Microbial enhanced oil recovery

Jimoh, Ismaila Adetunji

Publication date:
2012

Document Version
Early version, also known as pre-print

MICROBIAL ENHANCED OIL RECOVERY

Ph.D. Thesis

Submitted by

Jimoh Ismaila Adetunji

To the Faculty of Engineering and Science, Aalborg University

For the Degree of Doctor of Philosophy

Supervisor: Assoc. Prof. Erik G. Søgaard

Co-supervisor: Assoc. Prof. Svetlana N. Rudyk

Department of Chemistry, Biotechnology and Environment

Section of Chemical Engineering

CIChem Research Group

Aalborg University Esbjerg

Niels Bohrs Vej 8, DK-6700

Esbjerg

August 2012

Abstract

This Ph.D. dissertation dealt with microbial enhanced oil recovery focusing on adaptation of bacteria to some of subsurface conditions and the mechanisms of enhanced oil recovery through the use of microbes and their metabolic products.

The core of this thesis consists of eight articles mostly dedicated to combination of different chemical and physical laboratory methods for experimentation, analysis and interpretation. These include flow injection, fermentation process, chromatographic assay, spectroscopy and image analysis to provide new, simple and robust solutions to understanding of processes during microbial enhanced oil recovery. A review of microbial enhanced oil recovery was presented to better comprehend the problem. The importance of mathematical models used in predicting the structural and morphological of bacteria cells during adaptation stages has been qualitatively discussed. The roles of biogenic acids and gases in carbonate rock dissolution and re-pressurization during microbial fluid rock interactions were also highlighted. The adapted bacteria strain were tested in different in formation waters from the North Sea and also, evaluated for improvement in oil recovery from packed columns by injection of bacteria solution to mimic in-situ oil recovery.

Paper I and II presents models that explain the relationships between environmental parameters of pH, electrical conductivity, salinity and gas dissolution based on simple empirical models. This is valuable for understanding some of the interactions in the subsurface during the enhance oil recovery. The measured salinity is similar to those found in oil reservoirs.

Paper III gives an overview of the adapted strain of *Clostridium tyrobutyricum.* The main objective of this study was to investigate the growth and metabolic products capability of this adapted strain and the potential to enhance oil recovery at elevated salinity. It was elaborated that quantities of some of the metabolites, gas, acids and biofilms have direct relationships with salinity of the medium and recovery of 38 % from sandstone and 25 % from chalk was achievable.

Paper IV-V highlighted the microbial fluid rock interactions. It was found that porosity increase observed in all the rock samples was mainly due to significant dissolution of carbonate by the organic acids produced during microbial metabolism. The patterns of dissolution lead to reduction in the bulk volume of the chalk samples. The pore volumes were slightly reduced or generally remain the same and the release of Ca^{2+} ions into microbial medium.

Papers VI present a contribution of a simple quantitative measurement technique for monitoring microbial gas production by titrimetric method. It was shown that about 84 % of the gas produced during fermentation process of molasses is composed of carbon dioxide and the absorption rate of the gas decreases with increase salinity. The method gave an indication of a reliable estimate of the concentration of dissolved CO_2 in the fluid sample can be made which can be applied to practical purposes.

Paper VII outlined a simple spectroscopic and image analysis technique for characterization/discrimination of pure and adapted strains of bacteria. It also showed that quantitative prediction of gas production and pH variation with salinity can be carried out based on image analysis and multivariate data modeling.

Paper VIII objective was to demonstrate the potential of thermophilic anaerobic bacteria; *Thermoanaerobacter brockii* subsp. *lactiethylicus* strain 9801^T in microbial enhanced oil recovery processes. The result suggested that the strain was capable of producing organic acids and gas that can modify rock properties evident in dissolution of chalk samples. Cells were also able to migrate through pore spaces of carbonate rock sample suggesting possibility of high mobility when injected for microbial enhanced oil recovery purpose. Degradation of alkanes by biosurfactant produced was also highlighted.

The significance of these contributions to the continued development of microbial enhanced oil recovery will hopefully find good use in the applied technological and industrial sectors.

Synopsis

Denne afhandling omhandler MEOR (*microbial enhanced oil recovery*) med særlig fokus på bakteries adaptation til underjordiske levebetingelser og mekanismer for en forbedret olieudvinding opnået igennem udnyttelse af diverse mikroorganismer og deres metaboliske produkter.

