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MICROBIAL MODIFICATION OF CHALK SAMPLES AND IMPLICATIONS FOR ENHANCED OIL RECOVERY

Ismaila A. Jimoh[1], Svetlana N.Rudyk[2], and Erik G. Sogaard[3*]

[1,2,3,] Section for Chemical Engineering Aalborg University Niels Bohrs Vej 8 6700 Denmark, egs@bio.aau.dk

Laboratory scale experiment was used to investigate the effect of microbial treatment on porosity of chalk samples similar to those in the North Sea oil reservoirs by growth of a strain of *Clostridium tyrobutyricum* adapted previously to higher salinity in media supplemented with molasses for continuous supply of nutrient. The results showed that the amount of biomass accumulated on the surface correlates with the rock volumes (R^2= *0.95)*. However, the mean porosity of all the samples increase by 4% while density increased by 0.17 g/cm^3 after 28 days of immersion in microbial solutions, an indication that bacteria penetrated into the porous space of the samples too. The pH and electrical conductivity measurements over the period showed that the dynamics of the media was constantly changing because of release of calcium ions from the chalks. Measured concentration of calcium ions in the bacteria media increased from initial average concentration of 203 mg/l at the start of the experiment to 1178 mg/l in 28 days. All the bacteria media with salinity ranging from 40-100 g/l experienced a decrease in measured pH from average of 7.0 to 6.0 in the first week; this however started to increase again from second week with release of calcium ions suggesting a correlation between pH and calcium ions released. 3-D surface plot (pH, salinity and time) for the range of salinity measured revealed delineation of salinity into two groups with boundary between 70-80 g/l. The highest calcium ions concentration was measured at the highest salinities by the end of experiment which could be due to the lower values of pH as indication of that higher salt concentration facilitates more acid production by bacteria.

1. INTRODUCTION

Microbial enhanced oil recovery (MEOR) method has recently gained increased attention because of the dwindling oil reserves around the world and the need to find an alternative way to improve oil recovery. One of the many ways by which microbes can be useful in MEOR process is the alteration of physical properties of rock. During microbial growth, resulting from consumption and utilization of nutrients; the rock environment can be altered in many ways. These include modification of rock texture, pore size, pore shape and grain surface roughness, electrical properties of the rock and dissolution of rock matrix (Atekwana et al. 2006). The dissolution of reactive phases (such as soluble solids i.e. carbonates) may facilitate microbial colonization, leading to formation of biofilm that can enhances adhesion of bacteria to rock matrix (Banfield et al. 1999; Atekwana et al. 2006). In addition, metabolic products such as carbon-dioxide and organic acids can react with rock matrix further altering the geo-chemical properties of the rock.
The change in rock properties due to microbial process can have significant effect in reservoir properties especially in carbonate rocks, which are more susceptible to modifications by post depositional mechanisms. Carbonate rocks are important focus in microbial enhanced oil recovery because they contain more than 50% of the world oil reserves (Harbaugh, 1967). Also because it has been suggested that MEOR process are more likely to be successful in carbonate rocks than in sandstones since carbonate rocks are chemically formed sedimentary rocks and the injected bacteria spread wider and more quickly through fissures, fractures and pore canals (Wagner et al. 1992). Two of the most important rock properties affected by microbial process are porosity and permeability. Both permeability and porosity are related. While porosity is an essential factor in determination of water saturation; permeability is necessary for the flow of oil through the reservoir rock. Several workers have

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documented the plugging of permeability through modification by bacteria to seal the zone of high permeability thereby increasing sweep efficiency in low permeability region. For example, the work of Stepp et al. (1996) showed that applying permeability modification treatments can significantly extend the productive lives of active oil recovery projects, curtails the prospect of premature abandonment, and effective modification of reservoir sweep that can improve the economics of an oil recovery process. Meehan et al. (1978) have also demonstrated that additional oil recovery is possible if the channelling water in a water flood can be immobilized by selective plugging process using the in-situ growth of bacteria.

