81254 enzyme sample. When the drops were put underneath the rock, no difference in adhesion behaviour and contact angles were observed compared to the crude oil – SW – calcite system. After turning the mineral upside down adhesive forces were still predominant and oil drops remained stuck to the surface. However, the shape of drops and consequently the contact angle values were altered more significantly, and the drops became elongated in a vertical direction (Figure 3, 2b). The average contact angle increased

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from 39.5 to 49°. For this system the effect of buoyancy is quite significant and becomes comparable with the surface forces.

The surface tensions for given systems are equal to 19.9 mN/m for brine-oil, and 17.4 mN/m in the presence of the NS81254. Apparently, they are around a threshold value at which buoyancy becomes comparable with the surface forces.

Overall, buoyancy had some effect on the contact angles, but does not affect the type of the adhesive behaviour. This is also consistent with the calculated Bond number (see Section 2.1.3). In order to achieve the objectives of this research, it has been found to be sufficient to work solely with the drops beneath the mineral surfaces, as also suggested in the scientific literature (Nasiri *et al.*, 2009).

2.2.4. Interfacial Tension Measurements

Measurements of interfacial tension (IFT) were implemented by applying the drop volume method (Harkins and Brown, 1919). The method involves the following steps:

1. Generation of the oil drop using inverted needle (500 µl Hamilton syringe with inverted needle) immersed into the brine solution. It was found very important that the drop is created slowly and the last stage of the drop formation should take at least 1 – 2 minutes (Alpbaz *et al.*, 1988).
2. Determination of the oil drop volume at the moment of oil drop break-off from the needle tip (the volume of floating drop).
3. Measurement of liquid densities (Anton Paar, DMA 4100).
4. Calculation of IFT. This was done using in-house built algorithm, based on Tate's law with Harkins and Brown (1919) correction factor:

$$\gamma = \frac{g * (\rho_1 - \rho_2) * V}{2 * \pi * r * f(HB)},$$

where γ is the interfacial tension (N/m); g , the acceleration due to gravity (m/s^2); ρ_1 , the density of aqueous phase (kg/m^3); ρ_2 , the density of oil phase (kg/m^3); V , the average volume of the oil drop (m^3); r , the radius of the inverted needle (m); and $f(HB)$, the Harkins-Brown empirical correction factor.

The radius of the inverted needle was determined by applying the drop volume method for pure compound systems with known values of IFT. Based on measurements for *n*-decane – distilled water and *n*-octane – distilled water systems, the diameter of the inverted needle was found to be 0.39 mm. Using this value Harkins-Brown coefficient was determined as a function of $r/V^{1/3}$ ratio as one of the steps of the algorithm (Harkins and Brown, 1919).

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The experiments were carried out at 25°C and ambient pressure, in accordance with the adhesion/contact angle tests. The water bath was used to keep constant temperature. The value of IFT for each unknown system was determined based on 10 oil drops.

3. Results

3.1. Crude oil – SW – calcite system

The efficiency of water-flooding in a chalk reservoir (without additional agents such as enzymes) is largely determined by the wettability behaviour of the oil and brine on the mineral surface of the porous rock. Since water-flooding is a “reference” process for comparison of the EOR methods in petroleum engineering, the wettability state of the crude oil – SW – calcite system should be taken as a reference point. Hence, the influence of enzymes on the wetting properties of calcite was assessed relatively to this system.

Adhesion tests revealed that the initial wettability state of the crude oil – SW – calcite system corresponded to fully adhesive behaviour. The contact angles ($38^{\circ}\pm 7^{\circ}$), complies with the weakly water-wet state according to the classification by Anderson (1986). Therefore, the oil–brine–calcite system has a potential for de-adhesion of the oil.

3.2. Adhesion behaviour test

Addition of specific enzymes modified the behaviour described in the previous subsection. The adhesion map for the different enzyme solutions is given in Table 3. Initially each enzyme sample was tested at three concentrations (1%, 0.5% and 0.1%). For enzyme products that were found to change the original adhesion state, all enzyme concentration gave the same result. Hence for the final experiments using samples NS 44055, NS 81254 and NS 44071, for which oil adhered already at 1%, no study was made at two lower concentrations of the enzymes. However, there was one case of inverse effect: for NS 44110 experiments on grey calcite demonstrated the non-adhesion behaviour at 1%, temporary adhesion at 0.5%, which again changed to non-adhesion at 0.1%.

The results on adhesion tests revealed that each type of enzyme has a distinct behaviour. In accordance with previous studies (Nasiri *et al.*, 2009), esterases/lipases showed the highest ability to change wettability, implying the highest surface activity of this enzyme class. Most of the lipase samples turned calcite from an adhesion to a non-adhesion state at a concentration of 1%. At 0.5% the two samples NS 44034 and NS 44164 could keep the non-adhesion behaviour of the oil drops, while for other lipases temporary adhesion mainly occurred at this concentration. Decrease of the enzyme product content down to 0.1 % showed that few samples such as NS 44164 and NS 44035 could still provide temporary adhesion and sample NS 44034 could even provide the non-adhesion state, but for the rest of enzymes calcite adhered oil at the concentration of 0.1%.

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Table 3: Summarized adhesion behaviour of the calcite minerals in the presence of various enzyme products. The colours indicate: ■ – adhesion, ■ – temporary adhesion, ■ – non-adhesion; N/A – information is not available, N/R – not reasonable.

	1% (wt/wt) of product				0.5% (wt/wt) of product				0.1% (wt/wt) of product			
	Grey Calcite	Yellow Calcite	White Calcite	White Cleaved Calcite	Grey Calcite	Yellow Calcite	White Calcite	White Cleaved Calcite	Grey Calcite	Yellow Calcite	White Calcite	White Cleaved Calcite
Lipases/Esterases												
NS 44124												
NS 44129												
NS 81249												
NS 44034												
NS 44033					N/A				N/A			
NS 44164												
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				N/A				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												

Out of the lipase group (Table 3), samples NS 44033 and NS 44035 are the least desirable for further investigation, since for both of them non-adhesion behaviour was not reached at any concentration $\leq 1\%$. On the contrary, sample NS 44035 kept predominantly steady temporary adhesion state in the whole range of investigated concentrations. The rest of the samples exhibited a transient zone between 0.1 and 1%, where adhesion changed to non-adhesion via temporary adhesion state. The NS 44034 enzyme product also was not subjected to further studies, even though it performed well at low concentrations, because of a non-uniform response of pure calcite and calcite minerals with additives after addition of the enzyme sample.

Two esterase/lipase products – NS 44164 and NS 81249 – were found to be the most suitable for a more detailed examination. The advantage of NS 44164 is stable non-adhesion behaviour at concentrations equal or more than 0.5%, whereas NS 81249 is attractive due to its stable uniform response.

Addition of carbohydrases and oxidoreductases to the brine solution had no effect on adhesion behaviour of the oil drops. The only positive observation was the temporary adhesion state of the grey calcite at 1% for the NS 81252 sample.

Proteases performed better than carbohydrases and oxidoreductases, but the effect on adhesion behaviour was not as significant as for esterases/lipases. Addition of proteases NS 81253 and NS 44110 caused some positive changes in the wettability state of calcite, but responses were very non-uniform. For example, addition of 0.5% NS 81253 resulted in 50% of adhering and 50%

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of temporarily adhering oil drops for pure calcite with natural crystal face; 100% of adhering oil drops for cleaved calcite; 80% of temporarily adhering and 20% of non-adhering oil drops for grey calcite, and 50% of temporarily adhering and 50% of non-adhering oil drops for yellow calcite. The only observed trend was that grey and yellow calcite crystals were less “sticky” than the pure calcite with no additives. At an enzyme product concentration of 1% non-adhesion behaviour could be observed for the calcite with additives, while white minerals turned only to the temporary adhesion or adhesion state. Likewise, at 0.5%, crystals with additives demonstrated predominantly temporary adhesion, while white calcites mainly showed adhesion of the oil.

Samples NS 44034 (enzyme product content of 0.5% and 0.1%), NS 44033 (enzyme product content of 0.5%) and NS 81249 (enzyme product content of 0.1%) showed a similar trend. However, for one case a reverse effect was found: application of 0.5 % NS 44124 enzyme sample resulted in predominantly adhesion state of grey calcite as temporary adhesion occurred for other minerals. It might be proposed that interaction between the different enzymes and the mineral surface is a predominant effect, and that it depends on both enzyme and mineral composition. Even though proteases have some potential for EOR in terms of wettability change, their selective effect on different minerals makes them less desirable biological agents.

Multicomponent products which include several different enzyme types including cellulases, hemicellulases, amylases and proteases (NS 44055 and NS 44053), were also tested to examine possible synergistic effect of simultaneous application of several enzymes. However, no noticeable effect was observed: 1% NS 44055 was not capable of changing the adherence of oil to calcite, and NS 44053 kept a steady temporary adhesion state of the minerals at all the concentrations.

Two commercial enzyme-based mixtures, Apollo GreenZyme™ and EOR-ZYMAX™, were included into the enzyme screening list. Addition of EOR-ZYMAX™ did not influence the adhesion behaviour of the oil drops. On the contrary, application of Apollo GreenZyme™ resulted in absolute non-adhesion behaviour for all the calcite minerals at all the investigated concentrations. Based on adhesion behaviour, Apollo GreenZyme™ appeared to be a better product, but this will further be discussed in Section 4.2.

3.3. Contact angle measurements

The adhesion tests described above were subsequently complemented by contact angle measurements. Absolute values and relative decreases of contact angles for different enzyme product samples and calcite minerals are given in Table 4. Contact angle experiments generally correlated well with the results obtained on adhesion behaviour. Esterases/lipases were found to be the most surface active group of enzymes, reducing the water contact angle under both non-adhesion and temporary adhesion conditions – from 38° to 0°. A contact angle of 0° implies absolute water-wetness, which is favourable for oil recovery. At concentrations of enzyme product of 0.1%, when usually adhesion behaviour was observed, the decrease in contact angles was about 35%. Within the investigated range of enzyme concentrations it seems likely esterases/lipases can keep 0° water contact angle up to a certain threshold concentration, below which a decrease of the enzyme content causes increase in the contact angle values, as normally occurred at temporary

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Table 4: Absolute values with standard deviations and relative decreases of the contact angles for various enzyme concentrations and various calcite minerals (relative decreases were calculated as $(\theta_{ref} - \theta_{enz}) / \theta_{ref}$, where θ_{ref} is reference water contact angle, and θ_{enz} the contact angle after addition of an enzyme). If standard deviation value is not given, it equals to zero.

			1% (weight)				0.5% (weight)				0.1% (weight)			
			Grey Calcite	Yellow Calcite	White Calcite	White Cleaved Calcite	Grey Calcite	Yellow Calcite	White Calcite	White Cleaved Calcite	Grey Calcite	Yellow Calcite	White Calcite	White Cleaved Calcite
Esterases/ Lipases	NS 44124	Absolute	0°	0°	0°	0°	20°±5°	0°	0°	0°	28°±3°	27°±2°	26°±1°	27°±2°
		Relative	1	1	1	1	0.47	1	1	1	0.26	0.29	0.32	0.29
	NS 44129	Absolute	0°	0°	0°	0°	0°	0°	0°	4°±3°	23°±4°	27°±3°	28°±3°	28°±2°
		Relative	1	1	1	1	1	1	1	0.89	0.39	0.29	0.26	0.26
	NS 81249	Absolute	0°	0°	0°	0°	0°	0°	0°	0°	25°±1°	23°±2°	25°±1°	27°±2°
		Relative	1	1	1	1	1	1	1	1	0.34	0.39	0.34	0.29
	NS 44034	Absolute	0°	0°	0°	0°	0°	0°	13°±5°	0°	0°	0°	24°±2°	31°±5°
		Relative	1	1	1	1	1	1	0.66	1	1	1	0.37	0.18
	NS 44033	Absolute	0°	0°	0°	0°	-	0°	18°±4°	14°±5°	-	20°±3°	25°±3°	24°±4°
		Relative	1	1	1	1	-	1	0.53	0.63	-	0.47	0.34	0.37
	NS 44164	Absolute	0°	0°	0°	0°	0°	0°	0°	0°	-	12°±3°	13°±3°	22°±4°
		Relative	1	1	1	1	1	1	1	1	-	0.68	0.66	0.42
NS 44035	Absolute	-	0°	11°±3°	0°	-	0°	12°±3°	0°	-	0°	23°±2°	16°±2°	
	Relative	-	1	0.71	1	-	1	0.68	1	-	1	0.39	0.58	
Carbo-hydrases	NS 81251	Absolute	28°±4°	24°±2°	31°±3°	29°±4°	33°±4°	29°±2°	28°±2°	31°±4°	33°±4°	30°±3°	31°±3°	36°±5°
		Relative	0.26	0.37	0.18	0.24	0.13	0.24	0.26	0.18	0.13	0.21	0.18	0.05
	NS 81252	Absolute	8°±1°	29°±5°	30°±4°	29°±2°	29°±3°	32°±2°	33°±4°	32°±3°	31°±5°	33°±2°	32°±3°	30°±4°
		Relative	0.79	0.24	0.21	0.24	0.24	0.16	0.13	0.16	0.18	0.13	0.16	0.21

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Table 4 (continuation): Absolute values with standard deviations and relative decreases of the contact angles for various enzyme concentrations and various calcite minerals (relative decreases were calculated as $(\theta_{ref} - \theta_{enz}) / \theta_{ref}$, where θ_{ref} is reference water contact angle, and θ_{enz} the contact angle after addition of an enzyme). If standard deviation value is not given, it equals to zero.

			1% (weight)				0.5% (weight)				0.1% (weight)			
			Grey Calcite	Yellow Calcite	White Calcite	White Cleaved Calcite	Grey Calcite	Yellow Calcite	White Calcite	White Cleaved Calcite	Grey Calcite	Yellow Calcite	White Calcite	White Cleaved Calcite
Proteases	NS 81253	Absolute	0°	0°	28°±2°	8°±4°	0°	0°	22°±3°	20°±2°	15°±5°	0°±°	25°±3°	27°±4°
		Relative	1	1	0.26	0.79	1	1	0.42	0.47	0.61	1	0.34	0.29
	NS 44110	Absolute	0°	0°	6°±2°	4°±2°	0°	0°	16°±5°	20°±3°	0°	28°±3°	32°±6°	35°±4°
		Relative	1	1	0.84	0.89	1	1	0.58	0.47	1	0.26	0.16	0.08
Multiple components	NS 44055	Absolute	-	24°±3°	21°±2°	25°±3°	-	-	-	-	-	-	-	-
		Relative	-	0.37	0.45	0.34	-	-	-	-	-	-	-	-
	NS 44053	Absolute	-	0°	0°	0°	-	0°	18°±5°	15°±2°	-	10°±2°	24°±3°	26°±4°
		Relative	-	1	1	1	-	1	0.53	0.61	-	0.74	0.37	0.32
Oxidoreductases	NS 81254	Absolute	-	29°±2°	32°±3°	29°±5°	-	-	-	-	-	-	-	-
		Relative	-	0.24	0.16	0.24	-	-	-	-	-	-	-	-
	NS 44071	Absolute	-	23°±3°	24°±3°	25°±4°	-	-	-	-	-	-	-	-
		Relative	-	0.39	0.37	0.34	-	-	-	-	-	-	-	-
Commercial products	EOR-ZYMAX™	Absolute	27°±2°	28°±3°	29°±4°	30°±2°	30°±4°	32°±3°	34°±6°	33°±4°	32°±3°	28°±2°	29°±2°	29°±2°
		Relative	0.29	0.26	0.24	0.21	0.21	0.16	0.11	0.13	0.16	0.26	0.24	0.24
	Apollo GreenZyme™	Absolute	32°±4°	24°±3°	25°±5°	30°±4°	23°±2°	28°±2°	27°±7°	30°±4°	23°±1°	22°±2°	21°±2°	22°±2°
		Relative	0.16	0.37	0.34	0.21	0.39	0.26	0.29	0.21	0.39	0.42	0.45	0.42

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adhesion behaviour. Thus, a threshold where a contact angle changes from zero to a given value may be considered as a limiting value for desirable surface activity of an enzyme

Addition of carbohydrases did not change adhesion behaviour of the calcite, although the values of contact angles decreased by 32%, 19% and 16% in case of addition of 1%, 0.5% and 0.1% enzyme product, respectively. Similar behaviour was observed for oxidoreductases: for a 1% solution the reduction of contact angle values was 21% for NS81254 and 37% for NS44071, even though the same adhesion behaviour was maintained after addition of the enzyme. So carbohydrases and oxidoreductases had some impact on wetting properties of calcite, but not as significant as the group of esterases/lipases. It might be possible that by increasing the amount of carbohydrases and oxidoreductases the threshold concentration leading to absolute water state will be reached similar to esterases/lipases. However, this was not studied due to non-feasibility of application of larger amounts of the enzymes.

For proteases and multicomponent enzyme products similar to esterases/lipases the contact angle corresponding to non-adhesion and temporary adhesion was 0°. The only exception was enzyme sample NS44053. At concentrations of 1% it demonstrated temporary adhesion with 100% reduction of contact angle, while decrease of enzyme content to 0.5% and 0.1% caused about 57% and 34% reduction of the contact angle, respectively, even though temporary adhesion was still observed. This observation proves that it is most likely that the transition zone with the threshold value of enzyme concentration at which calcite becomes absolutely water wet occurs within the temporary adhesion behaviour.

Two commercial enzyme-based mixtures, Apollo GreenZyme™ and EOR-ZYMAX™, were included into the enzyme screening list. Decrease of contact angle after addition of EOR-ZYMAX™ was not more than 29% (21% on average). Combined with the results of the adhesion behaviour test it might be concluded that EOR-ZYMAX™ had no effect on the wetting state of calcite mineral. On the contrary, Apollo GreenZyme™ demonstrated absolute non-adhesion behaviour with a decline of the contact angle values by, on average, 60% (approximately 15°) for all the calcite minerals at all the investigated concentrations. Apollo GreenZyme™ was the only sample for which there was no correlation between contact angle measurements and adhesion behaviour.

4. Results - reference experiments

It was found that the group of enzymes representing esterases/lipases, can change wettability of the crude oil – SW – calcite system. However, the following questions should be answered in order to be certain of the conclusions from the experiments:

1. What component of the enzyme products causes an alteration in wettability: pure enzyme or stabiliser?
2. What are the possible mechanisms that underlie alteration of the wetting state of the calcite surface after addition of esterases/lipases?

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Wettability is a function of the interfacial tensions between oil – brine, oil – rock and brine – rock. Hence, the following potential mechanisms of the enzyme action might be discussed *a priori*:

1. Change of the oil composition. In the system, crude oil– [brine + enzyme]–rock, oil could act as a substrate, and water as a reagent. A specific enzyme might catalyse the hydrolysis reaction. For example, esterases represent a group of enzymes that potentially catalyse hydrolysis of ester fragments (which might be present in a particular crude oil) into the respective acids and alcohols. Consequently, application of the esterase in crude oil– [brine + enzyme]–rock system might produce an additional amount of surface active compounds. Alteration of the oil composition could result in changes to its properties (e.g. acidity) that could be reflected in a change of the type of interactions of the oil with the rock and with the brine solution. This might also change the oil viscosity.
2. Adsorption of enzymes on the rock surface. Being of proteinaceous nature, enzymes are surface active molecules (Hlady *et al.*, 1999). The adsorption potential of enzymes is due to the fact that their sites are physico-chemically very different, and some of them may be attracted to the mineral surfaces. To our knowledge, there is no data available on adsorption of enzymes on calcite, since most of the work on enzyme-mineral interactions has been focused on negatively charged mica (Demanèche *et al.*, 2009; Zaidan *et al.*, 2010), although there is an evidence of protein adsorption on the carbonate surface (Denisov *et al.*, 2008).
3. Adsorption of enzymes onto the oil-water interface. Surface activity of proteins can also result in formation of adsorbed protein films on the oil-water interfaces (Baldursdottir *et al.*, 2010; Beverung *et al.*, 1999). Hence, as with surfactants, enzymes might cause decrease of the interfacial tension between oil and brine, which could also change wettability.

In order to answer these questions and to test hypotheses made *a priori*, reference experiments with purified enzyme, stabiliser, *n*-decane, protein and surfactant were carried out.

4.1. Enzyme or Stabiliser?

The enzyme products applied in this study were all formulated with stabilising components, identical to those that would be used when applying enzymes in any industrial process. Typical formulations consist of enzyme protein, water, one or more polyols and a biocide to prevent microbial growth. This stabilising formulation secures stability and shelf-life of the enzyme product. The enzyme products provided by Novozymes A/S typically consisted of enzyme (2-5% w/w), stabiliser (25-30% w/w), water (63-75% w/w), as well as 0.2% w/w biocide. In some of the experiments, the enzyme fraction that was capable of changing the wettability of calcite to an absolutely water wet state was as low as 0.002%. The concentrations discussed in Sections 3.2 and 3.3 are those of the enzyme products, not of the enzyme proteins themselves.

Since applied enzyme products are not purely enzyme, it is highly relevant to test whether the enzyme itself causes the positive effect, or whether it is an effect of the stabiliser system. In order to do that, experiments with purified enzymes (i.e. enzymes with no stabilising and biocide additives) were conducted. A protein solution of the purified NS 44034 enzyme corresponding to an

enzyme concentration of 1% of NS 44034 was applied. Both adhesion behaviour and contact angle measurements with the purified NS 44034 showed equivalent results compared to the corresponding enzyme product. These experiments confirm that the observed changes in the wettability of calcite were indeed caused by the enzyme.

Wettability tests were also carried out with the stabiliser solution without enzymes (the amount of stabiliser corresponding to its content in a 1% enzyme solution). It was found that the stabiliser had no effect on the adhesion behaviour of calcite and had relatively minor influence on the value of the contact angle. The contact angle value for the crude oil – [SW+stabiliser] – calcite system was found to be 27°, whereas corresponding value for the pure SW was 38°. Considering that the stabiliser decreases contact angle by 11°, but does not affect adhesion behaviour, it can be concluded that it is enzyme that changes the wettability of calcite.

For commercial mixtures, the composition of the stabilisers was undisclosed, and therefore it was not possible to check whether the wettability improvement due to application of Apollo GreenZyme™ should be assigned to the enzyme or to the stabiliser.

4.2. Crude oil – [SDS + SW] – calcite system

The effect of a decrease of IFT on the wettability of crude oil – [enzyme + SW] – calcite system was tested by replacement of the enzyme with one of the most commonly used anionic surfactants (SDS). Behaviour of the crude oil – [SDS + SW] – calcite system (Figure 4a) was completely different compared to the performance of the enzyme systems. The only exception was Apollo GreenZyme™, whose behaviour resembled that of SDS (Figure 4).

As illustrated in Figure 4, in the presence of SDS the oil drops became flat. Addition of Apollo GreenZyme™ did not cause strong flattening of the droplets, but their shape was not as round as those in the presence of enzymes. These observations are in good agreement with the IFT measurements. At concentrations corresponding to 1% enzyme product, Apollo GreenZyme™ and SDS demonstrated drastic IFT decrease, down to 5.9 mN/m and 0.8 mN/m, relatively, while for the enzymes no significant decrease of IFT was detected. As discussed in Section 2.2.3, the decrease in IFT results in a significant (and visible) contribution to buoyancy, meaning that the oil drops are pressed to the calcite surface.



Figure 4: Oil drop shapes under the influence of IFT decreasing components added to the surrounding SW. a – SDS, b – Apollo GreenZyme™.

At concentrations higher than 0.05%, SDS turned calcite into a non-adhesion state, similar to that observed with the application of esterases/lipases and Apollo GreenZyme™ (Table 5). However, no substantial decrease of contact angles was observed (the maximum decrease was 29% compared to the initial value). It should be noted that at an SDS concentration of 0.003%, oil is adhering to calcite while some esterases/lipases at the corresponding enzyme protein concentration (0.1% of the enzyme product) provide a non- or temporary adhesion state. Simultaneous decrease of adhesion and invariability of contact angles means a proportional decrease of both liquid-liquid and liquid-solid tensions caused by surfactants. Interestingly, enzymes seem to affect only the liquid-solid interactions.