Afhandlingens kerne udgøres af otte videnskabelige artikler, der beskriver anvendelsen af diverse innovative kemiske og fysiske laboratorieanalysemetoder til undersøgelse og beskrivelse af den forbedrede mikrobielle olieudvinding. Formålet med anvendelsen af disse metoder, som omfatter *Flow Injection*, fermentation processer, kromatografiske og spektroskopiske undersøgelser og billedanalyse, var at skabe ny viden om rollen af bakterier i den mikrobielle olieudvinding. Afhandlingen starter med en gennemgang af litteraturen omkring emnet. Senere, bliver rollen af matematiske modeller beskrevet kvalitativt . Disse modeller vurderes uvurderlige for prædiktion af både morfologiske ændringer såvel som strukturændringer i de bakterielle celler. Afhandlingen lægger derudover fokus på rollen af biogene syrer og gasser i opløsning af karbonat bjergarter og i gendannelse af tryk under vekselvirkningen mellem et fluidum og en bestemt bjergart. Bakteriestemmerne, som blev brugt i dette projekt, kommer fra diverse oliereservoirer i Nordsøen, som hver især karakteriseres med unikke betingelser. Bakteriestemmernes evner til at forbedre olieudvindingen blev studeret in-situ i pakkede kolonner.

Artikler I and II i denne afhandling fastlægger sammenhængen imellem parametre som pH, ledningsevne, salinitet og opløselighed af gasser på basis af simple empiriske modeller. Denne viden er kritisk for forståelse af de underjordiske systemer, hvor olieudvindingen finder sted. De anvendte salinitet værdi afbilder salinitet værdier i oliereservoirer.

Artikel III præsenterer læseren med *Clostridium tyrobutyricum*, en bakteriestemme tilpasset til MEOR. Formålet med artiklen var at beskrive bakteriens vækst og metaboliske produkter samt at undersøge bakteriens potentiale for MEOR ved høje saliniet værdier. Resultaterne har vist at mængder af bestemte metaboliske stoffer, f.eks. gasser, syrer and biofilmer, kan relateres direkte til de tilstedeværende salinitet værdier. Derudover blev der fastlagt at udbytte af 38% og 25% kan opnås med hhv. sandsten og kalk som medium.

Artikler IV og V fokuserer på interaktioner mellem det tilstedeværende fluid og bjergarter. Ifølge resultaterne kunne der konstateres at prøvernes stigende porøsitet kan tilskrives opløsning af karbonater i metaboliske syrer og gasser. For kalk prøverne var dette forbundet med formindskelse af det samlede bulk volumen og frigørelse af calcium ioner.

Artikel VI præsenterer en simpel titrering metode til monitering af kuldioxid produktion. Det blev vist at 84% af gassen produceret under fermentering af melasse består af kuldioxid. Derudover tydede resultater på at kuldioxids absorption rate falder med stigende salinitet værdier. Disse konklusioner kan udnyttes til estimering af mængder af opløst kuldioxid i fluider.

Artikel VII beskriver en simpel og effektiv metode der kan skelne mellem tilpassede og ikke tilpassede bakteriestemmer. Resultaterne har også vist at kuldioxidproduktion og pH ændringer som funktion af salinitet værdier kan forudsiges ved hjælp af billedanalyse og multivariate data analyse.

Artikel VIII beskriver *Thermoanaerobacter brockii,* en termofil og anaerob bakteriestemme med et stort potentiale inden for MEOR. Resultater har vist at bakterien producerer organiske syrer og gasser som kan effektivt oplyse kalk. Celler af bakterierne har migreret igennem porer i prøverne, hvilket tyder på deres høj

mobilitet i forbindelse med en forøget olieudvinding. Analyseresultater har også vist at alkaner i olien bliver nedbrudt af overfladeaktive stoffer, som bliver fremstillet af bakterier.

Resultater af denne afhandling vil forhåbentlig bidrage til den fortsatte udvikling af MEOR og finde anvendelse i både den tekniske, såvel i den industrielle sektor.

Preface

This thesis is submitted in partial fulfillment of the requirement for the Ph.D. degree at the department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University, Denmark. The Ph.D. project was carried out under supervision of Associate Professor Erik G. Søgaard and co-supervisor Associate Professor Svetlana N.Rudyk from CIChem research group, Section for Chemical Engineering, Department of Biotechnology, Chemistry and Environmental Engineering, Aalborg University Esbjerg.