Moreover, the rock porosity can be altered by microbial process especially in carbonate rocks by acids produced during microbial process; these acids can dissolve the bed-rock with carbonate matrix. Rock dissolution is one of the desired products of MEOR as it has ability to increase oil release when porosity is increased. Also, porosity is an important factor for bacteria adhesion and migration through porous rock for application of microbial enhanced oil recovery and also for accumulation of biomass that can physically dislodged oil from the reservoir.

Not only is porosity increased, significant amount of calcium ions can be released into the microbial solution during this microbial fluid-rock interaction as a consequence of dissolution (Udegbunam et al. 1991; Adkins et al. 1992b). Divalent cations such as Ca^{2+} can influence biofilm formation directly through their effect on electrostatic interactions and indirectly via a physiology-dependent attachment process by acting as an important cellular cation and enzyme co-factor (Fletcher, 1996; Marshall, 1985).

Despite large volumes of work on microbial enhanced oil recovery, very few literatures have documented the modification of carbonate rock during the process of microbial enhanced oil recovery. Therefore, it is relevant that an investigation of the effects of microbial treatment on rock modification be conducted. This can be very useful in design and screening of reservoirs for MEOR purposes. The aim of this study therefore is to investigate effect of microbial treatment on chalk samples using *Clostridium tyrobutyricum* strain and to evaluate the modification with time. Understanding of how bacterial treatment and their metabolic products affects reservoir rock properties will allow for good application of microorganisms for enhanced oil recovery.

2. MATERIALS AND METHODS

The chalk samples used in this investigation were obtained from the supplier (Dan-Chalks). These chalk samples were cut into identical cylindrical shapes of 2.0 cm length and 2.4 cm diameter. They were characterized by whitish, fine textured and average porosity of 40%. The chalk samples were pre-treated by drying in the oven for 72 hours at 90 ^{o}C. The method of modification involved immersion of the chalk samples in growth chamber filled with microbial solution at different salinity. The salinity range used in the experiment was 40 -100 g/l (4-10 % w/v). The growth chambers were 500 ml fermentation bottles, sterilized, with cork consisting of a hole for sample withdrawal. Sodium chloride salt of different concentration was dissolved in 250 ml of de-ionized water to provide the desired values of salinity. 20 ml of molasses was then added to each flask to serve as source of nutrient. The medium were inoculated each with 10 ml of a strain of *Clostridium tyrobutyricum* designated as 90F cultivated on reinforced clostridia media (RCM). This strain has already adapted to grow till salinity range of 100 g/l. Detail of the adaptation process is given in Rudyk and Søgaard (2009). The whole set up for main experiments and control experiments (without chalk samples) and blank experiments (chalk and salt solutions only) to check background values were incubated at 37 ^{o}C for 28 days under anaerobic condition. Growth in the media was indicated by increase in turbidity. The pH and electrical conductivity measurements were made every 7 days and the fluid samples analyzed for calcium ions concentration by induction couple plasma (ICP).

2.1 Porosity Determination

For the determination of porosity, rock samples were pre-treated and dried in oven, the initial porosity was later determined using the principle based on introduction of a fluid of known density into the rock samples. The weight of the saturated sample (W_s) and the dry weight of the sample (W_d) were determined. The pore volume (V_p) was calculated by dividing the difference in weight between the saturated sample and the dry sample by the fluid density. The fluid in this case was water. The total volume of the rock samples (V_b) was determined by measuring the difference in level of the saturating liquid before and after the immersion of the saturated rock

samples in a graduated cylinder containing the same saturating fluid at specified level. The porosity of the chalk samples and total volume were determined before and after microbial treatment.

