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Study of wettability of calcite surfaces using oil–brine–enzyme systems for enhanced oil recovery applications

Alsu Khusainova [a], Sidsel Marie Nielsen [a], Hanne Høst Pedersen [b], John M. Woodley [a], Alexander Shapiro [a,*]

[a] Department of Chemical and Biochemical Engineering, Technical University of Denmark, Anker Engelunds Vej 1, DK-2800 Kongens Lyngby, Denmark
[b] Novozymes A/S, Krogshoejvej 36, DK-2880 Bagsvaerd, Denmark

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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.

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; Moon, 2008; He and Zhonghong, 2011; Ott et al., 2011). Meanwhile, the 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; Moon, 2008; Nasiri et al., 2009; He and Zhonghong, 2011; 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

* Correspondence to: Department of Chemical and Biochemical Engineering, Technical University of Denmark, Soeltofts Plads, Building 229, DK-2800 Kongens Lyngby, Denmark. Tel.: +45 45 25 28 81.
E-mail address: ash@kt.dtu.dk (A. Shapiro).

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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 ≥ 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
$MgCl_2 \cdot 6H_2O$	9.15
$CaCl_2 \cdot 2H_2O$	1.91
Na_2SO_4	3.41
Total dissolved solids	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 ≥ 99.5%) and were not subjected to further purification.

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.

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 ≥ 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

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_2O_2 as an electron acceptor. Activity: 10000 POXU/g
Commercial mixtures containing enzymes		
Apollo GreenZyme™	Undisclosed	–
EOR-ZYMAX™	Undisclosed	–

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:

$$Bo = \frac{(\rho 1 - \rho 2) g L^2}{\gamma},$$

where Bo is the Bond number, ρ_1 is the density of aqueous phase (kg/m^3), ρ_2 is the density of oil phase (kg/m^3), g is the acceleration due to gravity (m/s^2), L is the characteristic length of the drop (m), and γ is 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 the introduction of calcite minerals in 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 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 also indicates 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 to make this possible.

2.2.1. Adhesion test

Adhesion tests were carried out according to 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 (Turner and Vulfson, 2000; Cobianco et al., 2007). 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.

Calcite was immersed into the brine/enzyme solution in a glass container ($5 \times 5 \times 5$ cm^3 or $6 \times 6 \times 6$ cm^3 depending 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 μl) was carefully deposited on to the lower crystal face using a syringe with an inverted needle (Fig. 1). The oil drop was allowed to make contact with the mineral in the presence of brine for 2 min 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 (Fig. 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.

Each crude oil–brine–calcite system was tested at least twice. Adhesion behaviour of a certain system was determined on the basis of 12–24 drops. The response of adhesion behaviour after adding the 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; Yang et al., 2008; Shojai Kaveh et al., 2014). The procedure consisted of three steps: (1) placing a liquid drop on a solid surface; (2) recording the drop shape (image acquisition); and (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 in the adhesion test, as described in Section 2.2.1 and Fig. 1. After the deposition, a drop was allowed to settle for about an hour (60 min 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 100 mm F2.8L 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.

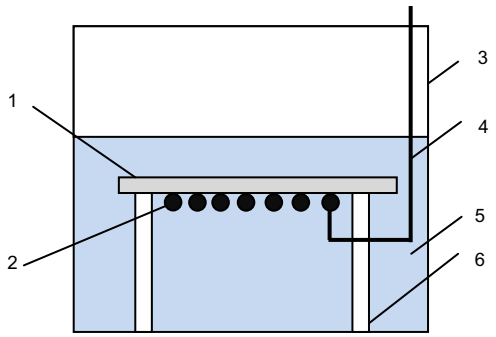

Fig. 1. Scheme of the experimental setup. (1) calcite mineral, (2) oil drop, (3) glass container, (4) inverted needle, (5) brine/enzyme solution, and (6) glass stand.

The contact lines between the two liquid phases and also between the liquids and the solid were established by applying edge detection techniques. Depending 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 the 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 12–24 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 2 min (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.

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 min. The result was a contact angle of $37 \pm 7^\circ$, 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 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.