The Ph.D. project was conducted within the field of enhanced oil recovery with the main focus on microbial enhanced oil recovery (MEOR). The project draws on different aspects to form the basis to describe the MEOR process in biogeochemical sense. In this thesis, the MEOR process is placed in the context of tertiary enhanced oil recovery which is the stage after primary and secondary enhanced oil recovery processes. MEOR as a technology tool for enhanced oil recovery is gaining momentum across the world because of its low capital cost and multiple oil recovery mechanisms available. However, there are still several areas which are still not resolved or adequately clarified by MEOR process and are often cited as major concerns by the oil industry when MEOR issues are discussed.

The challenges presented by this project are met through standard experimental approaches both quantitative and qualitative in nature. The project started with strain of *Clostridium tyrobutyricum* (DSMZ 663) purchased from the German Culture Collection (DSMZ) and during the course of this project, new strains of bacteria were developed with better abilities to withstand difficult reservoir conditions such as high salinity and high temperature which are often cited as some of the limitations for MEOR process. Studies also focused on assessment of metabolites production by the adapted strain to evaluate its potentials for production of the desired bio-products needed for enhancement of oil recovery through series of experiments such as biogenic gas production, activity at oil-water interface, modification of rocks, adhesion processes and rheological effects etc.

Also attempt was made to model some of the mechanisms for enhanced oil recovery during MEOR process. These models were empirical, but simple enough to describe some of the basic relationships between different environmental parameters. Also an image analysis technique was able to quantify morphological and physiological changes during adaptation process. Each paper has followed the guidelines and reference format for intended journal.

Finally, this project seeks to contribute to our understanding of the complex processes involved in MEOR. In addition, it is expected that new developments in MEOR will add even more advantages to the present technology and strengthen this approach for successful application in residual oil recovery that can increase our reserves for energy demand.

Acknowledgement

First of all, I wish to give glory to the Almighty, who has given me good health, strength and wisdom in the past decades and the grace to complete this project. During the course of this study, a number of people have provided help and support that have added value in no small amount to the completion of this work, to which I am very grateful. First of all, I would like to thank the Aalborg University for providing me the opportunity to carry out this Ph.D. research. It has been a wonderful technical and scientific experience that will forever remain in heart.

Most importantly, I would like to thank my supervisor Assoc. Erik Gydesen Søgaard and co-supervisor Assoc. Prof. Svetlana N. Rudyk. The dissertation you are holding at hand would not be possible at all were it not for their ebullient, enjoyable and inspiring instructions throughout my Ph.D. work.

A warm thank you to Assoc. Prof. Sergey Kucheryavskiy for kind assistance in experimental and data interpretation and also to Krzysztof Kowalski and Kazia Arturi for scientific contributions and discussions and all the jokes that took away the stress when my 'brain hurts'. It was a nice time being with you guys.

Moreover, many thanks to Daniel Ankrah, Morten Simonsen, Camilla Sønderby, Heidi Thomsen, Rudi Nielsen, Jens Muff, Pavel Spirov and the other members of CICHEM for scientific discussions and collaborations plus social activities that makes my stay at the university a pleasant one. I also acknowledge the help of the staff in the laboratories; Dorte Spansberg, Linda, Vivi, Henry, Miloz, for invaluable help with analytical and experimental work. I seized this opportunity to say thank you to Julaine Enas for many useful discussion and advice during the course of this project. I would also like to thank my dear colleagues at the Aalborg University Esbjerg for providing a fun and stimulating research environment.

To my friends Stephen Njoku, , Kyrian Okoroama, Seyi Odebode, Awele Nwaobi , Austin Ehime and all others guys that we weathered the cold storm in Esbjerg together, I say thank you all. To my folks and colleagues in Nigeria especially Wasiu Makinde for kind support and assistance at all times.

Finally my gratitude goes to my family, whose steadfast support, love, advice and encouragement have been invaluable since my childhood days and during my entire stay abroad, especially my mum and dad, Alhaji and Mrs. Y.A Jimoh and to my dear ones Yusrat, Faiz, Sumayat and Mariam. My special gratitude goes to my fiancée Georgiana Lina for her kind support, love and patience. I owed you all a lot and I say thank you for being there for me.

List of supporting papers and conference presentations

Papers

1. Jimoh I.A., Søgaard E.G., and S. N. Rudyk, 2012. Dissolution of carbon dioxide in aqueous sodium chloride solutions and its relationships to salinity, pH and electrical conductivity properties (Submitted, 2012).