2.2 Density and biomass determination

Calculation of the density of the rock samples before and after the microbial treatment was carried out using the dry density method. Densities were determined by weighing the samples after drying and dividing the mass by the total sample volume. In simple term, dry density (ρ_d) is equal to the dry mass (M_d) of the sample divided by the total volume (V) of the sample. Mathematically it can be expressed as:

$$\rho_D = \frac{M_D}{V} \tag{1}$$

For biomass accumulation (B) determination, dry weight of sample before immersion in bacteria solution (W_I) was subtracted from the dry weight after microbial treatment (W_F); the difference in weight gives the amount of biomass expressed as the dry weight in g/l. Samples were left in the oven overnight to dry complete at a temperature of 100 ^oC. The mathematical expression for biomass accumulation is given by:

$$B = W_F - W_I \tag{2}$$

3. RESULTS AND DISCUSSION

The experimental conditions and results before and after microbial treatment are given in Table 1 and 2.

Table 1: Result for porosity and weight before and after microbial treatment

Salinity (g/l)	Sample ID	ϕ_I (%)	ϕ_F (%)	$\Delta\phi$ (%)	(W_I) (g)	(W_F) (g)	B (g)
40	1	40.0	46.0	6.0	19.36	20.09	0.73
50	2	40.0	44.0	4.0	16.38	17.07	0.69
60	3	42.0	46.0	4.0	18.08	18.97	0.89
70	4	41.0	46.0	5.0	15.74	16.41	0.67
80	5	40.0	45.0	5.0	14.79	15.39	0.60
90	6	39.0	43.0	4.0	16.43	17.11	0.68
100	7	40.0	45.0	5.0	16.74	17.58	0.84

ϕ_I = initial porosity, ϕ_F =final porosity, W_I = initial weight, W_F = final weight, B= Biomass accumulation

Table 2: Result for total volume and density before and after microbial treatment

Salinity (g/l)	Sample ID	Initial V_{bi} (cm^3)	Final V_{bf} (cm^3)	Change in V_b (cm^3)	Initial Dens. (g/cm^3)	Final Dens. (g/cm^3)	Change in Dens. (g/cm^3)
40	1	8.80	8.59	0.21	2.20	2.33	0.13
50	2	8.75	8.59	0.16	1.87	1.99	0.12
60	3	9.30	9.00	0.30	1.94	2.09	0.15
70	4	7.90	7.69	0.21	1.99	2.14	0.15
80	5	6.78	6.43	0.35	2.18	2.43	0.25
90	6	7.92	7.49	0.45	2.07	2.28	0.21
100	7	8.93	8.59	0.34	1.87	2.05	0.18

V_{bi} = initial total volume, V_{bf} = final total volume, Dens. = density

From Table 1, it is evident that there is a change in porosity value of each rock sample after bacteria treatment. The average porosity increased from 40% to 44% by the end of experiment. The difference in average porosity before and after microbial treatment is 4% per sample. From the result, it is not indicated that change in porosity is related to salinity of the medium, rather it is directly related to the microbial activities, suggesting organic acid production and probably carbon dioxide formation both processes that leads to rapid dissolution of rock mass and subsequent increase in porosity. One of the major factors for feasibility of MEOR in reservoir rock is the transportation of bacteria. With increase in the porosity it can be assumed that more bacteria will be able to percolate the formation as the pore spaces becomes bigger. It has been shown that permeability in chalk depends on porosity and rock surface area (Mortensen et al. 1998; Rogen and Fabricius, 2002). Porosity increase can also result in increase of permeability, therefore reducing the formation resistance to bacteria movement and larger surface area to be exposed to bacteria treatment therefore enhance oil recovery by providing the required permeability to drain oil saturated low permeability rock matrix.

Moreover, increase in porosity can also have the advantage of internal pores which can be colonized by bacteria. The total volume of the rock samples was reduced after the treatment with bacteria culture; however the void ratio which is defined as the ratio of the volume of void space to the volume of solid in a given total volume (Hustrulid and Johnson, 1990) was increased. The increase void space can be filled with growing biomass, enabling bacteria adhesion to the rock through production of extracellular polymers as previously indicated by Huysman et al. (1983). Also, when there is large surface area to be colonized by bacteria, biofilm which provide a structural matrix for bacteria can develop on the porous rock and may lead to a change of surface properties and/or a decrease in permeability (Gandler, 2006).