The reference experiments with SDS show that the mechanisms of the action of the most of enzyme mixtures are different from those of surfactants. An exception is the commercial mixture Apollo GreenZyme™ exhibiting a surfactant-like action, indeed most likely explained by presence of surfactant in the product. These experiments also indicate that the contact angle measurements and the adhesion tests should be used in combination in order to completely describe the phenomenon of wettability. In some cases, similar contact angles could be observed with the different adhesion behaviours.

Table 5: Comparison of adhesion behaviour and contact angles when an SDS solution was applied as an aqueous phase. If standard deviation value is not given, it equals to zero.

SDS Concentration	White Cleaved Calcite			White Calcite			Yellow Calcite		
	Adhesion Behaviour	Contact angle		Adhesion Behaviour	Contact angle		Adhesion Behaviour	Contact angle	
		Absolute	Relative		Absolute	Relative		Absolute	Relative
0.5%		36°±4°	0.05		28°±2°	0.26		29°±3°	0.24
0.05%		38°±4°	0		27°±5°	0.29		29°±3°	0.24
0.003%		-	-		-	-		-	-

4.3. Crude oil – [BSA + SW] – calcite system

In order to find out whether the effect of enzymes was due to their catalytic activity or due to their proteinaceous nature, reference experiments with the enzymes substituted by BSA protein were carried out. The results were similar to those obtained for esterases/lipases (Table 6). Adhesion behaviour and contact angle values for the system crude oil – [BSA + SW] – calcite were strongly dependent on the protein concentration: a lower protein content resulted in a decrease of the calcite ability to repel an oil drop from the mineral surface. Similar to esterases/lipases, the transient zone from adhesion via temporary adhesion to non-adhesion behaviour occurred at the pure protein content between 0.001 and 1%.

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Formation of foams during preparation of the protein solution was also similar for BSA as for esterases/lipases. This serves as further evidence of the surface activity of BSA and the esterase/lipase group of enzymes, which most likely plays a significant role in altering the wettability of calcite.

Table 6: Comparison of adhesion behaviour and contact angles when a BSA solution was applied as an aqueous phase. If standard deviation value is not given, it equals to zero.

BSA Concentration	Corresponding Enzyme Product Concentration	White Cleaved Calcite				White Calcite				Yellow Calcite			
		Adhesion Behaviour	Contact Angle		Adhesion Behaviour	Contact angle		Adhesion Behaviour	Contact angle				
			Absolute	Relative		Absolute	Relative		Absolute	Relative			
											Absolute	Relative	
1%	~20%		0°	1		0°	1		0°	1			
0.1%	~2%		0°	1		0°	1		0°	1			
0.05%	~1%		0°	1		0°	1		0°	1			
0.01%	~0.2%		20°±3°	0.47		6°±2°	0.84		10°±3°	0.74			
0.005%	~0.1%		23°±4°	0.40		15°±3°	0.61		15°±3°	0.61			
0.001%	~0.02		22°±3°	0.42		22°±4°	0.42		18°±2°	0.53			

4.4. *n*-Decane – [enzyme + SW] – calcite system

In order to check the significance of the catalytic activity of enzymes, particularly of esterases/lipases, in one of the experiments *n*-decane was applied instead of crude oil. Using the enzyme as a catalyst requires the presence of specific bond types in the substrate (oil phase). For example, esterases/lipases require the esters, which while present in oil, are not found in a long chain alkane, such as *n*-decane. Therefore, if the hypothesis of esterases/lipases catalysing the hydrolysis of ester fragments of the crude oil is correct, no effect of change of wettability should be observed in those cases where *n*-decane was applied as the oil phase.

According to the adhesion behaviour tests *n*-decane is relatively strongly adhered to the calcite in presence of brine. This is supported by the contact angle measurements (32°±4°), which corresponds to a weakly water-wet state and is comparable to that found for crude oil.

Three enzyme samples were chosen for the reference experiments with *n*-decane: the best performing esterase/lipase samples NS 81249 and NS 44164, as well as an amylase sample NS 81251 that did not cause any wettability alteration. The results on adhesion behaviour and contact angle values are summarized in Table 7.

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Two out of three samples applied in the *n*-decane system (amylase NS 81251 and esterase NS 44164), gave the same results as if they were applied to crude oil, in terms of adhesion behaviour and contact angle values. However, the addition of lipase NS 81249 had a different effect in the cases of *n*-decane and crude oil. While wettability of crude oil – [SW + NS 81249] – calcite system was changed to absolutely water-wet state, the system of *n*-decane – [SW + NS 81249] – calcite maintained the original weakly water wet state with no change of adhesion behaviour and only a slight improvement of the contact angle value (33% on average).

Based on the results obtained, two important conclusions can be made. First, the catalytic activity of the enzymes may be important and, therefore, composition of the oil may affect the experimental results. Secondly, a particular mechanism of action may depend on the type of the enzyme. Surface and catalytic activity may work separately or in parallel. For example, in our experiment using the esterase NS 44164, the surface activity played a key role in changing the wettability of calcite, while for lipase NS 81249 the catalytic activity appears to be the dominant factor.

Table 7: Comparison of adhesion behaviour and contact angles when *n*-decane and crude oil were applied as an oleic phase. If standard deviation value is not given, it equals to zero.

<i>1 % solutions of enzyme products</i>		White Cleaved Calcite				White Calcite				Yellow Calcite	
		Adhesion Behaviour	Contact Angle		Adhesion Behaviour	Contact Angle		Adhesion Behaviour	Contact Angle		
			Absolute	Relative		Absolute	Relative		Absolute	Relative	
Amylase NS 81251	<i>n</i> -Decane		24°±5°	0.24		31°±3°	0.02		29°±2°	0.11	
	Crude Oil		29°±4°	0.23		31°±3°	0.18		24°±2°	0.36	
Esterase NS 44164	<i>n</i> -Decane		0°	1		0°	1		0°	1	
	Crude Oil		0°	1		0°	1		0°	1	
Lipase NS 81249	<i>n</i> -Decane		20°±1°	0.37		24°±4°	0.26		20°±3°	0.37	
	Crude Oil		0°	1		0°	1		0°	1	

5. Discussion

We have verified experimentally how addition of certain enzymes and their solutions modifies the adhesion properties of oil on a rock surface in a brine environment.

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While the original rock surfaces have proven to be weakly water wet, all the studied enzymes either behaved neutrally or modified the surface towards higher water wettability. This is supported by the results of contact angle measurements (presented in Section 3.3). Such a modification of the surface is not always considered to be advantageous for EOR. For example, the studies of Jadhunandan and Morrow (1995) (for Berea sandstones) and of Skauge and Ottesen (2002) (for North Sea reservoir cores) indicate that the residual saturations may be lower for nearly neutral-wet conditions. One of the reasons for that may be suppressing the mechanism of snap-off.

Hence a promising behaviour of an enzyme to improve the recovery would only be when it totally breaks bonds between the oil and the surface, thus overcoming the adhesion and making oil mobile in the flow. Apparently, the enzymes adsorb on the rock surface replacing oil. They are less active at an oil-brine interface (in contrast to surfactants). The possibility for an enzyme to make oil fully detach from the surface should be considered as the key property for its application to EOR, as well as for an explanation of the observed positive effect on recovery (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Nasiri *et al.*, 2009; Ott *et al.*, 2011). Measurements of contact angles provide additional information: a minimum concentration at which the contact angle decreases to zero may be considered as a threshold value for enzymatic action. It is also important that an enzyme behaves consistently, producing a reproducible effect even at low concentrations.

Only the group of lipases/esterases has been found to fulfil all these criteria. Moreover, only some enzymes of the group (like NS44164) have shown stable response under concentrations of the enzyme product as low as 0.5%. Such enzymes should be considered to be potentially suitable for practical applications.

Apart from breaking the bonds between the oil and the surface, two other mechanisms of enzymatic action have been considered in the scientific literature. As mentioned previously, these mechanisms are: decrease of the surface tension between oil and water; and modification of the oil viscosity due to catalytic action of enzymes on some of the components. Our experiments indicate that the first mechanism is probably irrelevant. Enzymes modify solid-liquid interactions, while their action on the liquid-liquid interface and the corresponding decrease of the IFT is insignificant. Here is a basic difference between the action of enzymes and surfactants, which are capable of modifying not only solid-liquid, but also liquid-liquid interactions.

Only one of the enzymes tested has shown an effect that might be interpreted as a modification of the oil composition (see Section 4.4). This effect might be more noticeable for viscous oils containing high amounts of extra-heavy components, but this effect needs a separate study.

The presented analysis of the mechanisms of the enzymatic EOR is not comprehensive for the task of finding out whether enzymes may be practically used for oil recovery. While we have studied static (equilibrium) behaviour of enzymes, their dynamic behaviour may also be of importance. There are also additional factors to be studied, such as the interaction of the enzymes with bio-environment of the reservoirs or chromatographic separation of enzymes and co-solvents by porous rocks. Laboratory flooding tests (similar to those carried out by Nasiri (2011)) may be

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required in order to confirm the efficiency of the chosen enzymes. Design of such tests (and others) will require the information about action mechanisms of enzymes, studied in the present work, which, thus, has an independent value.

6. Conclusions

We have studied the effect of enzymes on wettability of the surface of calcite representing the internal porous surface of a carbonaceous reservoir. Study of the contact angles of oil drops on the different mineral surfaces indicated that, while the original surfaces were found to be weakly water wet, addition of an enzyme modified the wettability by changing it towards a more water-wetting state. Some enzymes from the group of lipases/esterases were found to be capable of fully detaching the oil drops, even at concentrations as low as 0.1% of enzyme product (0.002 to 0.005% of pure enzymes). These enzymes hold the biggest potential for application to enhanced oil recovery. The effects of enzymes on the surface tensions (unlike the surfactants and the studied commercial products) were found to be insignificant. Reference experiments have also made it possible to verify that it is the enzyme, rather than any other constituents of the enzyme products, that produce the effect of de-adhering of the oil. The developed procedure may be used for screening of the enzymes in terms of their applicability to further EOR tests, and for identification of the static mechanisms by which the enzymes may participate in the EOR. Further studies (like flooding of the reservoir or outcrop cores) should be directed onto the dynamic mechanisms of the enzymatic EOR.

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Chapter 4. Study of Enzyme/Protein Adsorption for Chalk and Sandstone Reservoir Rocks

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Abstract

Enzymes have been considered as promising agents for enhanced oil recovery (EOR). Static adhesion tests and adsorption experiments on powders as well as dynamic flow-through experiments were carried out in order to assess the enzyme loss during their potential application to EOR. Amounts of adsorbed enzyme/protein were different in the various types of experiments, depending of the type of the solid substrate. The flow-through experiments have demonstrated significant loss of the enzymes due to the irreversible adsorption. This conclusion was confirmed in other types of tests, where the desorption times of enzymes were found to be significant. The chalk rock has demonstrated a higher affinity to the enzymes than the sandstone. Change of ionic strength and pH of the enzyme solution and displacing fluid, application of the various injection rates, as well as use of the enzyme stabiliser to desorb biomolecules did not decrease the adsorbed amounts. An alternative solution should be found in order to reduce protein loss in the rock and to make application of enzymatic EOR feasible on a field scale. A developed dynamic model, involving both reversible and irreversible adsorption, as well as normal and anomalous dispersion, demonstrated a reasonably good fit to the experimental flow-through data. The contribution of the anomalous dispersion was found to be insignificant.

Key words: enhanced oil recovery; enzymes; protein adsorption; dynamic model; chalk; sandstone.

1. Introduction

The microbial enhanced oil recovery (MEOR) is known since 1926 (see review in Ollivier and Magot, 2005). Recently a new branch of MEOR that applies enzymes, non-living derivatives of bacteria, was developed. Field and laboratory tests have been carried out, and the results have been quite promising (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Nasiri *et al.*, 2009; Ott *et al.*, 2011). Field tests include applications in China, Indonesia, Venezuela and USA (Apollo GreenZyme® official website; Feng *et al.*, 2007; Moon, 2008; He and Zhonghong, 2011; Ott *et al.*, 2011).

Laboratory displacement studies have confirmed that application of enzymatic enhanced oil recovery (EEOR) may result in up to 16% of additional oil recovery (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Nasiri *et al.*, 2009). Nevertheless, the background mechanisms of the enzymatic action have not been well studied. This may create problems when transferring the laboratory results on the field scale. Among the mechanisms of enzymatic action reported in the

literature, there are: surface wettability improvement towards more water-wet state (Nasiri *et al.*, 2009); decrease of the interfacial tension between oil and enzyme solution and subsequent emulsification (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Nasiri *et al.*, 2009); reduction of oil viscosity (He and Zhonghong, 2011; Moon, 2008); removal of high-carbon content paraffins (Moon, 2008). Feng *et al.* (2007) confirmed tolerance of the enzyme to the reservoir temperature, as well as to salts and bacteria contained in the produced water. However, some other potential problems related to enzyme application for EOR have not been studied in detail. One of them is the problem of enzyme retention by the porous medium.

Retention is usually studied in the static and dynamic adsorption tests. The static tests involve adsorption/desorption tests on the flat surfaces of the minerals characteristic of a petroleum reservoir under study; or adsorption on the powders. The adsorption experiments may be combined with the adhesion tests of the oil drops on the surfaces or between the grains of a powder (Buckley and Morrow, 1990; Hlady *et al.*, 1999). The dynamic adsorption tests involve the flow-through experiments of a single liquid (normally, brine) containing the active agent, and measurement of its recovery at the outlet of a rock sample.

In the previous study (Khusainova *et al.*, 2015) we used the static adhesion tests in order to select the group of enzymes that seems to be the most promising for the enhanced oil recovery. Adhesion of the oil drops on the mineral (calcite) surface in the environment consisting of the brine/enzyme solution was tested. Thus, the presumed mechanism of enzymatic action was changing the surface towards absolutely water wet and, correspondingly, detachment of the oil from the surface. The group of lipases/esterases was found to be the most promising for the wettability improvement. Other effects, like possible change of the oil-water interface or catalytic hydrolysis of the oil, were not detected.

Such a mechanism of enzymatic action may create some problems when applied under reservoir conditions. Adsorption of enzymes on the solid surface may be irreversible, or the time of desorption to be very large, as confirmed by the previous studies of protein adsorption on the solid surfaces (Kirchman *et al.*, 1989; Norde, W., 1986; J. Skujiņš *et al.*, 1974). This may result in retention of the injected enzymes in the porous medium. Since the enzyme concentration is rather small, there is a risk that the entire injected enzyme will be lost on the first centimeters of the rock and have no effect on the oil production from the rest of the reservoir. Therefore, it is important to study the mechanisms of enzymatic adsorption and its reversibility. A large effort has been applied to investigation of adsorption of surfactants (Kwok *et al.*, 1993; Wang *et al.*, 2015) and polymers (Cohen and Christ, 1986; Zaitoun and Kohler, 1987). However, to the best of our knowledge, the only work related to EEOR that has discussed adsorption of enzymes was that of He and Zhonghong (2011).

In the present work we carried out a number of static adsorption-desorption tests, as well as the flow-through tests in the outcrop rock samples of sandstone and chalk. Adsorption-desorption tests on minerals made it possible to examine times required for biomolecules to attach to and detach from the surface. Experiments with adsorption/desorption on powders were additionally

carried out, in order to check the effect of the high surface area. Adsorption-desorption of proteins was also studied in the oil-enzyme solution-powder environments, in order to investigate which interface, i.e. brine-rock or brine-oil, is more preferred by the proteins. Finally, penetration experiments in porous media produced information about, how enzymes/proteins propagate through the rock materials. Analysis of the production data was carried out on the basis of a model involving dispersion, as well as both reversible and irreversible adsorption of the enzymes.

The paper is organized as follows. In Section 2, materials and methods used in the study are described. Section 3 describes the analysis of the dynamic adsorption tests. Section 4 presents results of static and dynamic experiments as well as results of analysis of the flow-through tests. In Section 5, we discuss how our findings can affect application of EEOR on the reservoir scale. Finally, summary of the main results is given in Section 6.

2. Materials and Methods

2.1. Materials

2.1.1. Fluids

Synthetic North Sea water (pH=7.78; composition as given in Table 1) was prepared for both static and dynamic adsorption experiments. Chemicals for seawater (SW) preparation were purchased from Fluka (purity $\geq 99.5\%$) and were not subjected to any further purification. The brine was prepared by diluting salts in distilled water (DW).

Table 1: Composition of synthetic North Sea water used for experiments in present study.

Salt	Concentration, g/l
NaCl	18.01
NaHCO ₃	0.17
KCl	0.74
MgCl ₂ .6H ₂ O	9.15
CaCl ₂ .2H ₂ O	1.91
Na ₂ SO ₄	3.41
Total dissolved solids	33.39

n-Decane (purity $\geq 99\%$) used for static adsorption-desorption tests in the systems of oil-enzyme solution-particles and the Bradford reagent (product number B6916) used for quantification of proteins/enzymes were also purchased from Sigma-Aldrich. For adhesion tests light dead oil recovered from a chalk reservoir in the Danish sector of the North Sea was used.

2.1.2. Enzyme/protein samples

Bovine serum albumin protein (BSA, 98% purity) was purchased from Sigma-Aldrich. Two enzyme samples (NS81249 and NS44164) belonging to the class of lipases were kindly provided by Novozymes A/S as commercial products and also as purified enzymes. Enzyme/protein solutions

were prepared by dilution of a sample in SW. All the concentrations given below are weight-to-weight percentage concentrations.

NS81249 and NS44164 products consist of enzyme (2-5%_{wt}), stabiliser (25-30%_{wt}) and water (63-75%_{wt}). The actual concentration of an enzyme in a commercial product was discussed in detail in our previous work (Section 4.1, Khusainova *et al.*, 2015). The purified enzyme samples had higher concentrations of the enzyme (10-25%_{wt}) and almost no stabiliser (Novozymes personal information). The enzyme concentrations discussed in this work are those of the enzyme products (either commercial or purified), but not of the pure enzymes. Characteristics of enzymes/proteins used in this work are given in Table 2.

Table 2: Characteristics of enzymes/proteins applied in this work (BSA characteristics were found from Ge *et al.* (1998) and Barbosa *et al.* (2010); characteristics of NS81249 and NS44164 – from private communication with Novozymes A/S)

Protein/enzyme	Molecular size	Isoelectric point	Dimensions, Å	Working pH range
BSA	66463 Da	4.7	140 × 40 × 40	4-9
NS81249	≈ 30000 Da	4.8-5	Not available	5-10
NS44164	≈ 20000Da	7	Not available	6.5-10

2.1.3. Minerals

A calcite mineral sample was used in this work to represent a chalk reservoir. A sample of quartz represented a sandstone reservoir. The calcite sample with the surface created after cleavage of a larger mineral was kindly supplied by Center for Arctic Technology, Technical University of Denmark (Lyngby, Denmark). The quartz sample was kindly provided by the Geological Museum of Copenhagen, Denmark. Detailed preparation and cleaning procedure for the minerals can be found in our previous study (Khusainova *et al.*, 2015).

2.1.4. Cores

To investigate effect of the mineral composition on ability of protein /enzyme to propagate through the porous media, the sandstone and chalk cores were used. All the samples were outcrop cores drilled from Stevns Klint chalk (Denmark), Nordhorn sandstone (Austria) and Obernkirchener sandstone (Germany) blocks. Characteristics of the cores are given in Table 3.

2.1.5. Particles

Silica (5-10 μm) and carbonate (≤30 μm) particles were purchased from Sigma Aldrich (product numbers S5631 and 310034, respectively) and were not subjected to any further treatments. The silica particles were used to mimic a sandstone reservoir and the carbonate particles represented a chalk reservoir.

Table 3: Characteristics of the core samples used for the experiments in this work.

Sample name	Type	Origin	Diameter, mm	Length, mm	Weight of the dry core, g	Pore Volume, ml
C-13	Outcrop chalk	Stevns Klint, Denmark	37.17	71.59	110.53	35.61
C-18	Outcrop chalk	Stevns Klint, Denmark	37.15	70.45	109.6	34.85
C-57	Outcrop chalk	Stevns Klint, Denmark	36.97	75.77	118.09	37.03
C-8	Outcrop chalk	Stevns Klint, Denmark	36.96	76.1	119.83	37.26
S-145	Outcrop sandstone	Nordhorn, Austria	25.70	77.33	78.46	6.93
S-149	Outcrop sandstone	Nordhorn, Austria	25.56	76.15	77.26	6.98
S-153	Outcrop sandstone	Nordhorn, Austria	25.57	78.73	80.84	7.47
S-01	Outcrop sandstone	Obernkirchener, Germany	37.91	90.24	220.99	17.70
S-82	Outcrop sandstone	Nordhorn, Austria	25.48	77.2	78.06	7.35

2.2. Methods

Three sets of experiments were conducted in this study. First, principal occurrence of adsorption-desorption was estimated by static adhesion tests on the mineral surfaces. Second, adsorption tests on solid particles under presence and absence of the oil phase were carried out to investigate competitive adsorption between fluid-solid and fluid-fluid interfaces. Finally, dynamic flow-through tests were carried out in order to assess whether enzyme/protein can travel through the porous media and if this is possible, how much can be recovered.

2.2.1. Adhesion experiments

The mineral was soaked in enzyme/protein solution for 30 minutes. Enzymes were applied both in the form of commercial products and as purified samples in order to exclude the effect of additional components that can be found in commercial products. After an enzyme/protein interacted with the mineral, the mineral was taken out from the vessel, and the bulk protein solution was gently removed with paper. The mineral was then placed on stands in a glass container and immersed in SW. Countdown was launched immediately after immersion. An adhesion state of the mineral surface started being assessed as soon as possible. Determination of the adhesion state was

performed according to the method developed by Buckley and Morrow (1990). The adapted detailed procedure was described in our previous work (Khusainova *et al.*, 2015). Briefly, the drops of crude oil were formed by a syringe with an inverted needle and deposited on the lower crystal face. An oil drop was allowed to interact with the mineral surface for 2 minutes without detachment from the needle. Then the needle was moved down and, depending on a state of the mineral surface, three different events were observed: (A) The adhesion behaviour: the oil drop sticks to the mineral surface; (B) The temporary adhesion behaviour: initially the oil drop sticks to the surface, but upon lowering the needle it detaches and stays on the needle; (C) The non-adhesion behaviour: the drop stays on the needle, leaving clean mineral surface.

All the experiments were accomplished under ambient conditions and checked for reproducibility.

2.2.2. Static adsorption-desorption experiments with solid particles

The adsorption experiments were performed in the 15-ml plastic centrifuge tubes. 3 ml of commercial enzyme product solution of a desired concentration was added to 3 ml of *n*-decane and/or to 1 or 2 g of solid particles. The samples were mixed on a vortex mixer every 10 minutes for 1 hour at room temperature. The tubes were then centrifuged for 10-20 minutes at 4000 rpm and the enzyme concentration in the supernatant was measured using the Bradford assay (see Section 2.2.4). The amount of the enzyme adsorbed on the solid particles was calculated from the material balance. The desorption experiments were conducted by diluting the water phase with 3 ml of pure SW, without replacement of the original water phase. All the experiments were checked for reproducibility.

2.2.3. Flow-through tests

2.2.3.1. Experimental set-up

Dynamic protein adsorption tests were conducted on a coreflooding set-up (Figure 1). The experimental set-up consisted of the three main parts: the injection part, the coreholder, and the fraction collector.

The injection part included three stainless steel cylinders that were filled with the saturating and the displacing fluids and with the enzyme/protein solution. A high precision Teledyne ISCO syringe pump was used to inject the fluids. A pressure transducer was installed in order to monitor the injection pressure.

The Hassler type coreholder was used. A radial confining pressure was applied and kept constant by means of another Teledyne ISCO pump.

The effluent was collected using the X-Y BioFrac fraction collector 741-0002EDU (Bio-Rad Laboratories, Inc 2013).

The data acquisition was accomplished by the 34972A Data Acquisition/Data Logger Switch operated by the Keysight BenchLink Data Logger software.