2. Jimoh I.A., Søgaard E.G., and S. N. Rudyk, 2012. Laboratory investigation of pH, electrical conductivity and temperature relationships in aqueous high salinity sodium chloride solutions (Submitted, 2012).

3. Jimoh I.A., Søgaard, E.G and Rudyk, S.N., 2012. Microbial Enhanced Oil Recovery-Laboratory Experiments with Salinity Adapted Strain of *Clostridium tyrobutyricum* (Submitted 2012).

4. Enas J.T., Jimoh I.A., Søgaard E.G., and Rudyk S.N., 2012. Laboratory investigation of *Thermoanaerobacter brockii* subsp. *lactiethylicus* strain 9801^T for possible utilization in microbial enhanced oil recovery. J. Petroleum and Environmental Biotechnology 3:125. doi:10.4172/2157-7463.1000125.

5. Jimoh I.A., Søgaard, E.G and Rudyk, S.N. and Kucheryavskiy S., 2012. Spectroscopy and image analysis characterization of adapted strains of Clostridium tyrobutyricum for microbial enhanced oil recovery purposes. GSTF Journal of Biosciences

6. Jimoh I.A., Søgaard, E.G and Rudyk, S.N., 2012. Evaluation of produced volumes of carbon dioxide from the concentration of the gas absorbed in media during microbial fermentation for enhanced oil recovery purposes. Chemical Eng. Transactions, 27: 97-102.

7. Jimoh I.A., Søgaard, E.G and Rudyk, S.N., 2011. Microbial modification of chalk samples and implications for enhanced oil recovery. AIDIC Conference Series Pierruci, S. (Ed.). 10: 169-178.

8. Jimoh I.A., Søgaard, E.G and Rudyk, S.N., 2011. Microbial fluid-rock interactions in chalk samples and salinity factor in divalent Ca^{2+} ions release for microbial enhanced oil recovery purposes. Chemical Eng. Transactions, 24:889-894.

Conferences- oral presentations

1. Jimoh I.A., Søgaard E.G. and Rudyk S.N., 2010: Laboratory Experiments with a Strain of *Clostridium Tyrobutyricum*, European Student Conference on Microbial Communication 30 September 2010 Jena, Germany.

2. Jimoh I.A., Søgaard, E.G. and Rudyk S.N., 2010: Effect of Microbial Treatment on Chalk Samples, Reservoir Microbiology Forum, 30 1 December 2010, Energy Institute London.

3. Jimoh I.A., Søgaard, E.G. and Rudyk, S.N., 2011: Microbial Enhanced Oil Recovery: A Technology Tool for Sustainable Development of Residual Oil, Trends and Future of Sustainable Development Conference, 10 June 2011, Tampere, Finland.

4. Jimoh I.A., Sogaard E.G., Rudyk, S.N. and Kucheryavskiy S., 2012: Morphological and Physiological Changes during Adaptation to Higher Salinities of *Clostridium tyrobutyricum* for Microbial Enhanced Oil Recovery Purposes. 3rd World Annual Congress on Petromicrobiology, April 25-27, Xian, China.

5. Jimoh I.A., Søgaard, E.G. and Rudyk S.N., 2012. Reservoir Evaluation and Saturation Monitoring using RST Fluid Analysis for Improve Oil Recovery, EAGE Conference June 23-27, 2012, Copenhagen,Denmark.

Table of contents

Abstract ii

Synopsis iii

Preface v

Acknowledgements viii

List of supporting papers and conference presentations ix

Table of contents 1
Introduction 2

1.1 Background 2
1.2 The research objectives and scope of investigation 3
1.3 Thesis structure and content 4

2 Review of microbial enhanced oil recovery 7
2.1 General statement 7
2.2 Classification, mechanisms and constraints of MEOR 10
2.2.1 Classification of MEOR 10
2.2.2 The mechanisms of MEOR 11
2.2.3 MEOR constraints 15
2.3 Microbe selection for MEOR 18
2.4 Cases of MEOR 20

3 Paper I-VIII 29
Paper I 31-46
Paper II 48-67
Paper III 69-91
Paper IV 93-100
Paper V 102-115
Paper VI 117-125
Paper VII 127-137
Paper VIII 139-166

4 Conclusion and major findings 167
4.1 Conclusion ` 167
4.2 Major findings 169

5 Future perspectives 170

1 Introduction

1.1 Background

At present oil productions have been experiencing decline in many parts of the world due to oilfields maturity and example of such includes the major oilfields in the North Sea (Aleklett et al. 2010). Another major concern is the increase energy demand due to global population growth and mounting difficulty in discovering new oilfields. Therefore, there is a need to find alternative technologies to improve oil recovery from existing oilfields around the world. It is a fact that fossil fuels will still remain the key supply of energy source for many years to come despite the huge investments in other sources of energy such as biofuels, solar energy, wind energy etc. This fact is highlighted by the current global energy production from fossil fuels which currently stand at about 80-90% with oil and gas representing about 60 % (Graus et al. 2011).