When biomass accumulation is plotted against initial total volume (V_{bi}), there is a strong correlation between the two parameters as shown in Figure 1. The equation of the model is given by:

$$B = 0.12V_{bi} - 0.25 \quad (3)$$

where B is the biomass accumulation and V_{bi} is the initial total volume of the samples,

This suggests that the bigger the total volume of the rock the bigger the amount of biomass retained. The correlation between mentioned parameters can be calculated by equation (3) with R^2 = 0.95. Two of the samples deviating from the general trendline appear to have low biomass accumulation compare to others. These two samples were exposed to salinity 40 and 50 g/l. The possible explanation for this deviation could be that the strain of bacteria used in this study has been previously adapted to grow at high salinity (up to 100 g/l). Our previous observations showed that salinities lower than 70 g/l inhibit bacterial growth of this strain and therefore production of biomass that is low. In comparison, the sample at 100 g/l which is almost of the same volume with

the two deviating samples follows the general trend. Moreover none of the chalk samples experienced plugging as indicated by the increase of porosity, often, where microbial activity is involved, physical plugging by accumulated bacterial cells can be a source of concern.

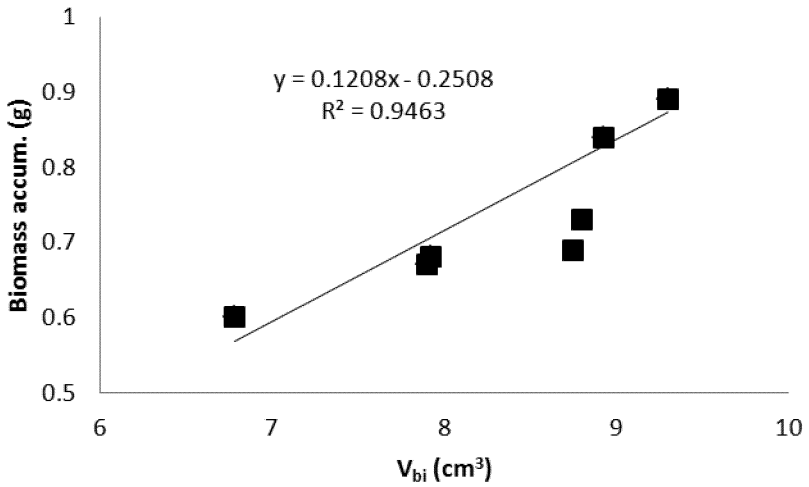

Figure 1: Biomass accumulation and total volume relationship for the chalk samples

Calculation of the density of the rock samples before and after the microbial treatment using the dry mass (Table 2) showed that there is increase in density of the rock samples as a result of amount of biomass retained on the rock samples. The pattern of the increase was not uniform, and the average increment for all the samples is about 0.17 g/cm^3. Difference in the pre-treatment and post treatment density and porosity can therefore arise only as a result of the modification effect of the bacterial medium on the chalk samples since no such effects were observed in the control experiment. It further confirms that bacteria penetrated or attached to the porous space of the chalk samples.

The bacteria solution at different salinity was analysed for calcium ion which is the main ion released into solution after dissolution of rock matrix of the samples. Figure 2 shows the plot of calcium ions in the microbial fluid over time at different salt concentration. The average concentrations at 7, 14, 21 and 28 days were 611, 822, 2100 and 3527 mg/l respectively.