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 was 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 (Figs. 3 and 2b). The average contact angle increased 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

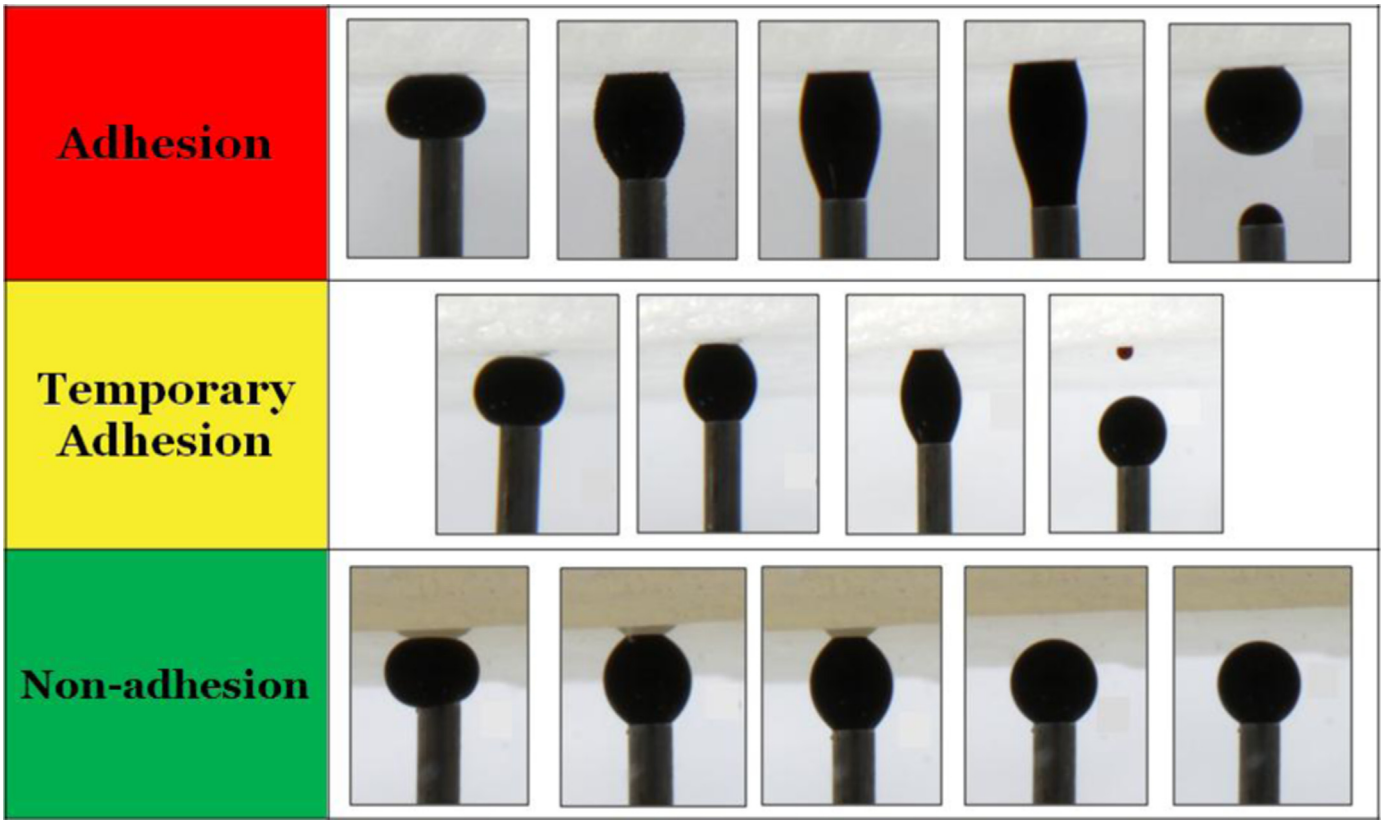

Fig. 2. Adhesion behaviour types.

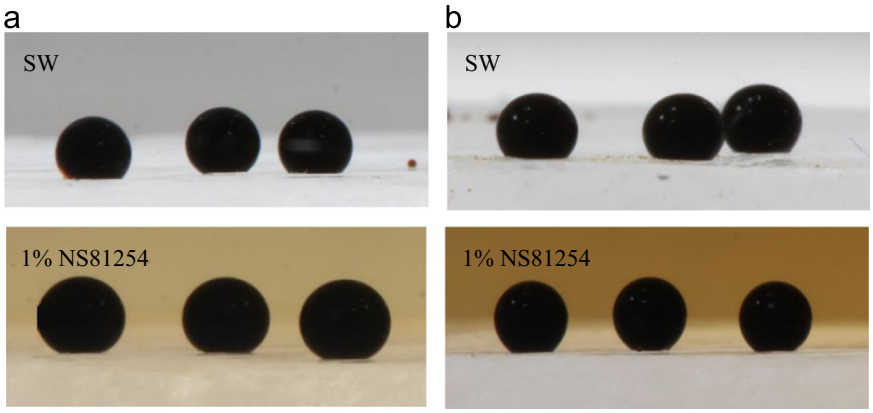

Fig. 3. Effect of buoyancy on shape of the oil drops: (1a) and (2a) – shapes of the drops placed on the bottom surface of the mineral in presence of SW and 1% NS81254 sample, respectively; (1b) and (2b) – shapes of the drops placed on the top surface of the mineral in presence of SW and 1% NS81254 sample,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