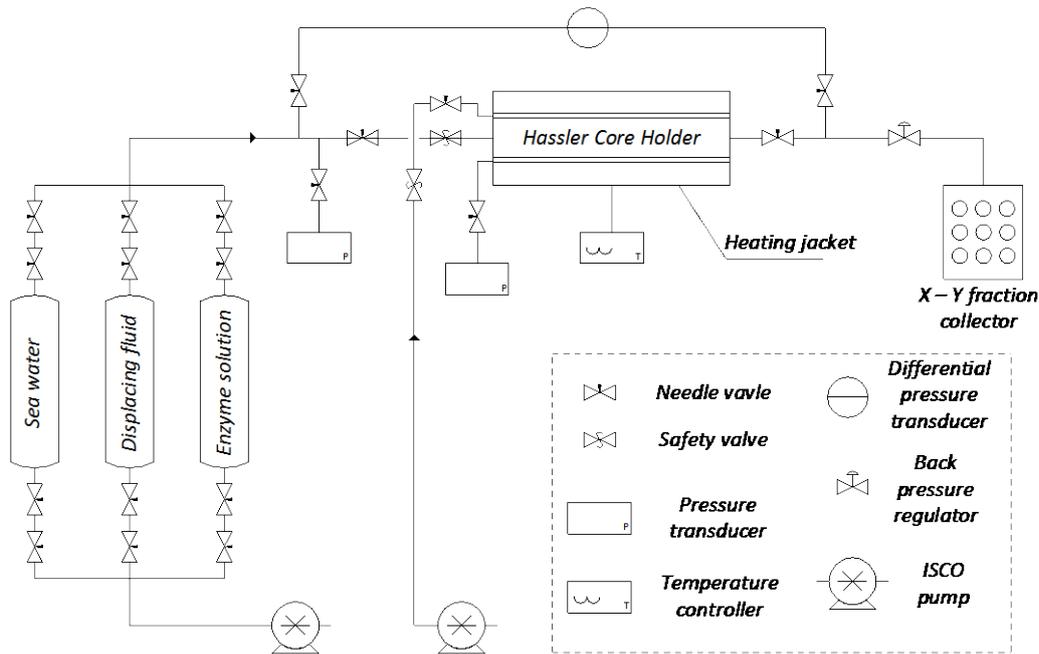


Figure 1: Coreflooding set-up used for dynamic adsorption experiments.

2.2.3.2. Preparation of the core samples

Prior to the experiments all the core samples were checked for integrity with the X-ray computed tomography scanner and then cleaned by flushing toluene and methanol. The cleaned samples were dried overnight at 90°C and scanned again.

In order to estimate the extent of core saturation with the brine, true porosity of the cores was measured by the steady state gas permeameter and porosimeter (Poroperm, VINCI TECHNOLOGIES) using nitrogen. Saturation of a core with the synthetic brine was carried out in three steps. First, the core was saturated under vacuum. Then the core was placed into a stainless steel cylinder filled with SW and pressurized to 100 bar for 48 hours. After saturation under pressure the core was rapidly transferred into a rubber sleeve and assembled into the coreholder. Finally, at least 10 pore volumes (PV) of SW were flushed through the core with the flow rate of 0.1 ml/min. The degree of saturation was then determined by the weight method. The results were compared with the value of the pore volume acquired by the porosimeter. A difference of less than 5% was considered to be acceptable.

2.2.3.3. Injection and displacement of an enzyme/protein

Dynamic penetration experiments were accomplished using the BSA and the purified enzyme samples. The protein concentrations were slightly higher than for the wettability tests (Khusainova *et al.*, 2015), in order to be able to quantify protein in the effluent. The flow-through experiments were started by flowing the BSA solution through a chalk core. The BSA was initially

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Table 4: Injection and displacement conditions and schemes of the flow-through experiments.

Experiment	Enzyme/ protein tested	Core sample	Sleeve Pressure, bar	Enzyme injection				Displacing fluid injection			
				Solvent used to prepare enzyme/ protein solution	Concentration of enzyme/protein solution		Volume injected, PV	Injection rate, ml/min	Displacing fluid	Injection rate, ml/min	Volume injected, PV
					% _{wt}	mg/ml relative BSA					
A	BSA	C-13	20	SW	0.5	4.45	0.5	1	SW	1	20
B	BSA	C-18	20	SW	1	8.82	0.5	1	SW	1	10
C	NS81249	C-57	20	SW	5	3.51	1	1	SW/DW/ propylene glycol	1	7/3/3
D	NS81249	C-8	20	DW*	5	3.70	1	1	DW*	1	8
E	BSA	S-145	20	SW	0.5	4.41	11	0.2	SW	0.1	4
F	BSA	S-149	30	SW	0.5	4.54	1	0.2	SW	0.1	4
G	NS81249	S-153	30	SW	5	3.60	1	0.2	SW	0.1	10
H	NS81249	S-01	50	SW	5	3.58	1	0.1	SW/3M NaCl	0.1	5/8
I	NS44164	S-82	50	SW	5	0.99	1	0.1	SW	0.1	3
J	NS44164	S-82- reused	50	SW	5	0.87	1	0.1	SW	0.1	4

*- DW + NaOH to adjust pH=8

used as a model protein, in order to develop experimental procedure. Then the results obtained with the BSA were compared with the enzymes.

The enzyme/protein was injected into a saturated core as a portion of 1 pore volume (PV). Then the enzyme/protein solution was displaced by SW or another displacing fluid. The results obtained during static adhesion tests were extended onto dynamic flow-through experiments: the flow rates were chosen based on the measured adsorption-desorption times. Injection and displacement conditions and schemes are given in Table 4.

The effluent was collected in the portions of 1/7 to 1/6 PV. The tubes with collected effluent were moved to the fridge in order to exclude denaturation of diluted enzyme/protein solutions. All the experiments were conducted at room temperature.

2.2.4. Quantification of the enzymes/proteins

The enzyme/protein concentrations were measured by Bradford Assay (Bradford, 1976). The 96 Well plate assay protocol from Sigma-Aldrich (2013) was followed. Briefly, 5 μ l of the protein standards and unknown samples were added to separate wells. 250 μ l of the Bradford reagent was added to each well. Afterwards the solutions were mixed on a shaker for about 30 seconds at room temperature. Then the samples were incubated at room temperature for 10 minutes and absorbance at 595 nm was measured. The protein-dye complex causes a shift in the absorption maximum of the dye from 465 to 595 nm.

The standard curve was built for the standard BSA solutions. The enzyme/protein concentrations of the unknown samples were determined by comparing the Net A600 values against the standard curve. Therefore, the obtained enzyme concentration values are not the absolute concentrations, but the concentrations relative to BSA.

Solutions of BSA for construction of the standard curve were prepared by dilution of the standard BSA in distilled water. It was found in advance that there is no interference from salts that were used to prepare the SW and there was no difference whether DW or SW was used to dilute the protein/enzyme samples.

3. Analysis of the results of the dynamic adsorption tests

Analysis of the enzyme/protein production history is necessary in order to evaluate characteristic parameters of their penetration through the porous media. Hence, a dynamic model accounting for important features of the observed experimental picture of penetration was developed.

Qualitative experimental observations (see discussion below) show that: 1) Distribution of the produced enzyme is significantly “washed out”; 2) Maximum of the enzyme production is postponed compared to the carrying liquid; and 3) Not all the enzyme has been produced back, even after great many porous volumes injected.

The first observation indicates that enzyme/protein dispersion is important for the process. Hence, the model should consider dispersion of the enzymes/proteins in the flow. We have considered not only traditional diffusion-like dispersion, but also the anomalous dispersion of the travel times, of the type of “elliptic diffusion”, as described in works of Shapiro (2007), Shapiro and Bedrikovetsky (2008); although, as will be discussed later, contribution of the effect of anomalous diffusion has turned out to be insignificant.

According to the second observation, reversible sorption of the enzyme/protein must take place. A delay of a protein molecule compared to the flow is explained by the fact that the molecule moves with the flux during a fraction of the time, while another fraction is spent on the surface, in the adsorbed state. We have incorporated equilibrium linear sorption characterized by the Henri constant Γ .

Finally, the fact that it is not possible to produce back the entire enzyme/protein may only be explained by its irreversible retention in porous medium. This retention may be described in a manner similar to the irreversible sorption, or the deep bed filtration. Simultaneous occurrence of both reversible and irreversible sorption has previously been detected for the polymer and surfactant solutions (Cohen and Christ, 1986; Kwok *et al.*, 1993; Wang *et al.*, 2015; Zaitoun and Kohler, 1987).

A linear flow equation incorporating all the mentioned effects has the form of (Shapiro and Bedrikovetsky, 2008):

$$\phi(1+\Gamma)\frac{\partial c}{\partial t} + V\frac{\partial c}{\partial x} = D_x\frac{\partial^2 c}{\partial x^2} + D_t\frac{\partial^2 c}{\partial t^2} - \Lambda Vc \quad (1)$$

Here ϕ is porosity, Γ the sorption constant rescaled to the porous volume, c concentration of the enzyme, V velocity of the flux, D_x, D_t the special and the temporal dispersion coefficients, and Λ the filtration coefficient taken per unit length. All the coefficients in Eq. (1) are presumed to be constants, to be adjusted on the basis of the experimental enzyme/protein production curves. For comparison, both data and equation have been reduced to the dimensionless form, with the dimensionless distance X in porous volumes, and time T in porous volumes injected (PVI):

$$\frac{\partial c}{\partial T} + \frac{1}{1+\Gamma}\frac{\partial c}{\partial X} = d_x\frac{\partial^2 c}{\partial X^2} + d_t\frac{\partial^2 c}{\partial T^2} - \lambda c \quad (2)$$

$$X = x / L; T = Vt / \phi L;$$

$$d_x = \frac{D_x}{VL(1+\Gamma)}; d_t = \frac{D_t V}{\phi^2 L(1+\Gamma)}; \lambda = \frac{\Lambda L}{1+\Gamma} \quad (3)$$

Equation (2) requires initial and boundary conditions. The initial condition corresponds to the absence of enzyme/protein in the rock sample before the experiment. The boundary condition reflects injection of the protein during one porous volume with a constant concentration C_0 :

$$\begin{aligned} t = 0 : c &= 0 \\ X = 0 : c &= c^0, 0 < T < 1; c = 0, T > 1 \end{aligned} \quad (4)$$

Presence of the second derivative in Eq. (2) would require, in principle, the second, “final” boundary condition in time. However, it has been shown by Shapiro (2007), Shapiro and Bedrikovetsky (2008), that this boundary condition is not required if a solution that is bounded at infinity is selected.

Eq. (2) with the boundary condition (4) allows for an exact solution, which is derived in Appendix A. The corresponding solution for the pure dispersion problem ($d_i = 0$) is described in Appendix B. The coefficients in these solutions have been optimized to fit the experimental curves. The minimization function S represented the deviations of the solution from the experimental values c_{exp}^i taken at the different times (PVI) T_i from the production stream at $X = 1$:

$$S = \sum_{i=1}^{N_{\text{exp}}} (c(X=1, T_i) - c_{\text{exp}}^i)^2 + A \cdot (Q - Q_{\text{exp}})^2$$

Here Q , Q_{exp} are the total amounts of the injected enzyme/protein, calculated and experimental, correspondingly. The constant A was selected to be large enough in order to assure these amounts to be (almost) equal.

4. Results

4.1. Adhesion tests

The adhesion experiments were conducted in order to get an idea about the characteristic times of adhesion and to predict some parameters for the dynamic adsorption tests. Based on the adhesion tests the flow rates and durations of the flow-through tests were estimated.

Both crude oil-SW-calcite and crude oil-SW-quartz systems demonstrated the adhesion behaviour: the oil drops attached to the mineral surfaces and stayed there. The adhesion state was taken as a reference point, and any further change in wettability of the mineral surface was ascribed to its exposure to the enzyme/protein solutions. In agreement with the previously established data (Khusainova *et al.*, 2015), enzymes/proteins used in this work could turn initial adhesion behaviour of the minerals to a non-adhesion state, mainly due to adsorption of the enzymes/proteins on the solid surface.

4.1.1. Crude oil-[SW+BSA]-mineral systems

Exposure of the calcite to the 1% BSA solution resulted in combined non-adhesion state due to adsorption of BSA molecules on the surface. Afterwards the calcite was immersed in the seawater and its wetting state was checked during 42 days (Table 5). No desorption of the protein was observed. In contrast, in case of application of 0.5% BSA solution there was rapid desorption of the protein with final desorption after 132 min. It should be mentioned that complete desorption never occurs for the proteins (Norde, 1986; Kirchman *et al.*, 1989). By “final desorption” we mean not complete desorption, but desorption of the significant amount that affects stickiness of the mineral surface to the crude oil.

These results can be correlated with the concentration dependent structure of the adsorbed protein layer and with the nature of the protein. When calcite was immersed into 1% BSA solution, there was an excessive amount of the protein molecules to cover the solid surface and adsorption occurred most likely in a multilayer mode. During desorption the molecules from the outer layers could detach, so that the adsorbed layer was loosened close to the surface. Due to the flexible nature of the BSA molecules, the molecules directly attached to the surface unfolded and bounded with each other via several sites preventing further desorption (Norde, 1986; Norde, 2008). This explains why complete desorption was not observed. For a lower concentration, there was a lack of protein molecules and the surface was only partly covered. Hence, temporary adhesion behaviour was observed when calcite was transferred to pure SW. After 132 min from the start of the experiment significant desorption of the BSA apparently happened. In this case, presumably, coverage of the surface was low and the adsorbed molecules could not form a network on the surface.

Experiments with quartz demonstrated different results compared to calcite. After the quartz surface with adsorbed proteins was immersed into the pure SW, the BSA molecules stayed adsorbed during the first forty minutes (Table 5). Then there was transition period, where the BSA started desorbing. Final desorption occurred after 75 min.

Table 5: Adhesion behaviour of calcite and quartz immersed in BSA solutions.

		1% BSA			0.5% BSA	
Calcite	Adhesion behaviour	[Green]			[Red]	[Red]
	Time	42 days			0 – 122 min	132 + min
Quartz	Adhesion behaviour	[Green]	[Red]	[Red]	N/A	
	Time	0 – 40 min	45 – 70 min	75 + min	N/A	

 - adhesion  - temporary adhesion  - non-adhesion

The adsorption-desorption results obtained for the BSA are in agreement with the expected electrostatic interactions between the BSA molecules and the calcite/quartz surfaces. The BSA is characterised by the isoelectric point of 4.7 (Ge *et al.*, 1998). The seawater used in this study had pH of 7.78, which means that BSA was negatively charged. The surface of the calcite crystals has a positive charge in SW (Somasundaran and Agar, 1967), while the quartz crystals are negatively charged (Jada *et al.*, 2006). Hence, due to electrostatic attraction the BSA binds stronger to calcite. As a result, desorption of the adsorbed BSA layer cannot be reached, whereas electrostatic repulsion between the BSA and the quartz surface enhanced desorption process.

4.1.2. Crude oil-[SW+NS81249]-mineral and crude oil-[SW+NS44164]-mineral systems

Adsorption-desorption patterns were quite similar for enzymes NS81249 and NS44164 (Tables 6 and 7), but generally opposite compared to the BSA (Table 5). Desorption of the enzyme molecules from calcite occurred rapidly, once the mineral was placed in enzyme-free SW, as adhesion behaviour was observed from the first measurement. Unexpectedly, after 25 - 35 min the adhesion state changed to combined temporary/non-adhesion behaviour. This means that desorbed enzyme molecules adsorbed again making calcite surface non-oil sticky. The secondary enzyme adsorption lasted for 110 and 65 minutes for NS81249 and NS44164, respectively. Then continuous adhesion state was established, indicating final desorption of the enzymes.

Table 6: Adhesion behaviour of calcite and quartz immersed in NS81249 enzyme solutions.

		1% NS81249 (product)			5% NS81249 (purified)	
Calcite	Adhesion behaviour				N/A	
	Time	0 – 30 min	35 – 145 min	155 + min	N/A	
Quartz	Adhesion behaviour					
	Time	48 days			0 – 83 min	92 + min

Table 7: Adhesion behaviour of calcite and quartz immersed in NS44164 enzyme solutions.

		1% NS44164 (product)			5% NS44164 (purified)	
Calcite	Adhesion behaviour				N/A	
	Time	0 – 20 min	25 – 90 min	100 + min	N/A	
Quartz	Adhesion behaviour					
	Time	39 days			0 – 202 min	215 + min

No significant desorption of the enzyme products was observed from the quartz surface, even though experiments lasted for more than a month. In contrast, final desorption of the purified enzyme samples was reached after 92 and 215 minutes for NS81249 and NS44164, respectively. An

enzyme product differs from a purified sample by presence of the stabiliser. Previously it was shown that stabilisers alone do not affect adhesion behaviour (Khusainova *et al.*, 2015). Moreover, the function of the stabiliser in the commercial product is to increase internal stability of the enzyme molecules to secure product shelflife. (Novozymes A/S private communication).

Most likely, reversibility of the enzyme adsorption can be related to the enzyme concentration. The 1% enzyme product contained 0.02 - 0.05% of pure enzymes, while the purified enzyme samples contained 4 - 5 times more of protein molecules (Novozymes A/S, private communication). Since the concentration of the purified samples was 5%, the concentration of pure enzymes in such samples was 0.4 - 1.25 %, that is comparable to concentration of BSA. Indeed, results obtained for purified enzyme samples are quite similar to data obtained for BSA.

With regard to reversibility of adsorption, the results shown by enzyme solutions (Tables 6,7) are opposite to the results for the BSA (Table 5). Enzymes produce adhesion, then non-adhesion or temporary adhesion, and then non-adhesion again of the oil drops on calcite, and temporary/non-adhesion on quartz, while behaviour of the BSA solutions with regard to the calcite and quartz surfaces is opposite. It should also be noticed that the enzymes produce these effects in much smaller concentrations: while the combined temporary/non-adhesion state of the quartz surface was reached using 1% of the enzyme products, i.e. 0.02-0.05% of the pure protein, for BSA the same state was reached using 1% of pure protein.

Both enzymes have isoelectric points below pH of the SW that means they are negatively charged in the solutions. Under presence of stabilisers, the enzymes possess strong internal stability and electrostatic interactions are the main factor governing adsorption-desorption processes, which means that the results should be similar to the BSA. The real picture of the interaction of the proteins with the ions in a solution is much more complicated, since different parts of the enzyme/protein molecules may have a different affinity to the different ions that are met in solution. The structures of the enzymes and BSA may also differ from each other. This probably explains the fact of varying behavior of the different proteins with regard to reversibility of adsorption, as described above.

4.2. Static adsorption-desorption experiments with solid particles

The results for static adsorption-desorption experiments with solid particles are summarized in Table 8. Different enzyme-particle ratios, as well as adsorption of enzymes on the particles under presence of the oil phase were examined. As expected, in both cases (with and without oil) the amount of the adsorbed enzyme on solid surface increased with increase of the enzyme concentration. Good reproducibility of the results was additionally confirmed by similar adsorption values obtained for 2:1 particles to enzyme ratio under the different concentrations of enzyme in solution (i.e. 2g of particles and 1% solution of NS81249 versus 1g of particles and 0.5% solution of NS81249).

Some experiments were carried out without solid particles, but in presence of the oil. For such experiments the adsorbed amounts were calculated per gram of oil (Table 8). Reduction of

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Table 8: Summary of the results for static adsorption-desorption experiments with solid particles (negative desorption values imply further adsorption of enzymes on solid particles when original water phase was diluted with 3 ml of pure SW).

		NS81249 concentration, mg/ml	No oil phase			Oil phase present			
			Adsorption		Desorption, mg/g-particles	NS81249 concentration, mg/ml	Adsorption		Desorption, mg/g-particles
			mg/g-particles	mg/m ² *			mg/g-particles	mg/m ² *	
No particles	1% NS81249	0.14				0.14			
		-	-	-	-	0.12	0.033	-	-0.036
	0.5% NS81249	0.06				0.06			
		-	-	-	-	0.05	0.013	-	-0.052
1g of particles	1% NS81249	0.14				0.14			
	CaCO ₃	0.10	0.058	0.052	-0.081	0.10	0.054	0.043	-0.080
	SiO ₂	0.05	0.250	0.054	-0.038	0.07	0.207	0.045	-0.094
	0.5% NS81249	0.06				0.06			
	CaCO ₃	0.05	0.045	0.041	-0.073	0.05	0.029	0.027	-0.059
	SiO ₂	0.01	0.142	0.031	-	0.03	0.096	0.021	0.144
2g of particles	1% NS81249	0.14				0.14			
	CaCO ₃	0.11	0.043	0.039	-0.021	0.12	0.029	0.027	-0.043
	SiO ₂	0.02	0.173	0.038	-0.009	0.05	0.126	0.027	-0.042
	0.5% NS81249	0.06				0.06			
	CaCO ₃	0.05	0.014	0.013	-0.044	0.05	0.014	0.013	-0.034
	SiO ₂	0.01	0.074	0.016	-	0.02	0.057	0.012	-0.022

*- Surface area of calcium carbonate and silica particles for calculation of adsorption per unit of surface area was taken from Huber Engineered Materials and Vandevanter *et al.* (2012), respectively.

enzyme concentration in water in such experiments was comparable to the corresponding values for adsorption of the particles without oil. This means that enzyme adsorption occurred both on solid-water and oil-water interfaces and the extent of adsorption was quite similar.

It might be expected that the adsorption values for the systems containing both oil and solid particles would be higher due to simultaneous adsorption on the two interfaces. However, as opposite, the total amount of enzyme adsorbed in such systems was slightly lower (Table 8). This phenomenon can be explained by formation of an *n*-decane layer on the particle surfaces (Brindza *et al.*, 2010; Schmitt *et al.*, 1988) and/or by formation of a layer of the particles on the oil-water interface (Fan *et al.*, 2011; Sharp *et al.*, 2014) that would prevent protein adsorption on solid-water and/or oil water interfaces, correspondingly. Microscopic photographs of the water-oil-particle emulsions (not shown here) confirm the second hypothesis: the particles tend to concentrate around water-oil interfaces.

The amounts adsorbed per unit surface area were found to be independent of a type of solid particles, but only on the particle-enzyme ratio and presence of oil (Table 8). An amount adsorbed per mass unit for silica was always higher than for calcium carbonate, but this was only due to the difference in particles sizes and, consequently, due to the different surface areas.

For all the studied systems no desorption was observed. Opposite, when the water phase was diluted with pure SW amount of the protein in the water phase decreased, which means further adsorption of enzymes occurred. This result is unexpected and needs further study.

4.3. Dynamic adsorption experiments

4.3.1. Filtration of enzymes/proteins through chalk

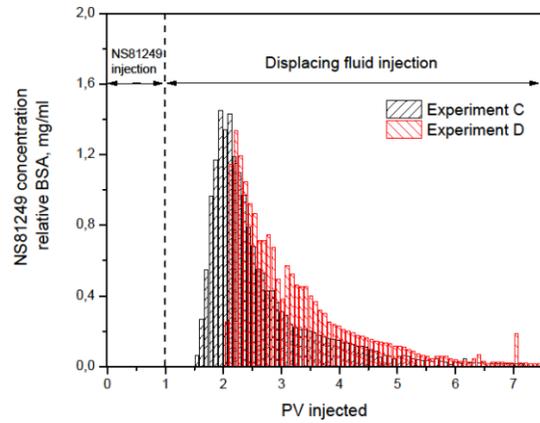
4.3.1.1. BSA

No penetration of the BSA through the chalk rock, even with a doubled concentration, and even after 20 PV of seawater injected, could be achieved (experiments A and B, Table 9). Further increase of the protein concentration was not investigated, since it was economically non-feasible for real field applications. It was concluded that all injected BSA molecules were irreversibly adsorbed to the chalk pore walls and could not be desorbed by dilution using SW. Adsorbed amount of BSA in chalk could be quantified as ≥ 0.563 mg/g-rock.