This amount is significantly different when compared to the average value of 230 mg/l without chalk samples (salt solutions and bacteria media) and average background value of 50 mg/l for blank sample (chalk sample and salt solution at 90 g/l without bacteria media). Wagner et al. (1992) have observed similar phenomenon in the water samples pumped from reservoirs after microbial treatment of carbonate reservoirs indicating about 5 times increase in calcium ions concentration. With increase ionic strength of the media, the relationship observed did not show a clear correlation between salinity and amount of calcium ions released even though it appears that highest concentration of Ca^{2+} ions due to higher acidity was observed at highest salinity. The presence of divalent ions can possibly enhance adhesion of bacteria to the rock particles in the medium because of presence of electrical charge on bacteria cells. In microbial enhanced oil mechanism, migration of these adhered bacteria to other parts of the reservoir rock can induce mobilization of oil in the new location.

Figure 3 shows the change in pH with time, for salinity 40, 50, 60 and 70 g/l, the pH is increasing from day 7 to the end of 28 days, while for salinity 80, 90, and 100 g/l, the pH is decreasing from day 14 suggesting more acidic condition at high salinities.

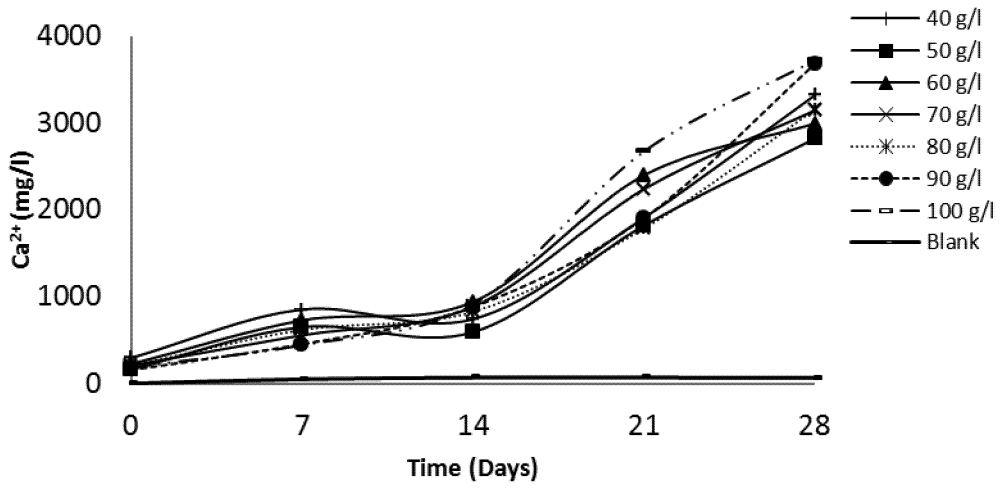

Figure 2:Ca^{2+} ions concentration variation with time at different salinity

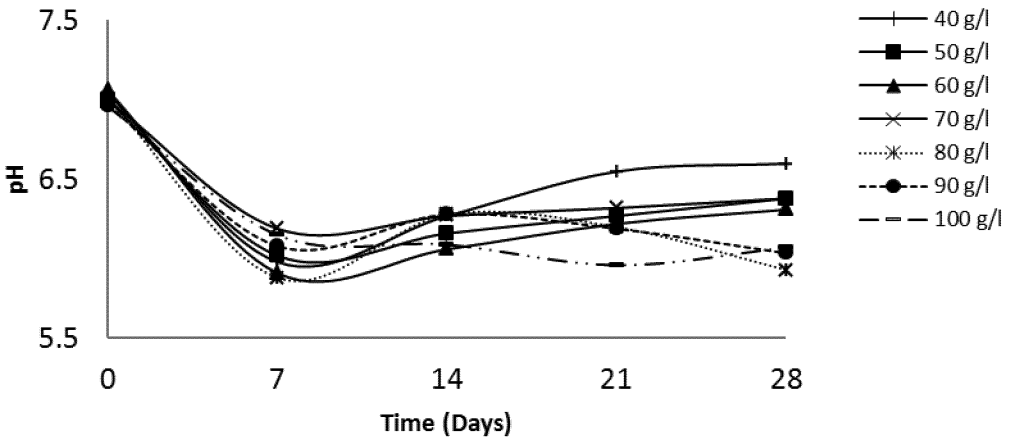

Figure 3: pH variation with time at different salinity

The release of calcium ions also has effect on the electrical conductivity of the media. The variation of electrical conductivity of the medium over time shows a slight increase when compared to the start value for each salinity measured (Figure 4). However the increase did not match the correlation between ions release and time suggesting a weak correlation between calcium ions and electrical conductivity. Electrical conductivity of solutions depends on the concentrations and the movement of charged particles in the solution. The bacteria culture is a heterogeneous and complex one that is constantly changing as seen from the pH variation. There is a direct correlation between conductivity measurements in relation to bacteria metabolism products (Parsons and Sturges, 1926). As it has been shown by Sierakowski and Leczyeka (1983), there is always some relationship between electrical conductivity and metabolic processes even though the direct cause for the alteration in electrical field strength may be obscure. In this present investigation, the electrical conductivity shows a weak correlation with calcium ions release. It is possible that the calcium ions in this media are not completely