During oil production, primary oil recovery can account for between 30-40 % oil productions, while additional 15-25% can be recovered by secondary methods such as water injection leaving behind about 35-55 % of oil as residual oil in the reservoirs (Cosse, 1993). This residual oil is usually the target of many enhanced oil recovery technologies and it amounts to about 2-4 trillion barrels (Hall et al., 2003) or about 67 % of the total oil reserves, (Bryant et al. 1993). Recovery of this residual oil is at present a big challenge for many oil companies and there is a continuous search for a cheap and efficient technology that can help in its recovery. Additional recovery from residual oil can lead to increase in global oil production as well as prolonging the productive life of many oilfields. The techniques employed for recovery of this residual oil are generally termed enhanced oil recovery (EOR) methods.

EOR methods are used in oil industry to increase the ultimate recovery of crude oil. This normally involves application of an EOR method (sometimes called tertiary recovery method) to a specific underground oil bearing reservoir. Examples of well-known tertiary recovery methods are chemical flooding, miscible CO_2 injection and thermally enhanced oil recovery that uses heat as main source of additional oil recovery (Lake, 1989). Significant volumes of residual oil in the depleted oil reservoirs could be produced by these EOR methods as current technology leaves about two third of the original oil in place in the reservoir. One of such method is microbial enhanced oil recovery (MEOR) which is the focus of this project. MEOR represents the use of microorganisms to extract the remaining oil from the reservoirs. This technology can be potentially implemented with an exceptionally low operating cost and also said to be capable of producing up to 50% of the residual oil (Lazar et al. 2007; Sen, 2008). The field trials have shown that normal projected oil production decline curve can be reversed or level off by MEOR and the reason is

because microbial growth and metabolites produced can have effects on the chemical and physical properties of reservoir rocks and crude oil (Hitzman, 1991).

1.2 The research objectives and scope of investigation

MEOR technology is composed of different mechanisms, consequently, the target of this study will be to investigate several aspects of MEOR in order to better our understanding of those factors that limit performance/or can enhance performance in the area of microbial enhanced oil recovery wherein referred to as the challenges in MEOR. The rationale is that, fundamental understanding of structure and function in microbial community and its linkage to biogeochemistry in reservoirs and understanding of mechanisms in MEOR environmental, microbial and physiological process is a prerequisite to developing microbial methods to enhance the quantity and quality of the HC recovery. Therefore, the scope of this project covered two main aspects namely;

(a). Microbiology in deep subsurface

(b). MEOR processes

The overall aim of this project is to come out with some answers by which microbial activity in hydrocarbon reservoir can be modified, improved or introduced to benefit oil recovery. Some of the project objectives include:

- Review of relevant literature available on microbial enhanced oil recovery to keep abreast with current knowledge and development
- Engineering of new strains of bacteria by proper adaptation to high salinity, temperature and pressure conditions to meet reservoir conditions in the North Sea oilfields
- Investigation of microbial fluid effect on rock properties
- Identification of good nutrient for the microbes for quick stimulation under reservoir conditions
- Investigation of oil releasing mechanisms /oil recovery process during MEOR
- Modelling of MEOR activities to develop predictive models that can enhance successful implementations
- Possible application or field test of the knowledge gain from the studies and the findings on field scale for enhanced oil recovery

Achievement of this research was based on intensive laboratory investigations and experiments, modelling, and statistical analyses among others; to engineer good microbes for MEOR and understand the metabolic and biogeochemical processes that are going on during microbial fermentation, interactions with chalk

samples and oil recovery processes. These investigations became necessary to shed more lights on MEOR mechanisms and improve forecasting abilities to enhance oil recovery in carbonate rocks which can later be transformed to field scale applications especially in the Danish sector of the North Sea and similar geologic formations.