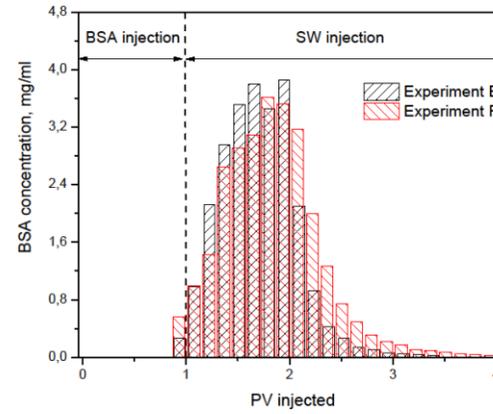
4.3.1.2. NS 81249

Enzyme NS81249 could partly be recovered from chalk. Figure 2 (a) shows production histories for experiments C and D, which had different displacing fluids. Both profiles show the unimodal distribution with concentration maxima at around 1.45 and 1.34 mg/ml for experiments C and D, respectively. The ratio of maximum to initial concentration (c_{\max}/c_0) was below 0.5. For Experiment C, the seawater was used as the initial displacing fluid. Displacement of NS81249 by SW (the black profile in Figure 2 (a)) resulted in 43.6% of the enzyme recovered. The first portion of enzyme in the effluent was detected after 0.6 PV of SW was injected. A major amount of the

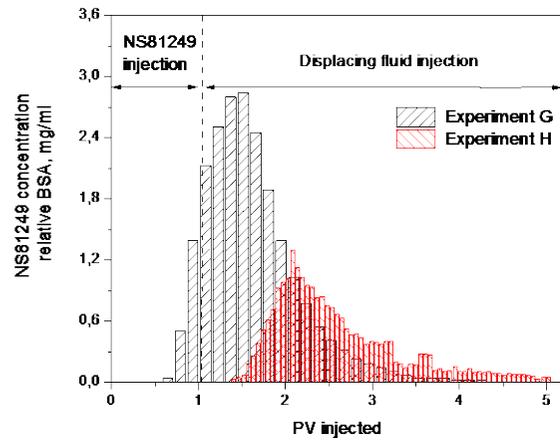
Chapter 4. Study of Enzyme/Protein Adsorption for Chalk and Sandstone Reservoir Rocks



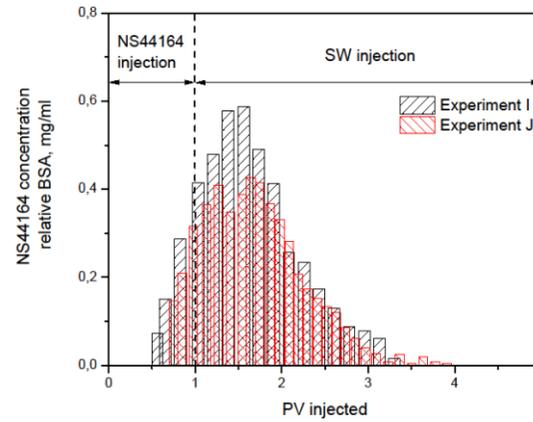
(a)



(b)



(c)



(d)

Figure 2: Production histories of injection of enzymes/proteins in chalk and sandstone cores and their following displacement using various displacing fluids.

enzyme was recovered after 4 PV injected, but a long tail of the enzyme could be detected even after 6 PV of SW injected.

Injection of the seawater in Experiment C was followed by injection of the distilled water (a solution with weaker ionic strength than the SW) and then by the enzyme stabiliser (propylene glycol). This was carried out in order to check possible ways of desorbing the enzyme, see analysis by Norde (1986). Injection of neither fluid has resulted in additional recovery of the enzyme.

In Experiment C enzyme NS81249 was injected in high ionic strength SW solution, that is, under adsorption-favourable conditions. On the contrary, in Experiment D enzymes were injected in a low ionic strength solution and displaced by it, to minimize initial adsorption. NaOH was added to DW to adjust pH, which, along with the ionic strength, is one of the main parameters influencing the protein adsorption. Since maximum adsorption occurs at isoelectric point of the protein, pH of the solution should not be close to this value in order to reduce adsorption. SW used in this study had pH of 7.78, which is higher than isoelectric points of BSA, NS81249 and NS44164 samples (see Table 2) and pH requirements was met. Original pH of DW was 5.5 and it is close to isoelectric point of NS81249, so pH was increased up to 8. Theoretically it was also possible to reduce pH of DW towards more acidic, but this would cause dissolution of chalk.

The history of enzyme production for Experiment D is shown in Figure 2 (d). The effluent concentration distribution is generally similar to that for Experiment C, but the two distinctions can be noted. First, the enzyme breakthrough occurs 0.4PV later than for Experiment C. Secondly, enzyme concentration decreases less rapidly. The total recovery of the enzymes, as well as adsorbed amounts were similar for both experiments (Table 9), hence, application of DW did not reduce the enzyme adsorption.

4.3.2. Penetration of enzymes/proteins through sandstone

4.3.2.1. BSA

The BSA penetration profiles are presented in Figure 2 (b). Experiment F was a duplicate of Experiment E, and showed good reproducibility of the results. In contrast to chalk, the BSA can penetrate through sandstone with a relatively low irreversible adsorption (total recovery of BSA were 82.0% and 86.9%). The adsorption values were also low (0.070 mg/g-rock and 0.054 mg/g-rock), while c_{\max}/c_0 ratios were rather high (0.8 and 0.87). It should be remarked that these values were obtained for relatively short samples, while on the reservoir scale the effect may be different (see analysis in the next section).

BSA was first detected in the effluent after 0.86 PV of protein was pumped (Figure 2 (b)). Taking into account that 1PV of BSA was injected in total, early protein breakthrough means that some BSA molecules were travelling faster than the carrying fluid. A majority of BSA was recovered after about 1.5PV of SW was flooded. A relatively short tail of the protein could be observed.

Table 9: Summary of the results of dynamic flow-through experiments.

Experiment	Protein/enzyme	Core internal surface area*, m ²	Total enzyme/protein recovery, %	Enzyme/protein recovery within first displacing PV, %	c_{max}/c_0	Irreversible adsorption mg/g-rock	Estimated irreversible adsorption mg/m ²
Chalk							
Experiment A	BSA	221.06	0	0	0	≥0.283**	≥0.141**
Experiment B	BSA	219.20	0	0	0	≥0.563**	≥0.281**
Experiment C	NS81249	236.18	43.6	13.4	0.41	0.620	0.310
Experiment D	NS81249	239.66	37.7	0	0.36	0.769	0.385
Sandstone							
Experiment E	BSA	132.60	82.0	55.9	0.87	0.070	0.041
Experiment F	BSA	130.57	86.9	59.3	0.80	0.054	0.032
Experiment G	NS81249	136.62	87.2	71.2	0.79	0.040	0.024
Experiment H	NS81249	291.71	40.5	9.5	0.36	0.167	0.127
Experiment I	NS44164	131.92	78.7	60.7	0.59	0.016	0.009
Experiment J	NS44164	131.92	69.0	51.9	0.55	0.026	0.015

* - Specific surface areas of outcrop chalk and sandstone core samples for calculation of adsorption per unit of surface area was taken from Puntervold *et al.* (2007) and Dubelaar and Nijland (2015), respectively.

** - The exact value of adsorption could not be calculated, as all injected BSA was retained in the core.

4.3.2.2. NS 81249

Penetration of NS81249 through sandstone was studied for two different sandstone cores: Nordhorn sandstone and Obernkirchener sandstone. The black profile in Figure 2 (c) demonstrates distribution of the enzyme in the effluent for filtration of NS81249 through the Nordhorn sandstone (Experiment G). The distribution is quite similar to the production history for injection of the BSA in sandstone. However, the NS81249 molecules were flowing even faster than BSA, and detection of the first portion of the enzyme in the effluent occurred after 0.6PV injected. The total enzyme recovery was the highest among all the experiments (87.2%). Adsorption was slightly less compared to BSA (0.040 mg/g-rock), while the ratio of c_{\max}/c_0 was almost the same as for Experiment F.

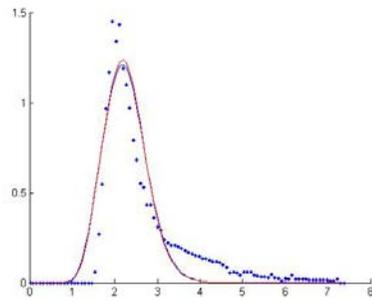
Adsorbents with a higher internal surface are usually characterised by a higher adsorption capacity. To investigate how increase of internal surface area can affect amount of adsorbed enzymes, the Obernkirchener sandstone core (Germany) was used (Experiment H). The core had an internal surface area of 291.71 m² compared to 136.62 m² of the internal surface area for the Nordhorn sandstone (Experiment G). The production history of NS81249 is given in Figure 2 (c). A general shape of the distribution is similar to penetration of NS81249 through chalk (Figure 2 (a)) rather than to penetration of NS81249 through Nordhorn sandstone (Figure 2 (c)). The same applies for the amount of the recovered enzyme (Table 9). The history plot is unimodal with a maximum at 1.30 mg/ml relative BSA. The ratio c_{\max}/c_0 is the same as for Experiment D. On the contrary, the adsorption value for the Obernkirchener sandstone was 3.6-4.6 times lower than for the Stevns Klint chalk, even though the sandstone core sample had an about 20% higher internal surface area (Table 9). Comparison of the experimental results for Nordhorn and Obernkirchener sandstones reveals that about 2 times increase of the internal surface area led to about 4 times increase of adsorption. Of course, this analysis is approximate, and a more precise analysis should be based on the results of modelling, as described in the next section.

4.3.2.3. NS 44164

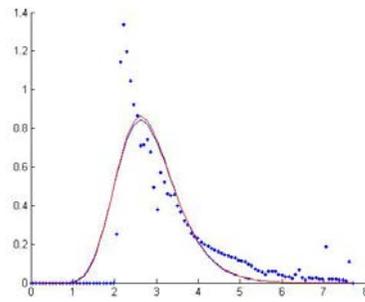
The production histories for NS44164 enzyme through sandstone are shown in Figure 2 (d). Experiment J was a duplicate for Experiment I. This was the only set of experiments where the core sample was reused. Reuse of the core in which enzyme/protein was injected was restricted by potential irreversible adsorption of proteins on core surface, and difficulties with cleaning of the used samples from the proteins. However, for this set the enzyme recovery in Experiment I was rather high (Table 9), so it was acceptable to re-use the core in the duplicate Experiment J. The results (Figure 2 (d)) are comparable to the results for BSA and NS81249 penetration through sandstone (Figure 2 (b), (c)). The adsorbed amounts are the smallest among all the experiments and enzyme recovery values are rather high (Table 9). The early breakthrough of NS44164 was also observed after about 0.5-0.7PV injected (Figure 2 (d)).

Comparison of Experiment I with Experiment J shows that reuse of the core leads to a slightly different enzyme concentration distribution in the effluent (Figure 2 (d)). However, the quantitative characteristics are similar for these two experiments (Table 9).

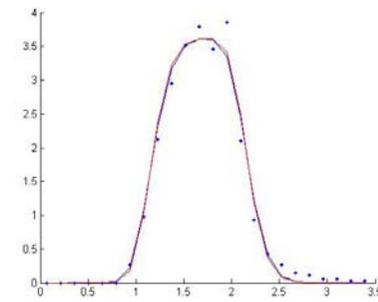
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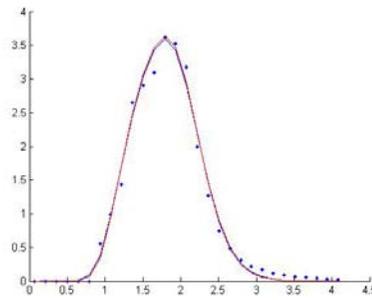
Experiment C



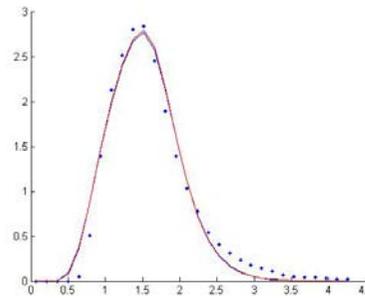
Experiment D



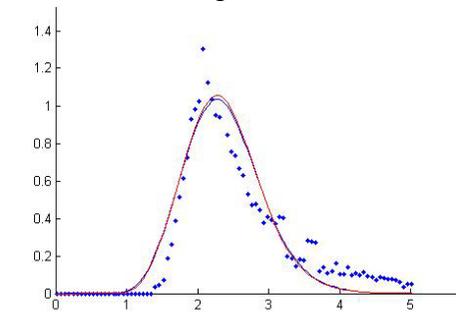
Experiment E



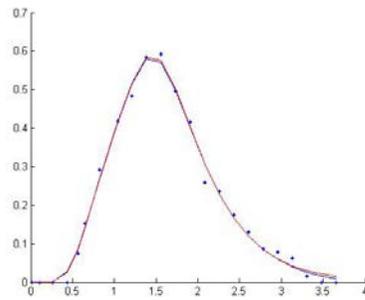
Experiment F



Experiment G



Experiment H



Experiment I

Figure 3: Results of fitting of the experimental data on enzyme production by the suggested model (red line – elliptic fitting, blue line – parabolic fitting).

4.3.3. The modelling results

Production of enzymes has been analysed for seven experiments: the two experiments with the enzyme NS81249 injection into the outcrop chalk (Experiments C and D); the two experiments with BSA injection into the outcrop sandstone (Experiments E and F); the experiment where the enzyme NS44164 was injected into the outcrop sandstone (Experiments I); the experiment with NS81249 injection into the outcrop sandstone (Experiment G); and the experiment with a long sandstone core where enzyme NS81249 was injected (Experiment H). Parameters of the cores and injection schemes are listed in Tables 3 and 4, respectively.

Comparison of the experimental data points and the model production histories, expressed in dimensionless time, as described in Section 3, is presented in Figure 3. The adjusted dimensionless parameters Γ , d_x , d_t , λ are shown in Table 10, while recalculated dimensional values in Table 11. The model curves for the elliptic and parabolic fitting are similar, as well as the fitting parameters. The elliptic dispersion coefficients are of the order of few seconds, while the experiments last for hours. Hence, contribution of elliptic diffusion in this case (and on this scale) is insignificant. We bring only the data for elliptic fitting.

Table 10: The adjusted dimensionless fitted parameters for elliptic fitting.

	d_x	d_t	$1/(1+\Gamma)$	λ
Experiment C	0.0152	0.0030	0.5488	0.4644
Experiment D	0.0185	0.0043	0.3930	0.3681
Experiment E	0.0076	0.0015	0.8477	0.1667
Experiment F	0.0201	0.0022	0.7642	0.1058
Experiment G	0.0572	0.0023	0.9613	0.1212
Experiment H	0.0159	0.0032	0.5062	0.4905
Experiment I	0.1078	0.0023	0.8337	0.1737

Table 11: Recalculated dimensional fitted parameters for elliptic fitting.

	Γ	Λ, m^{-1}	$D_x, 10^{-9}m^2/s$	D_t, s
Experiment C	0.835	11.25	32.8	5.57
Experiment D	1.563	12.40	56.4	11.18
Experiment E	0.182	2.587	4.465	0.660
Experiment F	0.312	1.795	13.07	1.039
Experiment G	0.044	1.639	15.08	1.975
Experiment H	0.989	10.813	4.216	11.743
Experiment I	0.201	2.703	32.71	2.273

Analysis of Figure 3 indicates that fitting of the experimental data on enzyme production by the suggested model is generally adequate, accounting for experimental imprecision. The data on the experiments with chalk cores are approximated less precisely than the data for sandstones. These data exhibit highly asymmetric production plots, unlike the standard diffusion models. Surprisingly, the anomalous diffusion expressed by the temporal dispersion term could not improve the model approximation. A step towards its further improvement could be implementation of the full-scale continuous time random walk models (Shapiro and Yuan, 2011). Alternatively, a nonlinear equilibrium or a non-equilibrium adsorption model could be checked. Since the goal of this work was to estimate the significance of the basic effects, but not to provide a full-scale model, we have restricted ourselves with the current model.

The values of Γ are not high, but still substantial, so that the delay caused by the equilibrium sorption cannot be neglected. These values are higher for enzyme/protein adsorption on chalk than on sandstone cores, in agreement with the fact that the internal surface is higher for the chalk cores. The only exception is the long sandstone core. The equilibrium sorption is generally lower for BSA.

A similar conclusion is valid for the irreversible sorption characterized by the values of Λ . The irreversible loss of the enzyme in chalk is much higher than in sandstone and as well higher than loss of BSA in chalk. The long sandstone core is the only exception. The obtained values of Λ are small to moderate on the laboratory scales, but they are rather large for the reservoir scales. The value of $e^{-\Lambda a}$ shows what fraction of enzyme is lost when it travels distance a . For our laboratory scales ($a \approx 8$ cm) this value varies from 1.15 to 2.7. However, already for the distance of 1m the value of $e^{-\Lambda a}$ varies from 5 to 75000. This indicates that all the enzyme will be retained on the first meters if injected into a petroleum reservoir. This makes it problematic to directly use the enzymes for enhanced oil recovery without additional technological adjustments, at least, with the rocks similar to those we have tested.

The values of spatial dispersion coefficient D_x are similar for parabolic and elliptic models. They are higher than the characteristic values of molecular diffusion, which indicates the effect of mesoscale heterogeneity and convection. The values of temporal dispersion D_t are in this case insignificant, which indicates also stability of the results. As shown by Sin and Yuan (2011), the value of D_t affects mainly the deposition profiles, but not production histories, which is in agreement with the present observations.

5. Discussion

Three types of experiments were carried out in order to investigate adsorption of enzymes/proteins to the rock surface. As it can be seen from Table 12, the static adhesion tests are in good agreement with the dynamic flow-through experiments, while the results obtained for the adsorption-desorption experiments with particles are different. The most plausible cause for this observation is a different shape and state of the substrates used. The same protein can adsorb differently on a flat and fixed surface, compared to surfaces of moving particles (Kim and Yoon,

2002). The static adhesion tests produced closer results to the dynamic flow-through experiments, even though a powder possesses a larger surface area. It should be highlighted that the adhesion tests give only qualitative data on the protein adsorption; therefore flow-through experiments cannot be substituted by any static test.

Table 12: Summary of static and dynamic adsorption experiments: + - complete desorption of enzyme/protein; +/- - partial desorption of enzyme/protein; - - no desorption of enzyme/protein, N/A – not available.

	Static experiments				Dynamic experiments	
	Adhesion tests		Adsorption on particles		Flow-through experiments	
	Quartz	Calcite	SiO ₂	CaCO ₃	Sandstone	Chalk
BSA	+	-	N/A	N/A	+/-	-
NS81249	+	+	-	-	+/-	+/-
NS44164	+	+	N/A	N/A	+/-	+/-

The flow-through experiments confirmed that adsorption and retention of the proteins may be a serious obstacle on the way of application of the enzymatic enhanced oil recovery. The production histories and the total amounts produced demonstrate that both reversible adsorption and irreversible retention take place during enzyme/protein slug injection and the subsequent SW displacement. Burghardt and Axelrod (1981) reported that for BSA three modes of desorption occurred: irreversible, slowly reversible and rapidly reversible. This observation is in line with the multilayer theory of the protein adsorption. Irreversible attachment of a protein occurs when it directly interacts with the surface. The strength of the protein attachment in upper layers of the adsorbate decreases with increase of the distance between the layer and the surface. The very upper layer can be rapidly desorbed, while for the intermediate layers desorption will take a longer time.

Irreversible adsorption is not the only reason for loss of enzymes/proteins in porous media. Another reason is that at low bulk concentrations enzymes/proteins can undergo conformational changes that may lead to strong binding between biomolecules and substrate and, consequently, may lower ratio of the reversible and irreversible adsorption (Evers *et al.*, 2008; Kirchman *et al.*, 1989). When an enzyme/protein is injected into a porous medium, a concentration gradient is formed along the core. This happens due to adsorption and dispersion of the protein (see the results above). Consider the stage where the already injected protein is displaced by brine. Its concentration just ahead of the displacement front is initially high. Hence, the adsorbed protein/enzyme exists as a multilayer film. As the displacing brine propagates, the upper layers get reversibly desorbed. With time, the water front becomes washed out due to dispersion, and the adsorbed film becomes thinner. The adsorption character changes towards more slowly reversible, and then to irreversible (according to the terminology of Burghardt and Axelrod (1981)). This may explain appearance of the long asymmetric tail on the production curve, which is not fully captured by the model described in Section 3. A more advanced model of nonlinear sorption is necessary in order to capture this phenomenon.

As it was found previously (Khusainova *et al.*, 2015), alteration of wettability of the rock surface is the main mechanism for enzyme as a successful EOR agent. The present study partly supports this conclusion. The enzymes/proteins demonstrate a high affinity to the solid rock surface. Only limited amount of enzyme should be injected in order to meet economic feasibility of the process. To minimize enzyme loss in the porous media, enzyme molecules should be able to adsorb and desorb continuously. During adhesion tests both NS81249 and NS44164 samples demonstrated secondary adsorption, which was considered as beneficial for the EOR purposes. However, additional technological advances are still needed in order to govern and to accelerate desorption of enzymes.

Apparently, the nature of a rock has also a certain effect on the enzyme adsorption and propagation rate. Comparison of the adsorption values (Table 9) demonstrates that significantly less adsorption occurs for sandstone compared to chalk, which means that adsorption capacity of carbonates is much higher. The maximum amount of enzyme adsorbed on chalk was 0.769 mg per gram of rock, which is 4.6 times higher compared to sandstone. The most plausible reason for that is the strength of the enzyme/protein-surface interactions. Apparently, interaction between enzymes/proteins and the chalk surface is much stronger than interaction with the sandstone surface, which, in turn, increases the ratio of the reversible and irreversible adsorption for chalk. Experiments with BSA support this hypothesis. BSA belongs to the group of soft proteins that are characterized with low internal stability. When BSA interacted with chalk it probably underwent conformational changes. Attachment of the biomolecules occurred via several sites, which enhanced irreversible adsorption. The conformational changes were so strong that BSA could not be desorbed at all. In contrast, when BSA was filtered through sandstone, interaction between protein and surface was not that strong and the BSA molecules could keep their structure. Hence, irreversibility was not that significant and majority of the protein molecules could desorb (Table 9). The value of the surface area had also some effect, but it was incomparable with the difference between the minerals. The enzyme adsorption on sandstone with about 20% higher internal surface area was 3.6-4.6 times less than on the Stevns Klint chalk (Table 9).

The enzyme adsorption values per gram of the sandstone are similar to those values for polymers and surfactants (Cohen and Christ, 1986; Wang *et al.*, 2015), but application of enzymes can be more advantageous. The enzymes are environmentally friendly substances due to their biological nature. The enzyme concentrations required to detach oil from the mineral surface are much lower, compared to the surfactant concentrations used for chemical flooding (Khusainova *et al.*, 2015). However, in present study it was found that, similar to surfactants, loss of enzymes during travelling through the reservoir rock is a large concern that should be taken into account.

Several parameters that can inhibit adsorption and enhance desorption were studied. First, the high-ionic seawater, which was used as displacing fluid and as a solvent for enzyme/protein solutions, was substituted by the low-ionic distilled water. Second, pH of the distilled water was adjusted, so that pH of the solution was much higher than isoelectric points of the proteins. Third, the solution of enzyme stabiliser was injected to test whether it can desorb enzymes. The different flow rates were also tried. However, the enzyme/protein recovery values were still much lower for

chalk than for sandstone and, generally, much lower than required to avoid enzyme retention under reservoir conditions. More work is required in this direction in order to make the EOR ready for practical applications.

6. Conclusions

Static and dynamic adsorption tests were conducted in order to test potential of enzymes as an EOR agents. The extent of adsorption was found to be dependent on the type of the solid substrate. For EOR applications, the enzyme adsorption should be investigated only using dynamic flow-through experiments. Similar to surfactants, irreversible adsorption plays an important role in ability of protein molecules to travel through the rock porous media. During displacement experiments the ratio of reversible to irreversible adsorption of enzymes/proteins decreased with time, as the type of adsorption was strongly affected by the bulk protein concentration. This makes application of enzymes for EOR quite challenging, especially for the rocks with high internal surface and affinity to the proteins. The chalk rock demonstrated much higher retention rate than sandstone. Change of ionic strength and pH of the enzyme solution and the displacing fluid, application of various injection rates as well as injection of the enzyme stabiliser to desorb biomolecules did not decrease the amount of irreversibly adsorbed enzymes/proteins. An alternative solution should be found in order to reduce the protein loss in the rock and to make application of EOR feasible at the field scale. The developed dynamic model demonstrated a reasonably good fit to the experimental flow-through data. Accounting for only traditional diffusion-like dispersion turned out to be sufficient to model the concentration wash-out, while contribution of the anomalous diffusion was found to be unimportant.