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 is very important that the drop is created slowly and the last stage of the drop formation should take at least 1–2 min (Alpbaz et al., 1988). (2) Determination of the oil drop volume at the moment the oil drop breaks off from the needle tip (the volume of floating drop); (3) measurement of liquid densities (Anton Paar, DMA 4100); and (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 is the acceleration due to gravity (m/s^2); ρ_1 is the density of aqueous phase (kg/m^3); ρ_2 is the density of oil phase (kg/m^3), V is the average volume of the oil drop (m^3), r is the radius of the inverted needle (m), and $f(HB)$ is 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, the 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).

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 ($38 \pm 7^{\circ}$) comply 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 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%.

Out of the lipase group (Table 3), samples NS 44033 and NS 44035 are the least desirable for further investigation, since for both 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

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, and 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									N/A			
NS 44035	N/A				N/A				N/A			
Carbohydrases												
NS 81251												
NS 81252												
Proteases												
NS 81253												
NS 44110												
NS 44055	N/A				N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
NS 44053	N/A				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												

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% 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 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 concentrations.

Two commercial enzyme-based mixtures, Apollo GreenZyme™ and EOR-ZYMAX™, were included in 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 calcite minerals at all 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

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 the reference water contact angle and θ_{enz} is 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
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
Oxidore-ductases	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

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 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 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 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?

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*:

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.

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).

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 (Beverung et al., 1999; Baldursdottir et al., 2010). 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.

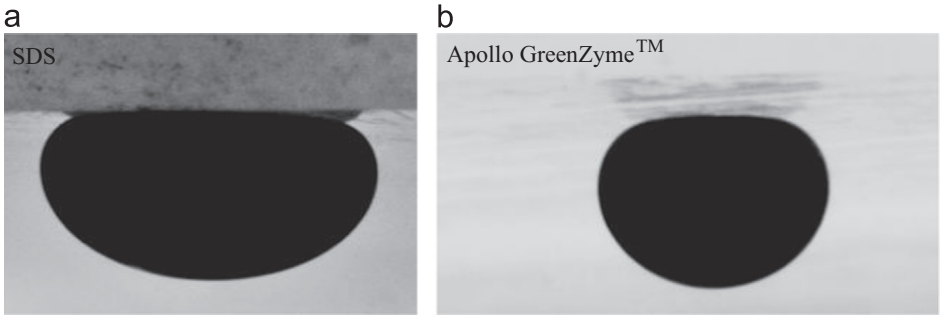

Fig. 4. Oil drop shapes under the influence of IFT decreasing components added to the surrounding SW. (a) SDS and (b) Apollo GreenZyme™.

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%		-	-		-	-		-	-

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 change 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 (Fig. 4a) was completely different compared to the performance of the enzyme systems. The only exception was Apollo GreenZyme™, whose behaviour resembled that of SDS (Fig. 4).

As illustrated in Fig. 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 was 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.

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 with 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 action of most 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 different adhesion behaviours.

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 crude oil–[BSA+SW]–calcite system 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

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 Absolute	Contact Angle Relative	Adhesion Behaviour	Contact angle Absolute	Contact angle Relative	Adhesion Behaviour	Contact angle Absolute	Contact angle 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

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.

1 % solutions of enzyme products		White Cleaved Calcite			White Calcite			Yellow Calcite		
		Adhesion Behaviour	Contact Angle Absolute	Contact Angle Relative	Adhesion Behaviour	Contact Angle Absolute	Contact Angle Relative	Adhesion Behaviour	Contact Angle Absolute	Contact Angle Relative
Amylase ***NS 81251***	*n*-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***	*n*-Decane		0°	1		0°	1		0°	1
	Crude Oil		0°	1		0°	1		0°	1
Lipase ***NS 81249***	*n*-Decane		20°±1°	0.37		24°±4°	0.26		20°±3°	0.37
	Crude Oil		0°	1		0°	1		0°	1

from adhesion via temporary adhesion to non-adhesion behaviour occurred at the pure protein content between 0.001% and 1%.

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.

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 ($32 \pm 4^{\circ}$), which correspond 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.

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.

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.

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; Moon, 2008; Nasiri et al., 2009; He and Zhonghong, 2011; 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/estherases 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 are 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 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 different mineral surfaces indicated that, while the original surfaces were found to be weakly water wet, addition of an enzyme did not modify the wettability to change 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 (0.002–0.005% 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 the enzymes in terms of their applicability to further conduct 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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