dissociated and therefore their low influences on the conductivity of the bacteria media. This suggests that some of the calcium ions that would have responsible for significant increases in electrical conductivity of the solution are probably bound by the bacteria.

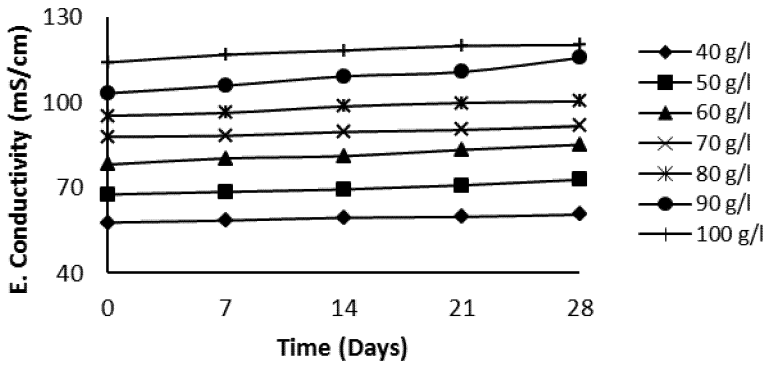

Figure 4: Electrical conductivity variation with time at different salinity

The dynamics of the rock dissolution and microbial activity can be inferred from the changes observed in the pH and the electrical conductivity measurement as they are indicative of the heterogeneous and constant changes taking place in the media. Increase in the pH of the media with time reflects the dissolution of the rock matrix into the medium and release of calcium ions as represented in equation (4) with formation of water soluble bicarbonates.

$$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2HCO_3^- \quad (4)$$

It is expected that as the concentration of HCO_3^- increases, the media will become more alkaline and the pH will increase. However, opposite effect was noticed at higher salinity (i.e. 80, 90, 100 g/l) from day 14 which shows lower pH values. An explanation for the observed difference can be attributed to effect of salinity on metabolism of bacteria that promotes more acid production and reduces the influence of HCO_3^- on pH. When compared with low salinity (40, 50, 60 and 70 g/l), the pH increases as the concentration of calcium increases. The negative correlation noticed between calcium ions and pH over time is thus an indication of increasing acidic environment with increasing salinity despite relatively higher concentration of calcium ions.

The dynamics of the media with chalk samples were compared with those of control experiments without chalk samples at different salinity for example as shown in Figure 5. It can be observed that the curve for 40 g/l (similarly for 50, 60 and 70 g/l) considered as low salinity is different from that of 100 g/l (similarly for 80 and 90 g/l) considered as high salinity by their orientation.

The result for all salinity measured when put together is shown in Figure 6. A strong correlation can be observed between the pH and calcium ion concentration with time. The equations show a negative correlation for different period of time and an almost equal gradient for dissolution of Ca^{2+} ions at every 7 days which is reflected in parallel and almost equal constants in the equations for each period. The system is limited with lines between 40 and 100 g/l showing low salinity group has increasing pH with increase calcium concentration while the high salinity group shows a decreasing pH with increase calcium concentration.