Acknowledgements

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Appendix A. Solution of the elliptic problem

Consider elliptic equation (MainD) with the boundary condition (Boun). Substitution

$$c = \exp(aX + bT)C; a = \frac{1}{2(1+\Gamma)d_x}; b = \frac{1}{d_t}$$

reduces the equation to the form of

$$d_x \frac{\partial^2 C}{\partial X^2} + d_t \frac{\partial^2 C}{\partial X^2} = \lambda_1 C; \lambda_1 = \lambda + \frac{1}{4d_t} + \frac{1}{4(1+\Gamma)^2 d_x}$$

Further, we rescale the variables

$$\tau = T\sqrt{\lambda_1/d_t}; \xi = T\sqrt{\lambda_1/d_x}$$

in order to obtain the standard equation

$$C_{\xi\xi} + C_{\tau\tau} = C$$

This equation is solved in the quarter-plane $\xi > 0, \tau > 0$. If it would be the whole plane, the fundamental solution (the Green function) of the equation would be expressed in terms of the modified zero-order Bessel function K_0 (Shapiro, 2007)

$$C_f(\xi, \tau; \xi_1, \tau_1) = \frac{1}{2\pi} K_0(r); r = \sqrt{(\xi - \xi_1)^2 + (\tau - \tau_1)^2}$$

The Green function for the quarter-plane may now be obtained by the method of reflections.

Assume all the values ξ, τ, ξ_1, τ_1 are positive, and consider the reflected radii

$$r_1 = r = \sqrt{(\xi - \xi_1)^2 + (\tau - \tau_1)^2}; r_2 = \sqrt{(\xi + \xi_1)^2 + (\tau - \tau_1)^2};$$

$$r_3 = \sqrt{(\xi - \xi_1)^2 + (\tau + \tau_1)^2}; r_4 = \sqrt{(\xi + \xi_1)^2 + (\tau + \tau_1)^2}$$

The Green function C_d consists of the solution C_f and its three reflections:

$$C_d(\xi, \tau; \xi_1, \tau_1) = \frac{1}{2\pi} (K_0(r_1) - K_0(r_2) - K_0(r_3) + K_0(r_4))$$

The Green formula for this region reads:

$$C(\xi, \tau) = \int_0^\infty C^0(\tau_1) \frac{\partial C_d(\xi, \tau, \xi_1, \tau_1)}{\partial \xi_1} \Big|_{\xi_1=0} d\tau_1 - \int_0^\infty C_0(\xi_1) \frac{\partial C_d(\xi, \tau, \xi_1, \tau_1)}{\partial \tau_1} \Big|_{\tau_1=0} d\xi_1$$

The second integral is equal to zero due to the boundary conditions. The first integral is simplified due to that, first, derivative of K_0 is K_1 ; and second, at the boundary $\xi_1 = 0$ we have $r_1 = r_2$ and $r_3 = r_4$. The solution in the transformed coordinates is:

$$C(\xi, \tau) = 2\xi \int_0^\infty C^0(\tau_1) \left(\frac{1}{r_1} K_1(r_1) - \frac{1}{r_3} K_1(r_3) \right) d\tau_1$$

Performing the back-transformations, we obtain finally:

$$c(X, T) = \frac{c^0}{\pi} \exp\left(\frac{X}{2d_x(1+\Gamma)} + \frac{T}{2d_t}\right) \frac{\lambda_1 X}{\sqrt{d_x d_t}} \int_0^{\min(T,1)} \exp\left(-\frac{T_1}{2d_t}\right) \left(\frac{1}{r_1} K_1(r_1) - \frac{1}{r_3} K_1(r_3)\right) dt_1$$

Variables r_1, r_3, λ_1 are found from the previous equations by back-substitution.

Appendix B. Solution of the pure dispersion problem

Consider equation (2) with the zero temporal dispersion term:

$$\frac{\partial c}{\partial T} + \frac{1}{1+\Gamma} \frac{\partial c}{\partial x} = d_x \frac{\partial^2 c}{\partial X^2} - \lambda c \quad (5)$$

The boundary conditions (4) remain valid for this equation. Its solution could probably be obtained from the solution of the corresponding elliptic problem (as described in the previous appendix) by tending d_t to zero. However, such a limiting transition is difficult. It is easier to solve directly Eq. (5) by the method similar to that applied in Appendix A.

Substitution

$$c = e^{aT+bx} C, \quad a = -\frac{1}{4d_x(1+\Gamma)^2} - \lambda, \quad b = \frac{1}{2d_x(1+\Gamma)}$$

reduces the original equation (5) to a standard form

$$\frac{\partial c}{\partial T} = d_x \frac{\partial^2 c}{\partial X^2}$$

The boundary conditions are transferred correspondingly. Solution with the corresponding Green function results in (Tikhonov and Samarsky, 1963)

$$C(X, T) = c^0 \frac{X}{2\sqrt{\pi d_x}} \int_0^{\min(T,1)} e^{(1/4d_x(1+\Gamma)^2 + \lambda)\tau} \frac{1}{(T-\tau)^{3/2}} \exp\left(-\frac{X^2}{4d_x(T-\tau)}\right) d\tau$$

The final answer is obtained by back-substitution of C :

$$c(X, T) = c^0 e^{X/2d_x(1+\Gamma)} \frac{X}{2\sqrt{\pi d_x}} \int_{T-\min(T,1)}^T e^{-(1/4d_x(1+\Gamma)^2 + \lambda)\tau} \frac{1}{\tau^{3/2}} \exp\left(-\frac{X^2}{4d_x\tau}\right) d\tau$$

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Chapter 5. Emulsion and Crude Oil-Brine Interaction Study

Formation of emulsions is one of the main mechanisms that might result in enhancement of oil production. In order to check whether this mechanism might be relevant for enzymatic enhanced oil recovery (EOR), formation and stability of enzyme/protein-stabilised emulsions were studied. This part of the work was conducted in collaboration with master student, Jakub Benicek, and in Section 1 of the chapter only the key findings are presented. For further details the reader is referred to the student's master thesis: Benicek, J., 2015. Application of emulsion-forming enzymes for enhanced oil recovery.

The second part of the chapter presents results from the crude-oil brine interaction experiments. It is an independent part that was conducted simultaneously with coreflooding experiments (Chapter 6). The experiments were inspired by the formation of black filter-cake observed during the oil displacement tests in chalk (Figure 2, Chapter 6). An initial hypothesis was that the formation is the viscous emulsion resulted from the interaction of crude oil and enzyme-containing brine under conditions similar to the reservoir conditions. Eventually, formation of the black substance was found to be, most likely, due to corrosion-induced asphaltene precipitation, but the crude oil-brine interaction experiments revealed some important findings presented in Section 2 of current chapter.

1. Emulsion Study

An experimental study of enzyme/protein-stabilised emulsions was carried out. Reproducible preparation of emulsions was found to be quite a challenging task. Therefore, a methodology of preparation of protein-stabilised emulsions allowing for handling a large number of samples and providing reproducible results was developed at the initial stage of the investigation. *n*-Decane was used as an oil phase in most of the experiments. Fraction of saturates was verified to be the main part of the crude oil involved in formation of the protein-stabilised emulsions. Long-chain alkanes provided formation of more stable emulsions, while short-chain alkanes were responsible for formation of the emulsions characterised by smaller drops.

Screening of several enzyme groups demonstrated that the group of lipases/esterases possessed the highest surface-active properties at the oil-water interface. The most stable emulsions were formed when lipases/esterases were applied as emulsifying agents. Of all the tested enzymes, proteases produced least emulsions. As it was expected, enzymes that belong to the same group formed similar emulsions. Results obtained in emulsion screening were in a good agreement with the wettability tests.

Emulsions stabilised by a surfactant (sodium dodecyl sulfate) were less stable than those stabilised by proteins. Emulsifying properties of the enzyme products were proved to originate mostly from the enzyme molecules, rather than from the stabilising ingredients. However, propylene glycol (the main stabilising ingredient) demonstrated some insignificant contribution to the surface activity of the enzyme products.

A possible role of enzymes in enhanced oil recovery (EOR) is acting as catalysts (Chapter 2). This possibility was tested by addition of an ester (ethyl decanoate) to the oil phase. The emulsions formed in ester-added systems were less stable compared to the emulsions in the corresponding ester-free systems. It was concluded that destabilising effect of the ester was more pronounced than the stabilising effect of the carboxylic acid and alcohol that were formed due to enzymatic hydrolysis of the ester.

Re-shaking of the samples after three months resulted in formation of the emulsions possessing a higher stability. Reasons for such behaviour might be long diffusion times and also a longer period required for protein conformational changes that occur during emulsification. This finding was quite important in the framework of the coreflooding experiments, since it demonstrated importance of the incubation periods. The stage of incubation was included in several coreflooding tests.

Combined application of enzymes and solid particles was tested at the last stage of the work. More stable and tighter emulsions were formed when calcium carbonate or silica particles were added to the enzyme solutions. Effect of silica particles was more pronounced, but, most likely, might be related to the size of the particles. The size of silica particles varied between 0.5 and 10 μm , while the size of calcium carbonate particles was $\leq 30\ \mu\text{m}$. The silica particles covered the drops providing stabilising effect. A smaller amount of the calcium carbonate particles was kept at the interface. Even though addition of solid particles resulted in the formation of larger volumes of more stable emulsions, the drop size distribution was not affected by addition of the particles.

2. Crude Oil-Brine Interaction Study

2.1. Materials and Methods

2.1.1. Materials

Light crude oil recovered from the chalk reservoir was used as an oil phase. The brine was synthetic seawater (SW); composition can be found in Chapters 3, 4 and 6.

Only enzyme NS81249 was applied in crude oil-brine interaction experiments. Novozymes A/S kindly provided the sample in the form of commercial product and as a purified enzyme. Concentration of the product solution used in the experiments was 1%_{wt}, while a higher concentration of 5%_{wt} was chosen in case of the purified sample in order to bring the experiment to the extreme condition. Solution of the enzyme stabiliser (without the enzyme) was imitated using propylene glycol (Sigma-Aldrich, purity $\geq 99.5\%$), as it was the main component of the stabilising ingredients. Typical Novozymes enzyme product consists of 2-5%_{wt} of enzyme, 25-30%_{wt} of the stabilisers is, 0.2%_{wt} of the biocide and the rest is water. So 1% solution of the enzyme product contains 0.3%_{wt} of propylene glycol. This was one concentration applied; another concentration of 10%_{wt} was chosen to bring the experiment to the extreme condition.

Nano-silica with the size distribution of 10-20nm and calcium carbonate particles ($\leq 30\ \mu\text{m}$) were purchased from Sigma-Aldrich and were not subjected to any further treatments. The

concentration of the nano-silica was 300ppm as in the coreflooding experiments (Chapter 6). The mass of the calcium carbonate was 1g. Effect of calcium carbonate was studied as formation of the filter-cake was only observed in chalk cores, so presence of calcium and/or carbonate ions might be a condition for formation of such filter-cake.

2.1.2. Methods: Crude oil-brine interaction experiments

12ml of crude oil was mixed with 12ml of SW containing enzyme product, purified enzyme or propylene glycol. Nano-silica and calcium carbonate particles were added to the enzyme/propylene glycol solutions to test effect of the presence of solid particles. Table 1 contain various investigated compositions of the water phase. The samples were placed in a shaker and mixed during 24 hours at 60°C (the temperature was chosen to be the same as in the coreflooding experiments). The obtained mixture was filtered through PTFE 0.45µm filters with the vacuum filtration technique. In order to keep constant temperature throughout the experiments, the filtration was accomplished in the oven. For the crude oil - 5%NS81249 (purified) in SW system the filter cake was analysed under the optical microscope. All the experiments were checked for reproducibility.

2.2. Results

Table 1: Summary of the results of crude oil-brine interaction experiments.

Set of experiments	System	Formation on the filter
1	Crude Oil - 1% NS81249 in SW	Gel-like structure in small amounts
	Crude Oil - 1% NS81249 in SW - SiO ₂ nanoparticles	Gel-like structure in large amounts
	Crude Oil - SiO ₂ nanoparticles	None
	Crude Oil - 1% NS81249 in SW - SiO ₂ nanoparticles - CaCO ₃ particles	Gel-like structure in large amounts
2	SW - NS81249	None
	Crude Oil - NS81249	None
3	Crude Oil - 5%NS81249 (purified) in SW	Viscous emulsion
	Crude Oil - 0.3% propylene glycol in SW	Gel-like structure in small amounts
	Crude Oil - 10% propylene glycol in SW	Gel-like structure in large amounts
	Crude Oil - 0.3% propylene glycol in SW - SiO ₂ nanoparticles	Gel-like structure in large amounts

The summary of the results of crude oil-brine interaction experiments is presented in Table 1. The first set of the crude oil-brine interaction experiments consisted of four different systems

(Table 1). Only for the crude oil - SiO₂ nanoparticles system the filter was left clean. During the rest of experiments a white semi-transparent gel-like filter cake was formed (Figure 1). In cases where there were particles (silica or calcium carbonate) the precipitation was larger.



Figure 1: The gel-like formation obtained during crude oil-brine interaction experiments.

As an explanation, it was initially suggested that protein denaturation occurred, since the obtained structures looked very much like an egg white after boiling. However, this was rejected after the second set of the experiments (Table 1). In order to achieve extreme conditions, the enzyme product was mixed with the crude oil without diluting in SW. The crude oil-brine interaction tests for NS81249 - SW and NS81249 -crude oil systems did not show any filter cake formation. Thus, formation of the gel-like precipitation cannot be solely attributed to the enzyme-SW or enzyme-crude oil interactions.

As discussed in Chapter 3, the enzyme products contain 20-30% of the stabilisers, which consist mainly of propylene glycol. Therefore, formation of the gel-like structure could also be attributed to interaction between propylene glycol, SW and the crude oil. It should be noted that formation of such filter cake requires presence of the SW, as nothing similar was observed when the enzyme product was directly mixed with the crude oil without dilution in SW. To test viability of the hypothesis, the third set of experiments was carried out (Table 1). In these experiments the purified enzyme with almost no stabilisers and propylene glycol was mixed with oil. These experiments confirmed that the gel-like filter cake was indeed formed of propylene glycol and not of the enzyme. The amount of the gel increased with the increase of the propylene glycol concentration and also when the nanoparticles were present.

An unexpected filter cake was obtained for the crude oil with 5% NS81249 (purified) in SW system (Figure 2a). The filter cake was found to be a viscous oil-in-water emulsion (Figure 2b). Microscopic images of the emulsion revealed two remarkable features. First, the oil drops had asymmetric shapes. Second, some oil-lined structures (the structure was very similar to the fly wings) were formed in SW.

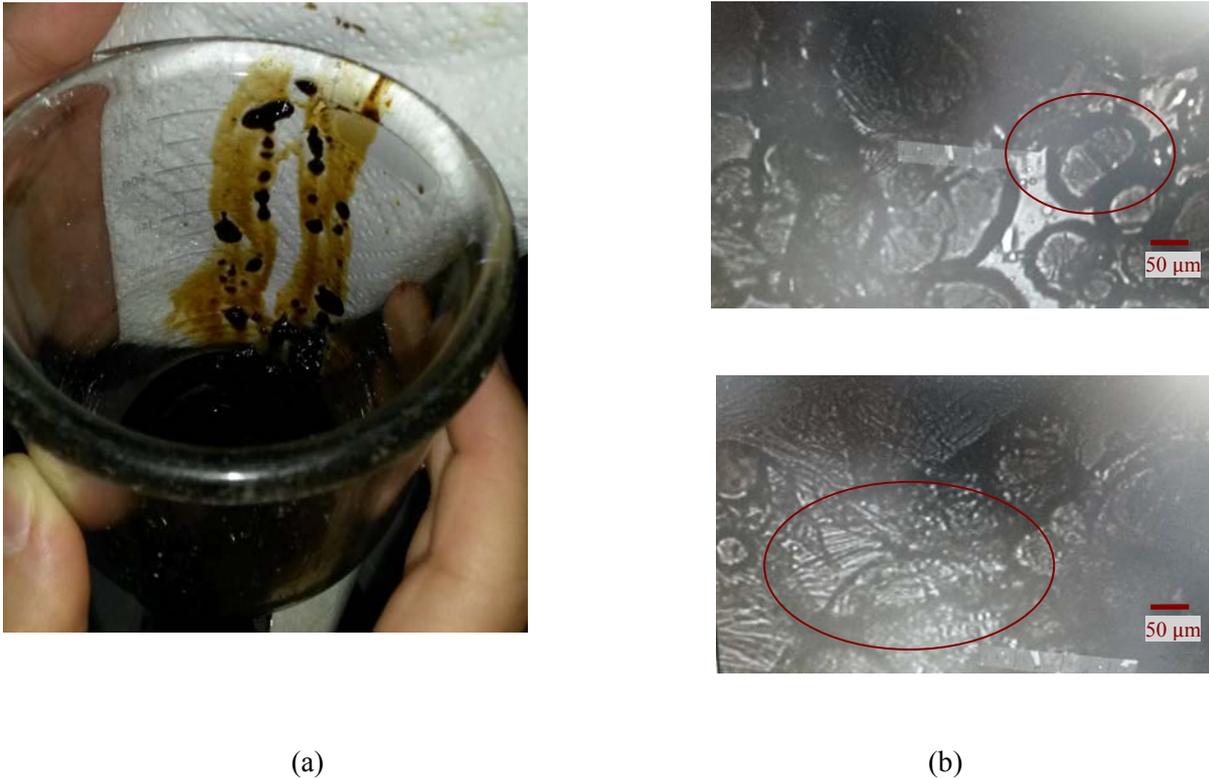


Figure 2: Viscous emulsion obtained after interaction and filtration of crude oil - 5%NS81249 (purified) in SW system. (a) - the emulsion left on the filter; (b) - microscopic images of the emulsion. Two circles on Figures (b) show asymmetric drop and some lined structures.

2.3. Discussion

The crude oil-brine interaction experiments revealed two more important findings that make it difficult to consider enzymes as EOR agents. First, interaction of the enzyme product with crude oil in the presence of SW can cause precipitation of the propylene glycol, which is the main constituent of the enzyme product stabiliser. Separation of the stabiliser from the solution may cause loss of the internal stability and even denaturation of the enzyme molecules. Stabilisers should hinder adsorption of an enzyme onto the rock surface (Evers *et al.*, 2011; Koo *et al.*, 2008; Timasheff, 1993). In the absence of the propylene glycol in the solution enzyme molecules would tend to adsorb even more, while irreversible adsorption onto the solid substrate was found to be a serious obstacle for the enzyme molecules to travel through porous medium. Therefore, enzyme products that are going to be used for petroleum industry applications should be thoroughly checked for stability after interaction with the crude oil, to ensure process-life of the product.

The structure of the gel-like filter cake was not studied in detail. It was only established that the filter cake consisted mostly of propylene glycol. The structure of the filter cake looked like gel, but it could also be the emulsion of the propylene glycol in SW, of a rather high viscosity. Such emulsion was formed only where the crude oil was present in the system, so apparently some crude oil component(s) served as emulsifier(s). The amount of the filter cake increased with increase of

the propylene glycol concentration and when nano-silica particles were present. The latter was most likely due to ability of solid particles to stabilize and enhance formation of the emulsions (Hassander *et al.*, 1989; Hunter *et al.*, 2008).

If the propylene glycol behaves in a similar way in the core during oil displacement experiments, formation of the gel/viscous emulsion can also contribute to the additional oil recovery. The formation could plug some water-bearing zones and redirect water to the trapped oil. Since the used cores were rather homogeneous, the effect of the plugging was not significant, which is in a good agreement with the pressure data and, consequently, with the observed low additional recovery values (Chapter 6).

The second important finding of the crude oil-brine interaction experiments was that a very viscous oil-in-water emulsion was formed when the purified enzyme interacted with crude oil. The shapes of the oil drops were random, while some net structures were also present (Figure 2b). Both characteristics of the formed emulsion, viscosity and oil drop shape, hinder the process of oil extraction. Thus, creation of such emulsions can be a disadvantage. On the other hand, emulsification could result in one-phase-like flow facilitating the recovery.

2.4. Conclusions

The crude oil-brine interaction tests revealed that interaction between crude oil and brine containing enzyme product causes precipitation of the propylene glycol, forming gel or a very viscous emulsion. This in turn might destabilise the enzyme molecules, as propylene glycol is the main constituent of the enzyme product stabilisers. Moreover, when the purified enzyme was used in the crude oil-brine interaction tests, formation of very viscous oil-in-water emulsion was observed. The oil drops had unusual asymmetric shapes; also some net structures were detected. The findings discover new limitations on the way of application of enzymes as EOR agents.

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Chapter 6. Experimental Investigation of Enzymatic Enhanced Oil

Recovery

The chapter has been prepared for a publication in Journal of Petroleum Science and Engineering:

Alsu Khusainova, Alexander Shapiro. Experimental Investigation of Enzymatic Enhanced Oil Recovery.

Abstract

Core flooding experiments were carried out under conditions similar to the conditions of the petroleum reservoirs, in order to check potential of the enzymes for enhanced oil recovery (EOR) application. Sandstone and chalk cores were used as reservoir material, light crude oils as an oil phase. Two types of the enzymes, lipase and amylase, were selected for testing, based on the static wettability and emulsion formation experiments. The enzymes were applied only during the tertiary recovery stage; various injection scenarios were tested. No significant enhancement of the oil production was observed due to the injection of the enzymes: the recovery factor for the stage of tertiary oil production was only 0.23-1.69% relative to original oil in place in sandstone, and no additional oil was recovered from chalk. Low recovery factors were combined with a delayed oil production. Change of wettability was proposed as the primary mechanism that governs enzymatic EOR, while formation of the emulsions plays most likely less important role. Overall, the enzymes performance demonstrated their low potential as EOR agents for sandstone and carbonate reservoirs containing light crude oil.

Key words: enhanced oil recovery; enzymes; tertiary recovery; core flooding; chalk; sandstone.

1. Introduction

Enzymes have been quite extensively used in the petroleum industry in the following areas: enzyme pre-treatment of biopolymers, gel breaking, desulphurization and enzyme-based production of acid (Harris and McKay, 1998). However, only recently they have been introduced as enhanced oil recovery (EOR) agents. Both laboratory and field studies demonstrated large improvement of the oil recovery after enzymes application (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Nasiri *et al.*, 2009; Ott *et al.*, 2011). The recovery factor was increased by up to 16% in the laboratory displacement studies and up to 269 barrels of oil per day more were produced in the field applications (Feng *et al.*, 2007; Moon, 2008; Nasiri *et al.*, 2009; He and Zhonghong, 2011).

The mechanisms responsible for the enhancement of the ultimate oil recovery have been reported to be: improvement of the rock surface wettability towards more water-wet state (Nasiri *et al.*, 2009); emulsification due to decrease of the interfacial tension (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Nasiri *et al.*, 2009); oil viscosity reduction (He and Zhonghong, 2011; Moon, 2008); removal of high molecular weight paraffins (Moon, 2008). However, these mechanisms require some verification with regards to the ingredient that causes improvement of the oil recovery process parameters. In almost all the studies, commercial mixtures of the enzymes were used; to the best knowledge, only in the works of Nasiri (2009, 2011) pure lipase was applied.

Such mixtures, apart from the enzymes, contain surfactants and stabilisers (see for example, Apollo GreenZyme™ Material Safety Data Sheet; Feng *et al.*, 2007). These ingredients can also attribute to the recovery.

This paper continues investigation of the applicability of enzymes for EOR. Previously various enzyme groups were screened to determine the group with the highest potential for EOR purposes (Khusainova *et al.*, 2015). Adhesion behaviour tests as well as contact angle measurements were employed. The group of lipases/esterases showed ability to detach the crude oil from the mineral surface and was selected for a more detailed investigation. Adsorption of the enzyme molecules onto the mineral surface and the related change of wettability were proposed as the main mechanism for the recovery improvement. It was also confirmed that enzyme molecules themselves, not stabilising ingredients, caused the change of the wetting state towards more water-wet conditions. Such mechanism could potentially have a significant disadvantage: retention of the protein molecules in porous medium. This was checked during the adsorption study (Chapter 4) that included both static and dynamic experiments. It was established that the adsorption capacity of chalk was much higher compared to sandstone. Simultaneously, emulsification studies were carried out, in which ability of the enzymes to form emulsions with crude oil was investigated (Chapter 5). The group of lipases showed positive results. Overall, conducted experiments revealed the principal mechanisms that might underlie enzymatic enhanced oil recovery (EEOR) and pointed out to the potential problems that might appear during field applications.

In this study the core flooding experiments were carried out in order to see how the results from the static experiments correlate with the dynamic tests of the enzymes application and to verify applicability of enzymes for enhanced oil recovery. The experimental procedure was developed in a way to approach more realistic conditions, to meet economical feasibility and to analyse the problems that might rise during the tertiary enzyme injection.

The paper is organized in the following way. In Section 2, materials and methods applied in the study are described. In this section we explain also the choice of the experimental plan, according to hypotheses to be tested. In Section 3 key experimental results are presented, while their analysis and general evaluation of the enzymes as EOR agents are given in Section 4. Finally, the main results are summarized in Section 5.

2. Materials and Methods

2.1. Materials

2.1.1. Fluids

For the core flooding experiments two types of light dead oil were used. One crude oil was recovered from a sandstone reservoir and was used for the experiments with sandstone, while another oil was from a chalk reservoir and was used in the experiments with chalk.

Synthetic North Sea water (pH=7.78; composition as given in Table 1) was applied as formation brine and displacing fluid in core flooding experiments. Chemicals for the preparation of

the SW solution were purchased from Fluka (purity $\geq 99.5\%$) and were not subjected to any further purification. To prepare the brine the salts were diluted in distilled water and the solution was mixed until all the salts were dissolved.

Table 1: Composition of the synthetic North Sea water used in the core flooding experiments.

Salt	Concentration, g/l
NaCl	18.01
NaHCO ₃	0.17
KCl	0.74
MgCl ₂ .6H ₂ O	9.15
CaCl ₂ .2H ₂ O	1.91
Na ₂ SO ₄	3.41
Total dissolved solids	33.39

2.1.2. Enzyme/protein samples

Two types of enzymes that were kindly supplied by Novozymes A/S were evaluated in this work (Table 2). Both enzymes were provided in the form of commercial products, and NS81249 was additionally provided as a purified sample. Apart from the pure enzyme, standard Novozymes A/S enzyme products contain stabilisers, biocide and water. Actual concentration of the enzyme in such products is only 2-5%_{wt}, concentration of the stabilisers is 25-30 %_{wt}, concentration of the biocide is 0.2%_{wt} and the rest is water. In the purified sample concentration of the pure enzyme was 4-4.5 times higher compared to the commercial product, and this sample was almost free of the stabilising ingredients (Novozymes A/S private communication). In order to exclude misunderstandings, we would like to stress that the term “enzyme concentration” used below implies concentration of the enzyme products (either commercial or purified), but not the concentration of a pure enzyme.

Table 2: Types and working parameters of the enzymes used in this study.

Enzyme name	Enzyme type	Enzymatic action	Working temperature range	Working pH range
NS81249	Lipase (esterase)	Hydrolysis of ester bond in lipid	30-85 °C	5-10
NS81251	Amylase (carbohydrase)	Hydrolysis of starch	30-100°C	5-9

The enzyme solutions were prepared by direct dilution of the enzyme product in SW. The commercial enzyme products were applied as 1%_{wt} solutions in the core flooding tests. For the purified product, concentration was 1%_{wt}.

The Bradford reagent (product number B6916, Sigma-Aldrich) was used for determination of the enzyme concentration in the effluent obtained during the core flooding experiments.

2.1.3. Cores

In order to check potential of EEOR for various types of petroleum reservoirs, homogeneous sandstone and chalk cores were used. The sandstone samples were outcrop cores drilled from an Obernkirchener sandstone block (Germany), while the chalk samples were obtained from a Danish North Sea reservoir. Characteristics of the core samples used in the work are presented in Table 3.

Table 3: Characteristics of the core samples used in the core flooding experiments (N/A - not available).

Sample name	Diameter, mm	Length, mm	Weight of the dry core, g	Klinkenberg permeability, mD	True pore volume	Actual pore volume, ml
S-2	37.31	77.02	184.46	2.309	14.96	14.52
S-1	37.28	78.00	187.60	2.479	14.64	14.31
S-5	37.34	76.63	185.34	4.088	14.18	13.70
S-4	37.30	77.00	183.55	6.897	15.01	14.71
S-3	37.94	89.90	218.95	N/A*	N/A*	18.82
C-1	37.60	62.90	127.18	0.523	23.84	23.65

* - The sample was too long to be assembled into the porosimeter and the permeameter.

2.1.4. Nano-particles

In the Experiment D application of the enzymes was combined with the application of the nanopowder. The silica powder with the particle size distribution of 10-20nm was purchased from Sigma-Aldrich and was not subjected to any further treatments. The concentration of the nano-silica was 300ppm. This concentration was found to be optimal for the enhancement of oil production in Berea sandstone (Skauge *et al.*, 2010). The nanopowder was mixed with the 1% NS81249 enzyme solution. The obtained solution was thoroughly shaken and then placed into the ultrasonic bath for 10min.

2.2. Methods

2.2.1. Core flooding experiments

Figure 1 shows the scheme of the set up that was used to carry out the oil displacement experiments. The Hassler-type coreholder was used for accommodation of the rock core samples. Confining pressure was created by Teledyne ISCO pump and was chosen to be 50bar throughout the experiments. Saturation and displacing fluids were placed in stainless steel cylinders with pistons. Injection was also accomplished using one syringe Teledyne ISCO pump. An X-Y fraction collector consisting of the automatically changing tubes was placed at the outlet of the coreholder to gather continuously the effluent. The effluent volume in each tube was 1/6-1/5 of pore volume (PV). Once the oil volume per tube was less than 0.2-0.3ml, the fraction size was increased up to 1/2 PV to increase accuracy of the oil volume measurements.

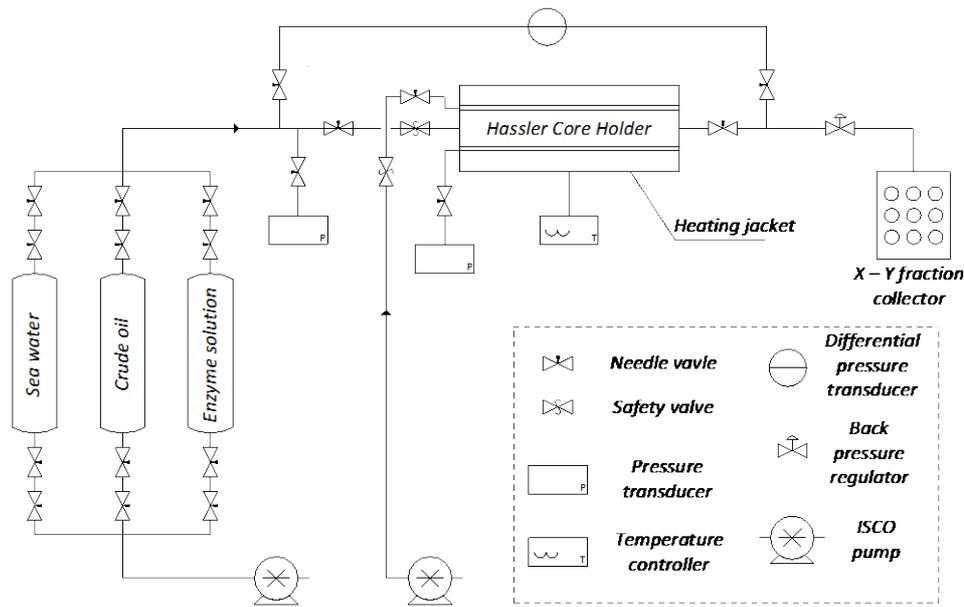


Figure 1: The scheme of the core flooding set up.

Detailed description of the experimental procedure is given in Appendix 1. Briefly, each core went through the stage of preparation, which consisted of CT-scanning to check an integrity and a homogeneity, cleaning and measuring porosity and permeability. The saturation with SW was carried out in three steps: under the vacuum (at ambient temperature), under the pressure of 100bar (at ambient temperature) and by injecting SW after the core was assembled into the coreholder (at 60°C, which was the experimental temperature). Original water and oil saturations were established by injecting the crude oil in the SW-saturated core. The injection speed was 0.1ml/min for all the fluids throughout the work. If aging had to be performed during the experiment, the flow rate was reduced to 0.002ml/min after the irreducible water saturation was reached, and the crude oil was injected at the low flow rate for three weeks.

Oil and water saturated cores were first waterflooded with synthetic SW until the residual oil saturation was achieved. Then 1 PV of the enzyme solution slug was injected (enzymes were always injected as tertiary agents). In Experiments B and C for the stage of incubation with addition of the enzyme the pulse size was 2 PV. Different injection schemes were applied during the tertiary recovery depending on the hypotheses tested (Table 4).

The oil content in the effluent was determined visually by reading volume marks on the graduated tubes (if the oil volume was more than 0.5ml) and using ultraviolet (UV) spectrophotometry (if the oil volume was less than 0.5ml) (Evdokimov *et al.*, 2003; Katika *et al.*, 2015). During EEOR, in addition to the oil phase, the produced water was also analysed for the protein content using Bradford assay (Bradford, 1976). The cores were discarded after the experiments, since the irreversible adsorption of the enzymes onto the rock surfaces was found to be quite large.

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Table 4: Hypotheses tested and injection schemes applied in the experiments.

	Core	Enzyme	Methods applied	Injection scheme during tertiary recovery
Experiment A	S-2	NS81249	<ol style="list-style-type: none"> 1. Injection of the lipase as an EOR agent; 2. Injection of two enzyme pulses. 	1PV enzyme injection - SW injection (until no more oil was produced) - 1PV enzyme injection - SW injection (until no more oil was produced)
Experiment B	S-1	NS81249	<ol style="list-style-type: none"> 1. Injection of the lipase as an EOR agent; 2. Incubation of the enzyme solution in the core 	1PV enzyme injection - SW injection (until no more oil was produced) - Incubation without injection of additional enzyme (1.5 months) - SW injection (until no more oil was produced) - 2PV enzyme injection - Incubation (1month) - SW injection (until no more oil was produced)
Experiment C	S-5	NS81251	<ol style="list-style-type: none"> 1. Injection of the amylase as an EOR agent; 2. Incubation of the enzyme solution in the core. 	1PV enzyme injection - SW injection (until no more oil was produced) - Incubation without injection of additional enzyme (1.5 months) - SW injection (until no more oil was produced) - 2PV enzyme injection - Incubation (1month) - SW injection (until no more oil was produced)
Experiment D	S-4	NS81249	Injection of the enzyme solution in the aged core.	1PV enzyme injection - SW injection (until no more oil was produced)
Experiment E	S-3	NS81249	<ol style="list-style-type: none"> 1. Injection of nano-silica particles in the enzyme solution. 2. Injection of the nano-particle-enzyme mixture in the aged core. 	1PV enzyme+nano-silica injection - SW injection (until no more oil was produced)
Experiment F	C-1	NS81249	Injection of the purified enzyme.	1PV enzyme injection - SW injection (until no more oil was produced)

2.2.2. Experimental plan: motivation and background

The previous works on EEOR attempted to establish a principal possibility to produce more oil with the enzyme flooding. We have tried to approach more realistic conditions and to identify the problems that might arise during application of EEOR on a field scale. That is why smaller concentration and injection of 1 PV of the enzyme solution flushed by water were used and the enzyme production was checked along the tertiary recovery stage.

The concentration of the enzyme solution was 1%_{w/v} in all core flooding experiments except Experiment F. The concentration was previously found to be sufficient to improve wettability of calcite from weakly water-wet to absolutely water-wet state (Khusainova *et al.*, 2015). Higher concentrations were not examined due to economic unfeasibility. Pulse-type injection of the enzyme was also employed in order to minimize the enzyme consumption.

During the tertiary recovery aqueous phase of the effluent was analysed for the presence of the enzymes. The protein content analysis was carried out to find out how presence of the crude oil affects ability of the enzyme molecules to travel through the porous medium. Some manufacturers (e.g. Apollo Separation Technologies Inc) claimed that enzyme solution is not consumed during EEOR and the solution can be reused after the process of oil production. This had to be verified.

The experimental study started with the application of sandstone as a porous medium and all main hypotheses were checked using sandstone core samples. Irreversible adsorption of the enzymes onto the rock surface was much lower for sandstone than for chalk (Khusainova and Shapiro, *submitted*). Therefore, it was considered to be more reasonable to start dynamic testing of EEOR for sandstone rocks.

The injection scenarios varied from one experiment to another (Table 4). This was done as the recovery values were quite low and we tried to change various parameters to increase effectiveness of the enzyme application and to establish an optimal injection scheme.

In the first part of Experiment A the simplest injection scheme, i.e. injection of the enzyme pulse followed by SW, was implemented. Then the second pulse of the enzyme solution was pumped in order to check whether the amount of the additionally produced oil depends on the volume of the injected enzyme solution.

Experiment B started as a duplicate of Experiment A. However, it was paused for 1.5 months for incubation after the first enzyme injection, while all the experimental conditions such as temperature and pressure were kept. There were two hypotheses behind carrying out the stage of incubation. First, in our previous adsorption study it was shown that the desorption time of a protein from the rock surface can be rather long. It might be assumed that during the injection scheme when enzyme injection was followed by immediate SW displacement, most of the enzyme molecules became adsorbed to the rock surface at the entrance of a core, and there was not enough time for them to desorb and to move further. Second, in the emulsion study it was also found that samples that were re-shaken after more than two months of storage created more emulsions with smaller mean size drops. This could be caused by a long diffusion time required for the protein molecules to

reach the oil-water interface. Therefore incubation of the enzyme solution in the core might be required in order to form an emulsion of the small drop sizes and to mobilise the trapped oil.

The first incubation was relatively successful even without additional injection of the enzyme, so it would be expected that incubation with freshly injected protein might be even more effective. Once there was no more oil production, 2 PV of the NS81249 enzyme solution was injected and the experiment was paused for 1 month at the experimental conditions (the second incubation).

The next tested possibility was application of the amylase NS81251. The group of lipases/esterases, unlike carbohydrases and, particularly, amylases, was claimed to be the most promising for EOR applications based on the wettability studies (Khusainova *et al.*, 2015). This finding was in a good agreement with another investigation (Nasiri, 2011). In contrast, the group of carbohydrases demonstrated very poor ability to change the wetting state of the calcite, which might mean low adsorption rate of the enzyme molecules onto the mineral surface. This in turn might mean a lower enzyme loss in the porous medium and a higher chance for the enzyme molecules to travel through the rock to longer distances. At the same time, emulsification studies revealed that under certain conditions (after incubation) carbohydrases could form very stable emulsions with micro size drop distribution. Therefore, application of carbohydrases, particularly, amylases might have been successful in terms of EOR. The injection scheme for the amylase NS81251 (Experiment C) was similar to injection of lipase in Experiment B.

For Experiment D it was decided to test how aging of a core can affect the overall performance of the lipase. Since the loss of the enzyme due to the irreversible adsorption was proposed as the main factor limiting improvement of the oil recovery, it was suggested that this loss could be much lower in an aged reservoir material as the pore walls will be “coated” with the surface active components deposited from the crude oil.

Overall, avoiding or decreasing extent of the irreversible enzyme adsorption was found to be quite challenging task. Hence, in the Experiment E occurrence of such adsorption was used as an advantage. Nano-EOR, i.e. injection of nano-particles such as nano-silica, was reported as a quite successful alternative to the traditional EOR techniques (Ragab and Hannora; 2015; Skauge *et al.*; 2010). At the same time, adsorption on solid substrate is one of the methods of support based protein immobilisation (Brady and Jordaan, 2009). Therefore, combination of nano- and enzymatic EOR by using enzyme-coated nano-silica particles could potentially result in a synergetic effect of the two methods.

It was also decided to test application of EEOR on chalk cores. In the two oil displacement experiments conducted in chalk some precipitation was observed at the inlets of the cores (Figure 2). The recovery values obtained in these experiments were considered to be unreliable and are not presented in this work. In the first experiment injection of 1 PV of 1% purified enzyme was followed by SW, while in the second experiment the injection scheme applied was injection of 1PV of the enzyme solution with silica nanoparticles followed by SW displacement. The precipitation was initially attributed to the viscous emulsion, potentially formed by the enzymes and crude oil,

placed in the core. In order to check this hypothesis, crude oil-brine interaction experiments were carried out. Even though the black formation was most likely to be caused by corrosion-induced asphaltene precipitation, the crude oil-brine interaction experiments revealed some important findings, which are presented in Chapter 5.



Figure 2: Black precipitation formed at the inlet of the cores in oil displacement experiments when using chalk as core material.

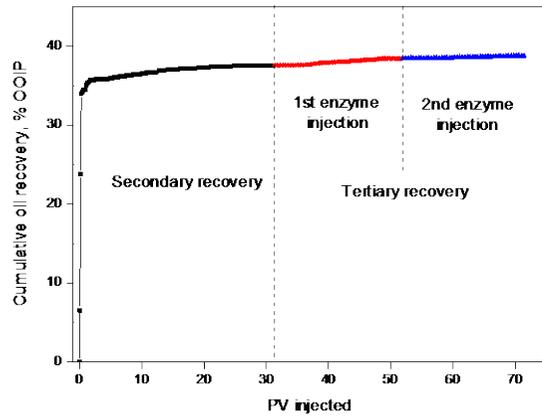
Finally, one more oil displacement experiment was carried out using chalk to test potential application of enzymatic EOR in carbonate reservoirs (Experiment F). Prior to this experiment all parts of the core flooding that potentially could introduce asphaltene precipitation were changed. In the experiment a solution of the purified enzyme product was applied as an EOR fluid, and concentration of the pure enzyme was higher than in the previous experiments where commercial product solutions were injected. This was done in order to check an effect of the increased pure enzyme concentration, to eliminate effect of the propylene glycol stabiliser precipitation and to improve stability of the enzyme molecules. No precipitation, as shown in Figure 2, was formed, so it was concluded that the black formation in the previous two experiments was due to asphaltene precipitation.

3. Results

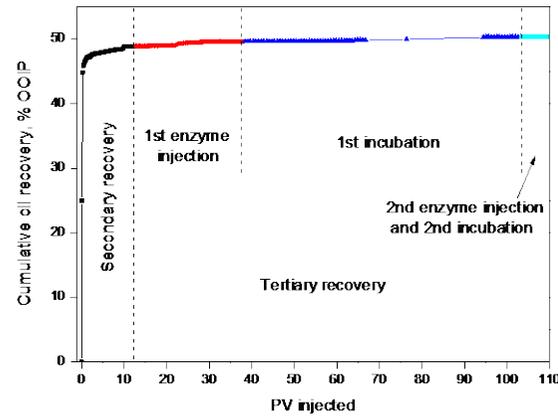
Eight core flooding experiments, five with sandstone and three with chalk, were carried out within the framework of the study. Summary of the experimental results is given in Table 5 and the recovery plots are presented in Figure 3.

Figures 3 (a) and (b) show oil production profiles for Experiments A and B. Application of 1% NS81249 solution did not result in a significant production of the additional oil. Results from both Experiments A and B were in a good agreement with each other. After the first enzyme injection 1.4% of additional oil relative to the residual oil after waterflooding was produced, while the recovery factor was below 1% of OOIP (Table 5). For both experiments the first portion of the additional oil was observed only after 4.71PV and 9.84PV of SW injected, for the Experiments A and B, respectively. This can be considered as a much delayed oil production. After the second enzyme injection in Experiment A, the recovery factor was twice less than for the first enzyme

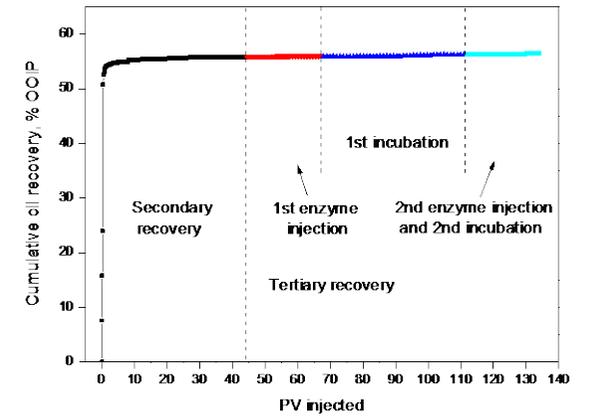
Chapter 6. Experimental Investigation of Enzymatic Enhanced Oil Recovery



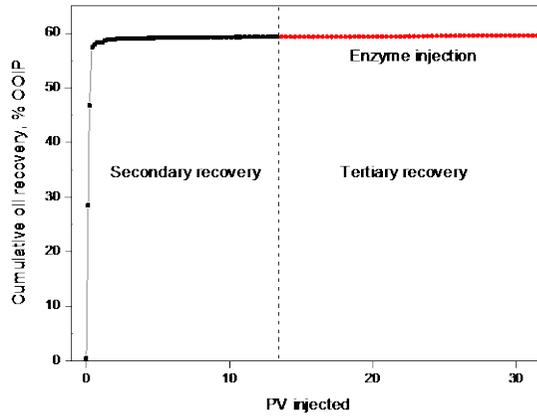
(a) Experiment A



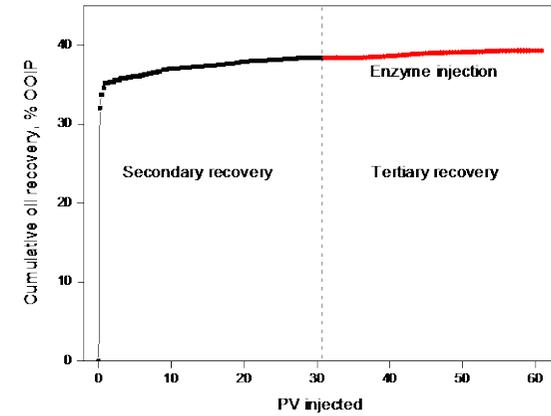
(b) Experiment B



(c) Experiment C



(d) Experiment D



(e) Experiment E

Figure 3: Production histories for the Experiments A - E.

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Table 5: Summary of the results of oil displacement experiments* (OOIP - original oil in place).

	Enzyme used	Original saturations		Secondary Recovery	Tertiary Recovery					Total oil recovery, %OOIP
		S_{wi}	S_{oi}		Recovery, %OOIP	EOR action	The PV of SW injected when the first portion of oil was detected	Additional recovery, % residual oil	Additional recovery, %OOIP	
Experiment A	NS81249	0.36	0.64	37.59	First enzyme slug injection	4.71	1.38	0.86	1.21	38.8
					Second enzyme slug injection	6.41	0.56	0.35		
Experiment B	NS81249	0.65	0.35	48.92	Enzyme injection	9.84	1.41	0.72	1.69	50.61
					Incubation without additional enzyme injection	10.96	1.30	0.65		
					Incubation with additional 2PV enzyme injection	4.19	0.64	0.32		
Experiment C	NS81251	0.51	0.49	55.82	Enzyme injection	8.13	0.40	0.17	0.73	56.55
					Incubation without additional enzyme injection	11.23	0.72	0.32		
					Incubation with additional 2PV enzyme injection	9.82	0.55	0.24		
Experiment D	NS81249	0.54	0.46	38.43	Enzyme injection (aged core)	4.89	1.45	0.89	0.89	39.32
Experiment E	NS81249	0.51	0.49	59.35	Injection of nano-silica–enzyme mixture (aged core).	5.15	0.56	0.23	0.23	59.58
Experiment F	NS81249	0.39	0.61	87.12	Purified enzyme injection	-	0	0	0	87.12

* - the results for only one core flooding experiment using chalk are presented as iron-induced asphaltene precipitation was observed during the first two experiments and their results were counted as unreliable.

injection (Table 5). Visual analysis of the effluent showed that oil after enzyme injection was produced as “flakes” (Figure 4)



Figure 4: Oil produced after the enzyme injection.