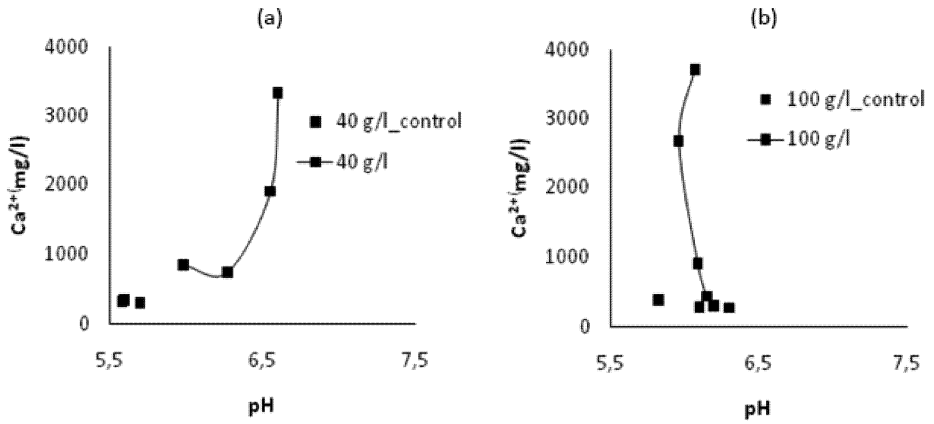

Figure 5: Comparison of media dynamics at (a) 40 g/l and (b) 100 g/l

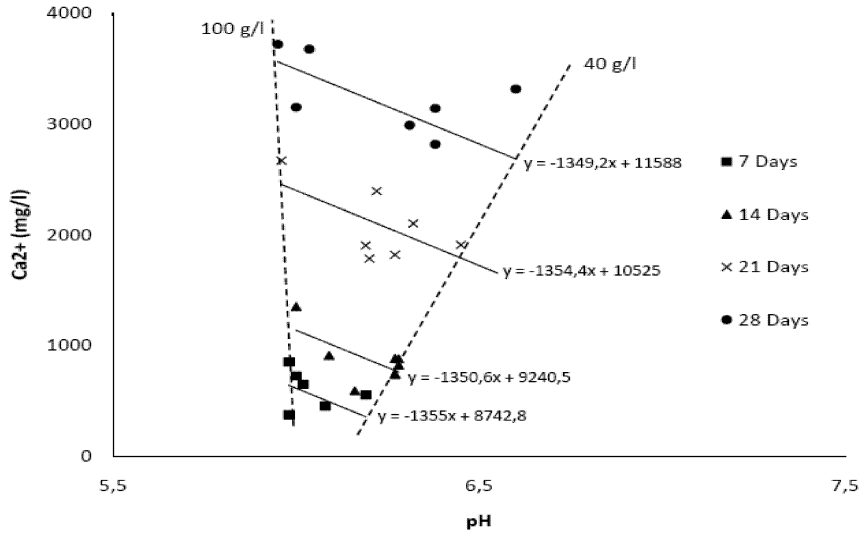

Figure 6: Overall dynamics of the media based on Ca^{2+} ions concentration and pH

Figure 7 below shows the 3D representation of this multivariate system representing all the measured salinity (40-100 g/l). As it can be observed from Figure 7, the plane angle of the salinity measured was delineated into two with a boundary between 70-80 g/l that separates the low salinity group (40, 50 60 and 70 g/l) and high salinity group (80, 90 and 100 g/l). The low salinity group when compared to high salinity group has an elevated plane surface that corresponds to higher pH while the high salinity group has a lower plane surface that corresponds to lower pH. The 3-D model thus confirms the deviation between the low salinity and high salinity group. This deviation is probably due to effect of salinity on bacteria metabolism resulting in different rate of metabolic process. Another possible explanation is that part of pH change might come from salinity and the resultant change in activity coefficients. The pH of aqueous sodium chloride was found to vary with increasing salinity due to different ionic strength (unpublished results) however with presence of microbes in the media this

observation might be different from a pure aqueous salt solution even though increase salinity can cause a decrease in bacteria population in the media.