In Experiment B the amount of the produced oil after the first incubation was comparable to the recovery factor after the first enzyme injection: 0.65% relative OOIP and 1.30% relative residual oil. So the step of incubation can be important and should be taken into account for the EEOR technology development (provided that a significant additional recovery could be achieved). However, after the second incubation and following SW displacement, the amount of the recovered oil was only 0.32% relative OOIP and 0.64% relative remaining oil, which is twice less than after the first incubation. Moreover, oil production was delayed compared to the SW flow: oil was first detected in the effluent after 4.19PV of SW was injected. Hence, the second incubation was not as effective as the first incubation.

Application of the NS81251 solution followed by immediate SW displacement (Experiment C) also resulted in very low additional recovery (Table 5). After the first incubation (no additional enzyme injected) recovery factor increased almost twice, but still the amount of the recovered oil was too small to consider the amylase as a successful EOR agent. The second incubation (with additional enzyme injected) did not improve oil recovery to a large extent. The recovery factors were 0.24% relative OOIP and 0.55% relative remaining oil, again lower compared to the first incubation. Similar to the lipase, oil produced due to the amylase injection was collected in the form of flakes (Figure 4) that might be the evidence for the formation of the oil-amylase complexes. Overall, the results were even lower for the amylase compared to the lipase.

In case of application of the aged sandstone core (Experiment D), additional oil recovery after the enzyme injection did not increase significantly and was only 0.89% OOIP. Application of the combined nano-enzymatic-EOR in aged core (Experiment E) did not make a big difference, either: the amount of the additional oil recovered was the lowest among the experiments with sandstone (Table 5).

For the chalk core samples (Experiment F), injection of the purified enzyme did not result in any additional amount of produced oil. The recovery factor after the secondary waterflooding was 87.12%.

For all the experiments water phase was analysed for the presence of the enzymes. However, only trace amounts of the proteins were detected. Another observation relevant for all the experiments is that there was no significant increase of the pressure drops across the cores.

4. Discussion

The results obtained in the study are different from the results of the previous works, in which recovery factors for the laboratory oil displacement tests were rather high - up to 16% (Feng *et al.*, 2007; He and Zhonghong, 2011; Moon, 2008; Nasiri *et al.*, 2009). However, in most of these studies enzymes were applied as components in commercial mixtures. Apart from the proteins, these mixtures contained other surface-active components that could also affect an amount of the additionally recovered oil. The observed effect was most likely synergetic and cannot be solely attributed to the enzymes. To the best knowledge of the authors, the work of Nasiri (2011) is the only where an enzyme (esterase/lipase) was applied without additional surface-active compounds. The recovery factors in Nasiri's work were 3% OOIP for sandstone and 1 to 7.3% OOIP for carbonate cores. These values are much higher than recovery values obtained in this study. The most probable reason for such a difference is the implemented injection schemes. We used pulse-type injection of the enzyme, when 1 PV of the enzyme solution was displaced by SW, while in the work of Nasiri the enzyme solution was continuously injected in tertiary mode (up to 13PV of 1% enzyme solution was pumped in). Continuous injection of the enzyme solution was not tested in our work due to economical unfeasibility of such an injection scheme. Since the enzymes in our work and in the work of Nasiri were similar (he also applied a sample obtained from Novozymes A/S), the difference in recoveries may only be attributed to the amounts injected.

Based on the static experiments change of wettability and formation of emulsions were proposed as the main mechanisms for EEOR (Khusainova *et al.*, 2015; Chapter 5). The results obtained during the core flooding experiments demonstrate that additional oil produced after the enzyme injection can rather be attributed to the change of wettability than to formation of the emulsions. The problem is that enzyme molecules become entrapped in the porous space instead of the released oil. Our initially proposed mechanism for EEOR implies that after release of the oil enzyme molecules should desorb and travel further detaching more oil from the rock surface. However, the irreversible protein adsorption was quite pronounced, so amount of the released oil was very low even though the NS81249 sample demonstrated high ability to form the emulsions.

Compared to the adsorption study, concentration of the pure enzyme in the core flooding experiments was much lower (Khusainova and Shapiro, *submitted*). It has been well established that low concentrations facilitate protein adsorption on solid surfaces (Evers *et al.*, 2008; Kirchman *et al.*, 1989). Meanwhile, the emulsion study of the oil-brine-enzymes systems revealed that enzymes could form very stable emulsions, which means that enzymes also adsorb to water-oil interface (Chapter 5). In case of the oil displacement experiments, both oil-water and water-rock interfaces

were present and competitive adsorption took place. The enzymes would have better chance to travel through the porous media if they would prefer the oil-water interface. Then the main mechanism of the EEOR would be similar to surfactant flooding.

However, protein adsorption on the rock pore walls prevailed over the adsorption on the oil-water interface. This was supported by the second enzyme injection in Experiment A, for which the recovery factor was twice less than for the first enzyme injection (Table 5). When the first slug of the enzyme entered the core, enzyme molecules could access both the rock and the water-oil interfaces. So the oil could be mobilised both due to adsorption of the enzyme molecules, which detached the oil from the rock surface, as well as due to formation of the oil-enzyme structures in water. The mechanism of oil substitution by the protein molecules is in a line with static wettability tests (Khusainova *et al.*, 2015). Visual analysis of the effluent showed that after enzyme injection the oil was produced as “flakes” (Figure 4). Similar structures were detected during the emulsion study (Chapter 5). Hence, the emulsion formation most likely also took place. When the second pulse of the enzyme was injected more rock surface was available at the inlet part of the core (as some oil was released), so more enzyme molecules adsorbed to the pore walls and less enzyme could penetrate through the core and reach the oil phase. This explains a lower recovery factor after application of the second enzyme slug. Even though some enzyme-oil structures can be formed in the rock, their formation is limited by the protein adsorption on solid substrate, so that the enzyme molecules simply cannot reach the oil in sufficient amounts.

Prevalence of the enzyme adsorption mechanism is supported by the results of the experiment with amylase. Previously, no significant adsorption was observed for the NS81251 amylase (Khusainova *et al.*, 2015), while ability to form the oil-water emulsion was rather high. Hence, it was expected that the amylase could perform better than the NS81249 lipase. However, the recovery factor after the application of the amylase was even lower than for the lipase. This evidences that for the enzymes formation of the emulsions is not the main mechanism for the EOR applications.

Predominance of the wettability change over emulsion formation explains why no additional oil was produced in the experiments with chalk after incubation (Experiment F). Chalk demonstrated much higher adsorption capacity (Khusainova and Shapiro, *submitted*) and even though 1% of purified NS81249 solution that contained about 4-4.5 times more pure enzyme was used, production of additional oil was not observed.

Out of the various recovery attempts, the highest effect was observed after the stage of incubation during 1.5 months without addition of enzyme. Surprisingly, the second incubation after injection of the additional enzyme was less effective. It should be noted that the first incubation was longer. It appears that time plays more important role than even injection of the additional portion of the enzyme. It could also be that the first incubation resulted in recovery of almost all the additional oil, which could be recovered at all by this method. In further studies of enzymatic EOR, incubation should be taken into account as an important step. Anyway, so far amount of the additionally produced oil for sandstone during enzymatic tertiary recovery was relatively small.

EEOR trials in the aged core did not reveal any effect of the aging. However, it should be highlighted that only the light crude oils were applied in this study. The fractions of the heavy components in these oils were relatively low compared to the light fraction of saturates. Probably, there was not enough material to “coat” the pore walls to such extent that it would prevent the protein adsorption. Importance of the aging might become more apparent for heavier oils, where amount of the deposited heavy fractions could be much more significant.

As was pointed out by some enzyme product manufacturers (e.g. Apollo Separation Technologies Inc), one of the advantages of application of enzymes as EOR agents is regeneration of the enzyme solutions. However, analysis of the water phase showed that only trace amounts of the proteins were contained in the effluent. Adsorption of enzymes prevented their regeneration and reuse. Another potential reason for the loss of the enzymes that was not checked in the framework of this work could be interaction with the crude oil. The oil is quite aggressive liquid from the biochemical point of view, while enzymes are biological substances sensitive to the solvents. Therefore, enzyme/protein-crude oil interactions could potentially cause degradation of the enzyme molecules. In the adsorption study the enzyme content was measured after the solution interacted with the crude oil. Reduction of the concentration was 14-17% and it was attributed to the enzyme adsorption onto the oil-water interface. However, further studies need to be conducted to establish effect of the long-term interaction between crude oil and enzyme-containing brine on enzyme molecules stability at elevated pressures and temperatures.

5. Conclusions

The results obtained during the study show that that the probable application of enzymes as EOR agents may be doubted, at least under conditions close to the experimental conditions of the present work. Application of neither lipase nor amylase could not provide large enhancement of the ultimate oil recovery. For the sandstone the recovery factor during tertiary oil production varied from 0.23% OOIP up to 1.69% OOIP, while no additional oil production was observed when chalk sample was used. Apart from the small recovery factors, oil production after application of the enzymes was always much delayed.

Change of wettability was confirmed to be the main mechanism that governs EEOR, while formation of the emulsions might be the secondary mechanism. However, loss of the enzymes due to the irreversible adsorption onto the rock surface limits successful implementation of the EEOR technique.

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Chapter 7. Conclusions

The study of the enzymes for enhanced oil recovery (EOR) applications was carried out. The main question addressed in the investigation was whether enzymes can be utilised as effective EOR agents. To answer the question, the project was accomplished in four steps: the wettability study, the adsorption study, the emulsion and crude oil-brine interaction study and the core flooding study. In the wettability study, a number of enzyme groups was screened in order to select the most promising samples. The possible mechanisms of enzymatic EOR were proposed. In the adsorption study, the enzyme behaviour at water-rock interface was investigated. Production problems that might arise during field application of enzymatic EOR were revealed. During the emulsion and crude oil-brine interaction study, formation of the enzyme-stabilised emulsions was tested. Finally, in the core flooding study the enzyme performance under conditions similar to reservoir conditions was investigated.

Wettability Study

- The group of lipases/esterases was found to be the most promising for further investigations in terms of EOR, since they are capable of changing the wetting state of calcite surface from weakly to absolutely water-wet state. For some esterases/lipases the threshold concentration of enzyme product from which they were capable to improve wettability to absolutely water-wet state was as low as 0.1%_{wt}, which corresponds to 0.002 – 0.005 %_{wt} of pure protein.
- Influence of the stabilising compounds of the pure enzyme product on improvement of wettability was excluded and effect of the enzyme was confirmed.
- Possible mechanisms underlying wettability alteration under influence of esterases/lipases are adsorption of enzymes onto the mineral and/or formation of additional interfacially active oil compounds.
- Proteases also had some ability to improve wetting state of calcite, however, the effect could be non-uniform depending on the impurities in the calcite.
- Carbohydrases, oxidoreductases and EOR-ZYMAX™ had no effect on wettability of calcite at concentrations of enzyme product from 0.1 to 1%_{wt}.
- The commercial product Apollo GreenZyme™ improved wetting state of the calcite minerals at the whole range of investigated concentrations, but results could be produced by both enzyme and other components of the mixture (presumably, surfactants).
- Adhesion behaviour tests in conjunction with contact angle measurements may be applied as a screening tool for selection of the enzymes capable of changing the wetting properties of calcite. At investigated concentrations, results on adhesion behaviour and contact angle measurement are strongly dependent on the type of enzyme.

Adsorption study

- Irreversible adsorption significantly hinders the enzyme propagation in the rock material.
- Extent of irreversible adsorption increases with time, as the ratio of reversible to irreversible protein adsorption is strongly dependent on the bulk protein concentration. Such dependence might become a serious obstacle for the protein transport in the reservoir rocks that are characterised by both high internal surface and strong affinity to the protein.
- The retention rate of chalk was much higher than of sandstone. So sandstone possesses better quality from the enzymatic EOR application point of view.
- None of the applied methods (change of ionic strength and pH of the enzyme solution and the displacing fluid, application of various injection rates and injection of the enzyme stabiliser) was able to promote desorption of the enzymes. For the development of viable EEOR technology, an alternative solution that is able to keep reasonable balance between reversible and irreversible adsorption should be established.
- Application of various substrates such as minerals, particles and outcrop core rocks, demonstrated that the amount of the enzyme adsorbed was dependent on the type of the solid substrate. In order to obtain the most reliable results for EOR applications, enzyme adsorption behaviour should be estimated using dynamic flow-through experiments.
- A reasonably good agreement between the model results and the experimental flow-through data was achieved. Addition of the anomalous diffusion to the traditional diffusion-like dispersion did not result in any significant improvements of the model.

Emulsion and crude oil-brine interaction study

- Fraction of saturates was found to be responsible for the formation of protein-stabilised emulsions.
- The group of lipases/esterases demonstrated the best performance as emulsifying agents. High surface activity of the lipases/esterases at oil-water interface was in a good agreement with the wettability tests results.
- When an ester was added to the oil phase and the esterase was applied, the emulsion was less stable than for ester-free system. Destabilising effect of the ester outweighed stabilising effect of the carboxylic acid and alcohol that were formed due to enzymatic hydrolysis of the ester.
- Incubation of the oil-enzyme systems results in formation of more stable emulsions. Therefore, the stage of incubation should be taken into account when the methodology of the core flooding experiments is designed.
- Enzyme molecules themselves, not stabilising ingredients, possess surface-active properties that result in formation of the emulsions.

Chapter 7. Conclusions

- Combined application of an enzyme and solid particles resulted in creation of more stable and tighter emulsions compared to the application of enzymes alone.
- Methodology of protein-stabilised emulsions preparation was developed. The method allows handling large number of samples and provides reproducible results.
- The crude oil-brine interaction experiments revealed that exposure of the enzyme solution to the interaction with crude oil causes precipitation of the propylene glycol (main stabilising ingredient) in the form of gel/viscous emulsion.
- A very viscous oil-in-water emulsion was formed when the purified enzyme in higher concentration was applied in the crude oil-brine interaction test. The oil drops had unusual asymmetric shapes; also some “net structures” of oil in water were detected.

Core flooding study

- The study determined relatively low potential of enzymes as EOR agents, at least under conditions close to the experimental conditions of the present work
- Application of neither lipase nor amylase did not result in significant improvement of the ultimate oil recovery. However, in general, performance of the lipase sample was better than of the amylase.
- Additional recovery after application of the enzymes in tertiary mode varied from 0.23% OOIP up to 1.69% OOIP in sandstone, while no enzyme effect on oil recovery was observed for chalk.
- Change of wettability has been a primary mechanism of EEOR. Formation of the emulsions was not that pronounced, but it might be the secondary mechanism that is responsible for the enhancement of oil recovery after application of enzymes.
- Effective realisation of the wettability mechanism is strongly limited by the loss of the enzyme molecules due to the irreversible adsorption. Permanent deposition of the enzyme molecules on the porous rock surfaces was claimed to be the main restriction for successful implementation of the EEOR technique.

Chapter 8. Recommendations for Future Work

- If enzymes are going to be further developed as EOR agents, the first problem that needs to be solved is reduction of the enzyme irreversible adsorption onto the rock surface and increase of the ratio of the reversible to irreversible adsorption. Among others this might be achieved by (a) addition of stabilising co-solvents, e.g. glycerol and sucrose (Evers *et al.*, 2011; Koo *et al.*, 2008; Timasheff, 1993); (b) varying amount/addition of kosmotropic (e.g. $(\text{NH}_4)_2\text{SO}_4$ and Na_2SO_4) and chaotropic (NaSCN and $\text{Ca}(\text{SCN})_2$) salts (Evers *et al.*, 2009); (c) injection of polymers prior enzyme slug injection (similar to polymer pre-treatment during surfactant flooding (Wang *et al.*, 2015)).
- Only light crude oils were applied in the study. EEOR might perform completely in a different way for heavy oils. For example, asphaltenes and lipids have similar structures; therefore there is some chance that lipase NS81249 would interact with the asphaltene fraction of the crude oil. Therefore, crude oils with various properties should be tested for EEOR applications.
- In order to realise catalytic properties of the enzymes, an enzyme that can interact with crude oil (or a certain fraction of the oil) should be selected/“designed/”built”. A chemical reaction that would be facilitated with the enzyme injection should be beneficial from the EOR point of view. For example, it can be the chemical reaction that can “break” asphaltenes into smaller hydrocarbon molecules and an enzyme that will catalyse this reaction. This process can serve as in-situ crude oil refining and could probably be applied in heavy oil reservoirs.
- More crude oil-enzyme solution interaction experiments should be carried out in order to find out long-term effect of the crude oil on enzyme stability, activity and molecular structure. The experiments should be conducted under the conditions similar to the reservoir conditions (i.e. elevated temperature and pressure).
- Combined application of the enzymes and nano-particles did not result in large amount of additionally produced oil. This was only the first trial. A more detailed investigation should be carried out in order to test the synergetic effect of enzymes and nano-particles on the oil recovery and to establish an optimal particle concentration.
- The established mechanisms underlying EEOR are based on the surface activity of the enzymes and are determined by their protein nature. The catalytic effect was not observed so far. Therefore, further investigations may be focused on application of proteins, which, in turn, might also be more economically feasible. To the best of our knowledge, there is only one study that has presented application of a protein (particularly, of the aqueous solution of a common bean protein, *Phaseolus vulgaris* Linn) for improvement of oil production (Gbonhinbor and Onyekonwu, 2015).
- The following improvements are suggested for a better performance of the core flooding set-up:

(a) As described in Appendix 1, after change of an injection fluid, the pressure of the ISCO-pump raised up to P1 (P1 is the injection pressure shown by the ISCO-pump at the end of injection of the previous fluid) before opening the inlet valve of the coreholder. This was done in order to exclude entering the fluid from the core to the injection cylinder as the pressure in the coreholder is always higher than the atmospheric pressure. However, this adjustment of the pressures using pressure recordings of the ISCO-pump was sometimes quite rough, and pressure fluctuations were observed at the beginning of the experimental stage. This can be avoided, if a pressure transducer can be incorporated into the coreholder inlet line. Then, when an injection fluid is changed the injection pressure should first increase up to the value of the pressure in the coreholder inlet line, and only afterwards the inlet valve of the coreholder should be opened.

(b) The methodology that was used to measure small quantities of crude oil in the effluent (see Appendix 1, Section 8b) was quite time- and labour-consuming. For conduction of a large number of oil displacement experiments, application of an automated crude oil detector may be quite beneficial. An oil content meter using the solvent extraction/infrared analysis method that is widely used for the oil content measurements during oil spills and wastewater treatment is a good example of the device that can be adapted for the analysis of the effluent from core flooding experiments (Global Environment Centre Foundation website). The basic principle of the oil content meter is similar to the methodology that was used during the study. However, the steps of solvent addition, mixing, separation of the solvent phase and oil concentration measurements are accomplished automatically, that makes the process of oil content determination more effective.

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Appendix 1. Step-wise Description of an Oil Displacement Experiment and What We Have Learned along the Experimental Journey

1. General overview

An oil displacement experiment can be divided into five steps: selection and preparation of a core, saturation with a synthetic brine, saturation with a crude oil, secondary, and tertiary recovery (Figure 1). Most of the steps comprise of sub-steps and will be discussed in details below. Dynamic adsorption tests (flow-through experiments) discussed in Chapter 4 were carried out in a similar way, but excluding the step of saturation with a crude oil.

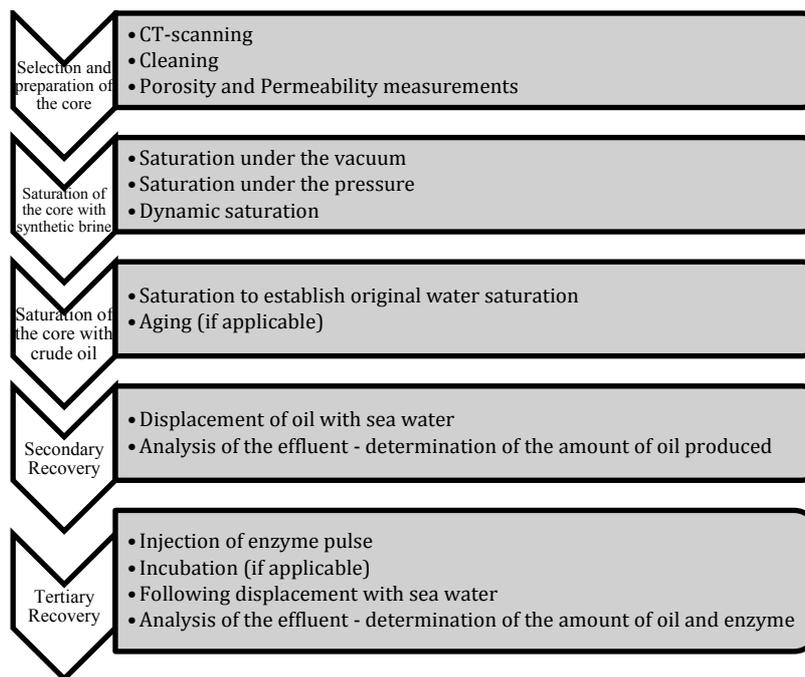


Figure 1: Flowchart of Oil Displacement Experiment.

2. Preparation of the coreflooding set-up

Since our experiments implied use of enzymes, which are biological substances, an attention was paid to the cleanliness of the equipment. A coreflooding set-up (including injection cylinders) was cleaned with toluene and ethanol prior to every experiment. Description of the set-up can be found in Chapters 4 and 6.

The lines going to pressure transducers were always filled with distilled water. It was found that use of sea water (SW) in these lines could interfere pressure readings.

3. Selection of a core

Depending on the aim of an experiment and type of the rock required, the core samples were taken from outcrop or a reservoir. The outcrop samples were used at the beginning of the study, where general methodology was at the stage of development. In order to investigate the effect of the different rock types sandstone and chalk cores were used. It was found that use of the outcrop chalk

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samples for oil displacement experiments is limited due to their fragile nature. Our in-house protocol suggests three steps of saturation (see Section 5) that introduce a rather long rock-brine interaction. As a result, we experienced several tests where outcrop chalk cores were fractured during the experiment. So for chalk only reservoir cores were used in the oil displacement experiments.

All the cores were initially dried in the oven for several days (at least overnight) and then CT-scanned in order to check integrity and homogeneity of the core and discard fractured samples as application of fractured cores was out of the scope of the work. For each core scans of three cross-sections were done: two along the longitudinal axes and one along the lateral axis (see example on Figure 2). This combination and number of cross-sections were found to keep good balance between number of pictures required and amount of information that can be extracted.

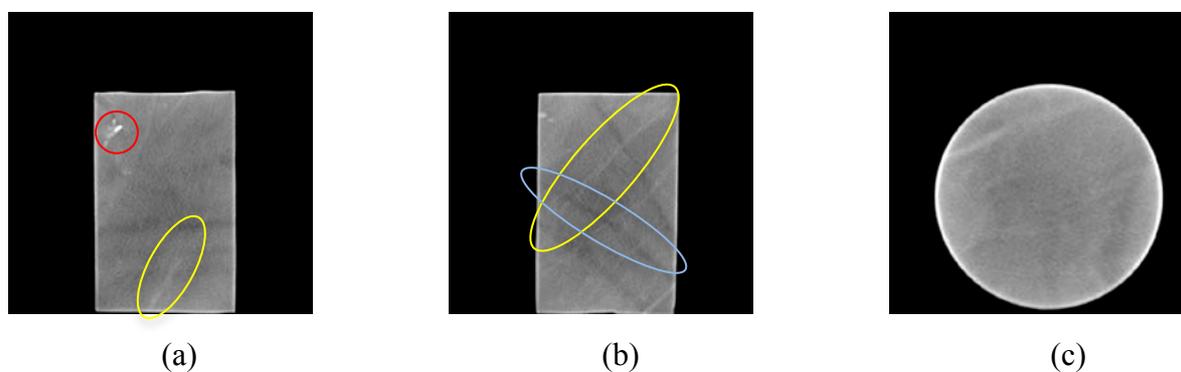


Figure 2: Pictures obtained from the CT-scanner for one of the reservoir chalk cores. (a) and (b) are cross-sections obtained along the longitudinal axes and (c) is the cross-section obtained along the lateral axis. The red circle highlights a high-density inclusion; the yellow ovals demonstrate high-density layers, while the blue oval shows a low-density layer. Overall, these pictures show that the sample is heterogeneous.

4. Preparation of the core

a. *Cleaning of the core samples*

Both chalk and sandstone core samples were cleaned with toluene and methanol. Toluene was used to remove organic compounds, which might be present in the rock, while methanol was used to remove polar compounds and toluene (as those two solvents are very miscible). Besides, methanol was used after the toluene, as it is more volatile compared to toluene that makes it easier to remove the cleaning solvent from the core sample. At the beginning of the study ethanol was used instead of methanol. However, solubility of sodium chloride that is the main component of the naturally occurring brine in the rock is 25 times higher in methanol than in ethanol (Pinho and Macedo, 1996). Therefore, it was decided to use methanol despite its high toxicity.

A cleaning apparatus was a simplified coreflooding set-up (Figure 4). A core was placed into the rubber sleeve and then in the coreholder. The sleeve pressure of 20 bars was applied both

Appendix 1. Step-wise Description of an Oil Displacement Experiment and What We Have Learned along the Experimental Journey

for chalk and sandstone samples. Toluene and methanol were injected in 3 cycles with the flow rate of 1 ml/min (Table 1). If the effluent was colourless and contained no Cl⁻ ions at the end of the third cycle, cleaning was stopped. Otherwise, additional cycles were run until there was no colour and no Cl⁻ ions in the effluent. Presence of Cl⁻ ions was checked by adding AgNO₃, which is a reagent for qualitative analysis of chloride ions. The cleaned sample was removed from the coreholder and dried at 90°C at least overnight, up to a constant weight. The CT-scans of the core were taken again in order to make sure that the core could keep the integrity during the cleaning process. If no fractures/damages were created during the cleaning procedure, it was assumed that the core is strong enough to withstand further experimental procedure.

Table 1: Solvents injection scheme for cleaning of the cores.

Rock Type	Cleaning Scheme	
Chalk	First cycle	300 ml of toluene followed by 300 ml of methanol
	Second cycle	200 ml of toluene followed by 200 ml of methanol
	Third cycle	100 ml of toluene followed by 100 ml of methanol
Sandstone	First cycle	200 ml of toluene followed by 200 ml of methanol
	Second cycle	100 ml of toluene followed by 100 ml of methanol
	Third cycle	50 ml of toluene followed by 50 ml of methanol

b. Porosity and permeability measurements

True porosity and Klinkenberg permeability of the core were measured by the steady state gas permeameter and porosimeter (Poroperm, VINCI TECHNOLOGIES), using nitrogen. The true porosity was used for further estimation of the extent of saturation with the brine.

5. Saturation with the synthetic brine

The process of saturation of the core consisted of three stages. The first stage was five-step saturation under vacuum; the second stage was saturation under the pressure; and finally, dynamic saturation with a constant flow of the brine was carried out. In the original in-house protocol the saturation consisted of only dynamic saturation. However, it was found that for some samples, especially for Nordhorn sandstone (Austria), the overall brine saturation was below 60% when the core was saturated only by injecting brine. The following parameters were changed in order to approach complete saturation of the core: increase of the injection pressure; different directions of the flow and vertical injection; decrease of the flow rate. However, none of these modifications helped to improve the degree of saturation. Therefore, the procedure of saturation under the vacuum was investigated. At the beginning the cores were fully covered with the brine and saturated during different periods of time. Nevertheless, the difference between true porosity and the porosity relative SW was still quite high. Air bubbles trapped in the middle of the core were suggested to be the main reason for incomplete saturation. Therefore, a five-step saturation under the vacuum was proposed. This procedure provides gradual saturation of the sample with the brine, while there is always some path for the air to be removed. As a result, a sufficient brine saturation (close to true porosity) could be reached.

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a. Five-step saturation under the vacuum

1. A cleaned core sample and SW were deaerated separately for 2 hours (Figure 3, a)
2. One third of the degassed core was immersed into the degassed SW for 1.5 hours (Figure 3, b)
3. Two thirds of the degassed core were immersed into the degassed SW for 1.5 hours (Figure 3, c)
4. Almost full coverage of the core with SW for 1.5 hours (Figure 3, d)
5. The core was turned upside down and fully covered with SW overnight (Figure 3, e)

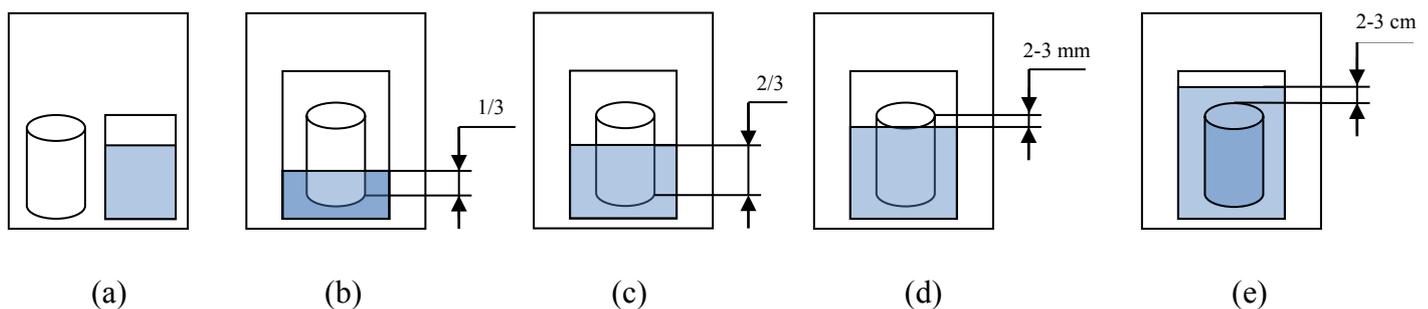


Figure 3: 5-step saturation under the vacuum.

After saturation under the vacuum the core was weighed and its pore volume was determined based on the weight method:

$$PV = \frac{m(\text{core} + \text{SW}) - m(\text{core})}{\rho(\text{SW})}$$

The level of saturation was determined by comparing the pore volume obtained by the weight method with pore volume value acquired by the porosimeter.

b. Saturation under the pressure

After the saturation under the vacuum the core was put into a stainless steel cylinder to be pressurized under 100 bars in SW for 48 hours (the surrounding SW was pre-degassed for 2 hours). The reason for saturation under high pressure is that if there any air bubbles were still left in the core, under high pressure they became smaller and it was easier for them to be released from the porous media. It was experimentally observed that for chalk this step is more important than for sandstone. For the chalk cores, saturation under the pressure can give up to 5% of additional brine in a core, while for the sandstone this value was below 1%. At the end of saturation under pressure the core was weighed again and the level of saturation was established. The difference between true and actual pore volume of less than 5% was counted as acceptable. The actual pore volume (PV) calculated in this step was assumed to be final and was used for all further calculations.

c. Dynamic saturation with the constant flow of the brine

After saturation under pressure the core was quickly transferred into the rubber sleeve and assembled in the coreholder (Figure 4). For chalk cores, stainless steel filters were put on both sides

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in order to exclude indentation of the end caps of the coreholder into the cores and to provide uniform distribution of the injecting fluid. The sleeve pressure applied was 50bar for outcrop sandstone and reservoir chalk and 20bar for outcrop chalk.

The coreholder's heating jacket was switched on. The working temperature was 60°C in all the oil displacement experiments, as this is the most optimal temperature for the applied enzymes (see Chapter 6), The system was left with no flow for 24 hours. This was done in order to establish uniform temperature in the core and to equilibrate the system. After that at least 3 PV and 7 PV of SW were run through the core with the flow rate of 0.1 ml/min for chalk and sandstone, respectively. Injection and differential pressures were monitored; their stabilities were used as an indication that the core kept its integrity. Just before stopping brine injection the pressure of injecting ISCO-pump was observed and written down as P1.

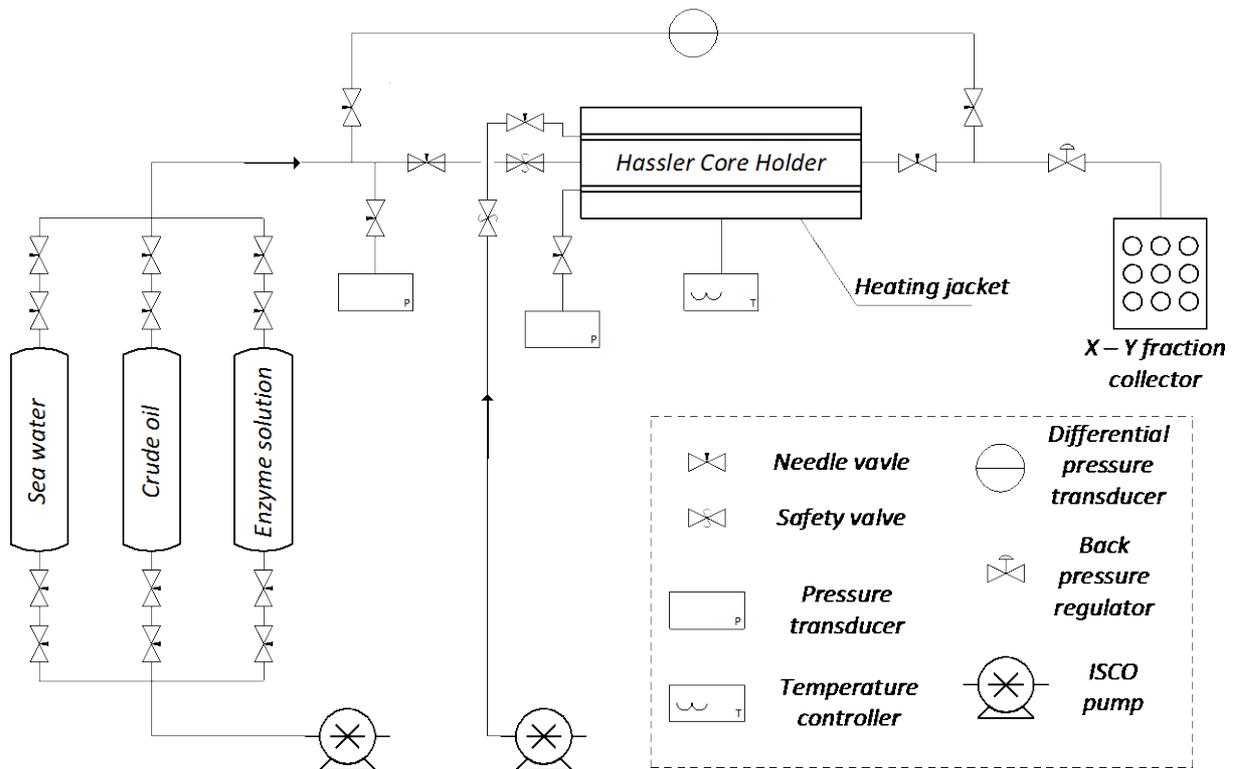


Figure 4: Coreflooding set-up used for dynamic adsorption experiments.

6. Saturation with crude oil – establishment of original water saturation

Prior the crude oil injection the inlet line from the injection cylinder up to the inlet valve of the coreholder was filled with the crude oil to reduce the dead volume. The outlet line connecting the outlet valve of coreholder with the fraction collector was cleaned from SW and left empty. These two actions were always made when the injected fluid was changed. Before opening the inlet valve of the coreholder the pressure was raised up to P1. This was done in order to exclude SW entering the injection cylinder with the crude oil as the pressure in the coreholder is always higher than atmospheric pressure. The pressure adjustment was always done when injecting liquid was

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changed or injecting pump was refilled (this will not be mentioned for the further steps). Then the flow rate was set to be 0.1ml/min and crude oil was injected until no more SW was produced. The effluent was collected using the X-Y fraction collector with the fraction size of 14ml. Similar to brine saturation, injection and differential pressures were monitored.

The original water saturation and the volume of oil in place were calculated from the volume of SW produced, taking into account dead volume of the coreholder. SW volumes above 0.5ml were determined by reading volume graduation on the tube (all the tubes for the effluent collection in all steps were with volume graduation). For the samples with SW below 0.5ml the volume of water phase was determined by comparison, i.e. similar tubes with pre-known volume of SW were prepared and compared to the tubes filled during crude oil injection.

If there was no aging according to the experimental plan, the pressure P1 was noticed again at the end, and the crude oil injection was stopped.

7. Aging (if applicable)

After the original water saturation was established, the flow rate was changed to 0.002ml/min and the core was aged for 3 weeks under dynamic flow. At the end of the aging step the core was re-flooded with the flow rate of 0.1ml/min for 24 hours.

8. Secondary waterflooding – SW waterflooding

a. Oil displacement

The SW was injected until no more oil was produced. The applied flow rate was 0.1ml/min. In cases where the injection pressure was too high, the flow rate decreased up to 0.01-0.05ml/min. The fraction size was 3ml (1/6-1/5 PV) at the beginning, to be able to detect the point of water breakthrough. Once the amount of oil in the effluent was less than 0.2-0.3ml, the fraction size was changed to 0.5 PV of the core, so that there were at least two points for every pore volume injected. This was done to reduce amount of the samples to analyse and also to increase accuracy of the oil content measurements.

In practice it was found to be impossible to reach a complete stop of an oil production. The line connecting the outlet valve of the coreholder and the fraction collector was made of transparent plastic. It could be noticed visually that a small fraction of the crude oil was stuck to the walls. When no more oil could be produced as a secondary recovery, some oil in the effluent was still observed. This was found to be oil from the outlet tube walls. Therefore after some time when the amount of produced oil stabilised to trace amounts, the outlet plastic line was cleaned with 3ml of toluene (the toluene effluent was collected and kept, see 8b) and ethanol (the effluent was discarded) and dried. Then SW injection continued overnight to confirm that there was no more oil production from the core. Before cleaning the line, injection and differential pressure profiles should be checked. If they are stable, trace amounts of the produced oil are most likely to be the oil that detaches from the tube walls, as discussed above. However, if there are some fluctuations in pressure profiles, the core is probably fractured.

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b. Analysis of the effluent

The oil volume in the effluent tubes was determined visually (i.e. using graduation on the tube) when the volume of oil produced was more than 0.5ml. The rest of the samples were analysed using the UV-vis method adapted from Evdokimov *et al.* (2003). This method was found to be applicable and reliable for analysis of the oil produced during coreflooding experiments (Katika *et al.*, 2015).

It was found that the oil content and the optical density (OD) of the oil solution in a certain amount of toluene are linearly dependent (Katika *et al.*, 2015). 3ml of toluene was chosen as a fixed toluene volume. First, the two calibration curves for the oil content determination were built using samples with pre-known oil volumes. Due to stickiness of the oil that caused difficulties during oil transferring, the mass of the oil was measured and then converted to the volume. The first curve was built for the samples that contained up to 1ml of crude oil, while second curve was for 0-100 μl volumes (Figure 5). A reason for building the “micro” calibration curve was that for this range the slope of Net absorbance=f(oil content) graph was slightly sharper than for the 0-1ml range. For both cases, three replicates were performed in order to minimise the experimental error. Moreover, for the case of the “micro” curve an additional version that used enzyme solution as water phase was constructed. This was done in order to confirm that enzymes do not interfere to the absorbance measurements. Standard curves were built once at the beginning of the experimental work and then they were used for the whole work.

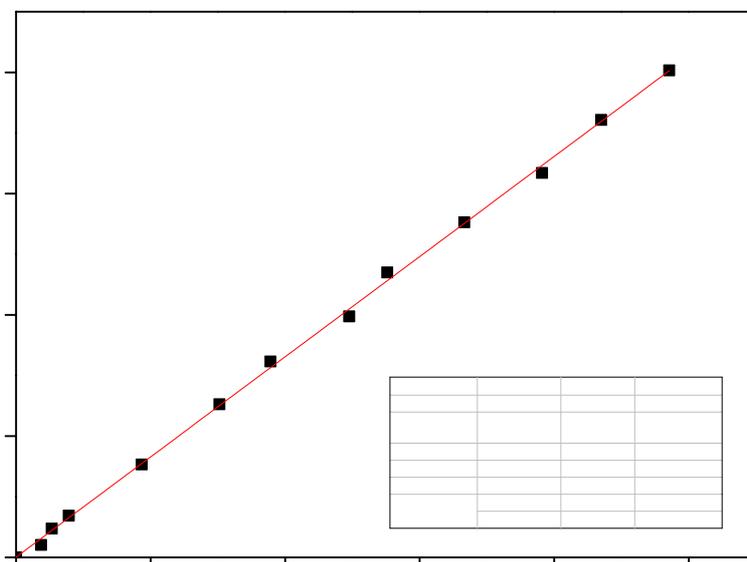


Figure 5: The “micro” calibration curve for oil volume determination.

The experimental steps for an OD measurement were as follows. 3ml of toluene was added to a vial containing oil. The solution was thoroughly shaken in order to dissolve all the oil and left

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for a few minutes until two phases were completely separated. Then toluene phase was transferred to the glass cuvettes and net absorbance at 750nm was measured. OD of the solutions was always measured within 10-15 min after solution preparation; otherwise net absorbance values were decreasing and unstable over time. Three values of net absorbance for each sample were determined.

A toluene solution obtained from tube washing (see Section 8a) was analysed in a similar way. The obtained oil volume was added to the volume of the oil in the first effluent tube.

9. Tertiary waterflooding – Enzymatic Enhanced Oil Recovery

a. Injection of enzyme pulse, incubation (if applicable) and displacement with SW

In all the oil displacement experiments enzyme was injected as a 1 PV pulse of the 1%_{wt} enzyme product solution (more details about enzyme concentration can be found in Chapter 3). The solution was prepared by dilution of the enzyme product in the same synthetic SW that was used throughout the experiment. The flow rate of 0.1ml/min was applied (unless injection pressure was too high), the size of the effluent was 0.5 PV of the core. A small amount (5-10ml) of the original enzyme solution was kept for the Bradford assay to determine initial enzyme concentration (see Section 9b). In experiments where effect of incubation was investigated, enzyme was injected as 2 PV pulse and was left in the core for 1 month.

Injected and incubated (if applicable) enzyme was displaced with SW until no more oil was produced. During tertiary production the process of oil production was captured by a web camera applying the Yawcam software. This method was introduced by Halim *et al.* (2015) and was used in order to be capable of establishing precisely when the first portion of additional oil was produced. As it was discussed in section 8a, oil stuck to the walls of the plastic tube that connected outlet valve of the coreholder and the fraction collector. This issue became quite significant during tertiary recovery as up to 1% of original oil in place (OOIP) could be retained in the tube. Therefore, the production history was built based on the photo data. At the end of tertiary waterflooding the outlet plastic tube was washed with 3ml of toluene. This amount was equally distributed during production time.

During tertiary recovery both water and oil phases were analysed. Oil phase was analysed according to the Section 8b, while water phase was checked for enzyme content (see Section 9b). For cases, where both oil and water phases were present, 3ml of water phase were first sampled using syringe with the needle (to make sure that toluene did not destroy potentially present enzyme molecules). Afterwards 3ml of toluene was added in a way, so oil attached to the sampling needle was washed into the toluene solution.

b. Analysis of the water phase of the effluent

Enzyme concentrations were measured using Bradford Assay (Bradford, 1976). Standard Sigma-Aldrich protocols were used in this work. At the beginning the standard 3.1ml Bradford assay (that utilises 1:30 protein - Bradford reagent ratio) was chosen, however, no enzyme was detected in the effluent solutions. Therefore, the method was switched to the Micro 2 ml Assay Protocol, which can determine protein concentration in the range of 1–10 µg/ml. Briefly, the

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standard curve covering protein range of 1–10 $\mu\text{g/ml}$ was built using a BSA standard. Therefore, all the determined enzyme concentrations were concentrations relative to BSA. In contrast to the standard curve for oil content analysis, the BSA standard curve (Net absorbance = $f(\text{protein concentration})$) should have been rebuilt for every set of measurements. 1ml of an unknown sample (or a BSA standard sample) and 1ml of the Bradford reagent was mixed in a falcon tube using vortex. The mixture was incubated at room temperature for about 10min. Then the sample was transferred to the cuvette and absorbance at 595nm was measured. The enzyme concentration was determined by comparing the net absorbance values against the standard curve. Presence of certain salts in the solution might interfere to the absorbance measurements. The produced SW containing enzymes was a high ionic solution containing six different salts. However, it was established that these salts do not affect final results, and SW was used as a buffer solution.

10. Recovery plots

The recovery plots expressed cumulative percentage of the oil produced as a function of pore volume of displacing fluid injected (Figure 6). Two features should be kept in mind, when constructing the recovery plot. First, the pore volume value that should be used is the one that was determined after the step of saturation with SW under pressure (see Section 5b). Second, during determination of the effluent volume the dead volume should be subtracted. This should be done after each change of the injected fluid.

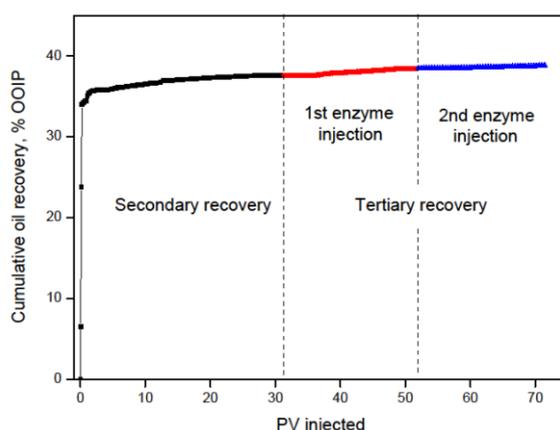


Figure 6: Recovery plot for the Experiment A (see Chapter 6).

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Appendix 2. Joint Author Statements

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A representative sample of authors is comprised of

- Corresponding author and/or principal/first author (defined by the PhD student)
- 1-2 authors (preferably international/non-supervisor authors)

Titel of the article	Study of Wettability of Calcite Surfaces using Oil-Brine-Enzyme Systems for Enhanced Oil Recovery Applications
Author(s)	Alsu Khusainova, Sidsel Marie Nielsen, Hanne Høst Pedersen, John M. Woodley and Alexander Shapiro
Journal/conference <small>* if applicable</small>	Journal of Petroleum Science and Engineering
Name of PhD student	Alsu Khusainova
Date of Birth	08.05.1988

Description of the PhD student's contribution to the abovementioned article

1. PhD student carried out all experimental work.
2. PhD student wrote the majority of the article.

Signature
of the PhD student

Date 07.01.2015

Signatures of co-authors

As a co-author I state that the description given above to the best of my knowledge corresponds to the process and I have no further comments.

Date (DD/MM/YY)	Name	Signature
07.01.2015	Sidsel Marie Nielsen	
18.02.2015	Hanne Høst Pedersen	
07.01.2015	John M. Woodley	
07.01.2015	Alexander Shapiro	

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A representative sample of authors is comprised of

- Corresponding author and/or principal/first author (defined by the PhD student), and if there are more authors:
- 1-2 authors (preferably international/non-supervisor authors)

Titel of the article	Study of Enzyme/Protein Adsorption for Chalk and Sandstone Reservoir Rocks
Author(s)	Alsu Khusainova, Alexander Shapiro
Journal/conference <small>* if applicable</small>	Submitted for a publication in Journal of Petroleum Science and Engineering
Name of PhD student	Alsu Khusainova
Date of Birth	08.05.1988

Description of the PhD student's contribution to the abovementioned article

1. PhD student carried out the experimental work.
2. PhD student wrote majority of the manuscript.

Signature
of the PhD student

Date 25.01.2016

Signatures of co-authors

As a co-author I state that the description given above to the best of my knowledge corresponds to the process and I have no further comments.

Date
(DD/MM/YY)

Name

Signature

25.01.2016

Alexander Shapiro

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- 1-2 authors (preferably international/non-supervisor authors)

Titel of the article	Experimental Investigation of Enzymatic Enhanced Oil Recovery
Author(s)	Alsu Khusainova, Alexander Shapiro
Journal/conference <small>* if applicable</small>	Prepared for a publication in Journal of Petroleum Science and Engineering
Name of PhD student	Alsu Khusainova
Date of Birth	08.05.1988

Description of the PhD student's contribution to the abovementioned article

1. PhD student carried out the experimental work.
2. PhD student wrote majority of the manuscript.

Signature
of the PhD student

Date 25.01.2016

Signatures of co-authors

As a co-author I state that the description given above to the best of my knowledge corresponds to the process and I have no further comments.

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