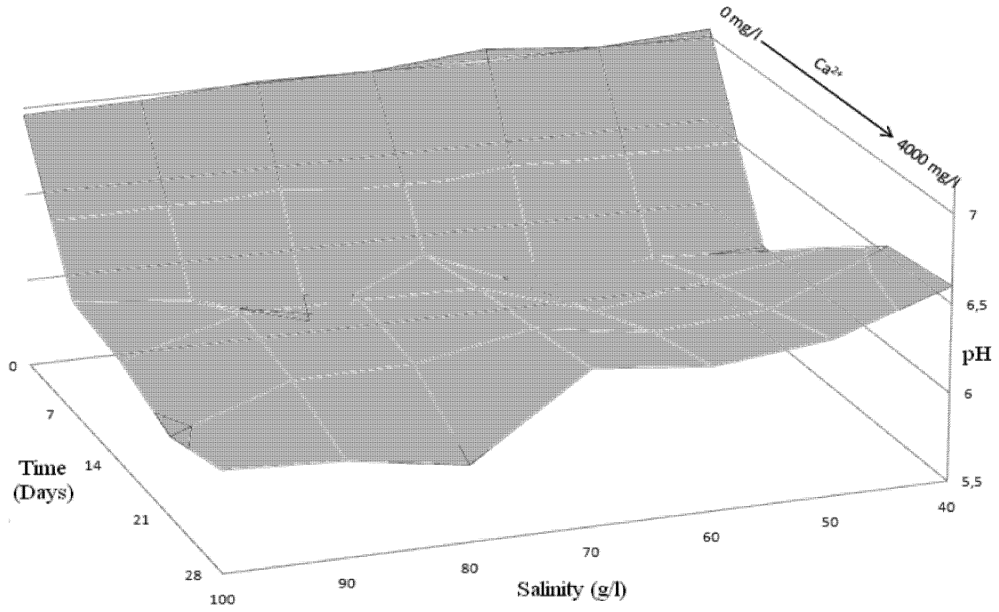

Figure 7: Surface representation of the media parameters

4. CONCLUSION

Successful application of microbial enhanced oil recovery requires a lot of understanding of not only the reservoir properties but also an understanding of the effect of microbial treatment on reservoir rocks in order to get a desirable result. The experiment with treatment of chalk samples with a media inoculated with a strain of *Clostridium tyrobutyricium* indicated microbial modifications. The porosity increased by an average of 4 % for the chalk samples and was mainly due to dissolution of rock matrix. The pattern of dissolution also leads to reduction in the total volume by an average of 3.57%. The mechanism of dissolution can be attributed probably to the organic acids and carbon dioxide produced during microbial metabolism. The densities of the chalks were also increased by an average of 0.17g/cm^3 an indication of that bacteria existed as attachment to the rock surface. Further results suggests that incongruent dissolution of chalk samples by microbial solution at different salinity enriched the solution with Ca^{2+} ions about 5 times more than the initial value. No clear correlation was however established between concentration of Ca^{2+} in the media and salinity. The highest concentration of Ca^{2+} ions due to higher acidity was observed at highest salinity and the 3-D surface plot was able to distinguish a possible partitioning of pH along two salinity groups. The effect on pH is clearly related to the concentration of Ca^{2+} ions in the media over time due to bacteria metabolism; however the electrical conductivity shows a weak correlation with calcium ions release suggesting that the calcium ions in this media are not completely dissociated. Since modification of rock properties during microbial process can be positively applicable in different forms; increasing the reservoir porosity can expose larger fraction of the reservoir to bacterial treatment causing a change in surface properties of the reservoir rock and ultimately enhancing oil recovery.

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