The recent oil decline in the Danish sector of the North Sea and the need to identify the most plausible technology to enhance oil recovery make this research an ideal one. This is because most of the reservoirs rocks in the Danish sector are made up of carbonate rocks and MEOR has been postulated to be more effective in carbonate reservoirs (Wagner, 1991). In Danish sector of the North Sea, oilfields were estimated to have recoverable hydrocarbon reserves of 240 million m^3 of oil and 120 billion m^3 of gas at January 1 2007 (Danish Energy Agency, 2006). According to this report, the total production of oil and natural gas in Denmark will exceed consumption up to and including 2015 and the remaining reserves in these fields are estimated to provide oil self-sufficient to 2035. In the future, additional recovery from these reserves demands the use of innovative ideas for improving recovery methods to sustain production. For example in Denmark, an increase of just 1% in the oil recovery from Danish fields would be sufficient to cover the country's demand for two years (Danish Energy Agency, 2006). Therefore, enhanced oil recovery methods such as microbial enhanced oil recovery can provide alternative and optimum sources of recovering these remaining hydrocarbons.

1.3 Thesis structure and content

The present thesis is organized as 3 submitted articles and 5 published articles in different journals together with a review of microbial enhanced oil recovery and extended summary and future perspectives on the research conducted in this Ph.D. study. The introduction part (Chapter 1) provides brief information on the need for enhanced oil recovery including the objectives and scope of the current thesis. Chapter 2 covers the review on microbial enhanced oil recovery that gave an overview of microbial enhanced oil recovery development from early stages, the mechanism and examples of applications over the past 40 years. Chapter 3 covers the published and submitted articles that cover different aspect of microbial enhanced oil recovery such as adaptation, MEOR mechanisms, metabolites production, microbial modification of rock, gas production and evaluation, oil recovery from packed columns among others. Chapter 4 is the conclusion and highlights the most significant results achieved and relates the findings to the current state of the art. It is mainly based on the findings and results from the articles listed in chapter 3 but presented with an extensive knowledge of the microbial enhanced oil recovery and it also incorporates additional

unpublished data from laboratory studies performed as part of this thesis. The last chapter (Chapter 5) provides the future perspectives on microbial enhanced oil recovery based on the outcome of this study.

References

1. Aleklett, A., Höök, M., Jakobsson, K., Lardelli, M., Snowden S., Söderbergh, B., 2010. The peak of oil age –analyzing the world oil production scenario in world energy outlook 2008. EnergyPolicy, 38 (3): 1398-1414.

2. Bryant R.S., Stepp, A.K., Bertus, K.M., Burchfield, T.E., and Dennis, M., 1993. Microbial enhanced waterflooding field pilots. Dev. Pet. Sci. 39:289-306.

3. Cosse, R., 1993. Basics of reservoir engineering, editions technip, Paris, 343pp.

4. Danish Energy Agency, 2006. Facts about Danish oil and gas production 2006. Danish Energy Agency (DEA), http://www.ens.dk viewed on 12-08-2009

5. Graus W., Roglieri, M.,, Jaworski, P., Alberio L., and Worrell, E., 2011. The promise of carbon capture and storage: evaluating the capture-readiness of new EU fossil fuel power plants. Climate Policy 11:798-812.

6. Hall, C.,Tharakan, P., Hallock, J., Cleveland, C., and Jefferson, M., 2003. Hydrocarbons and the evolution of human culture. Nature, 426:318-322.

7. Hitzman, D.O., 1991. Microbial enhanced oil recovery-the time is now. In: Microbial enhancement of oil recovery-recent advances, Donaldson E.C. (Ed.), Elsevier, Amsterdam.

8. Lake, L. W., 1989. Enhanced Oil Recovery, Prentice Hall, Englewood Cliffs New Jersey,

9. Lazar, I. Petrisor, I.G., and Yen, T.F., 2007. Microbial enhanced oil recovery, Petroleum Science and Technology, 25: 11, 1353-1366.

10. Sen, R., 2008. Biotechnology in petroleum recovery: the microbial EOR . Progress in energy and combustion Science, 34 (6): 714-726.

11. Wagner, M., 1991: Microbial enhancement of oil recovery from carbonate reservoirs with complex formation characteristics. Dev. Pet. Sci. 31, 387-398.

1 Review of microbial enhanced oil recovery

2.1 General statement

Microbial enhanced oil recovery is a collection of techniques that utilizes microorganisms and their metabolic products to improve the recovery of crude oil from reservoir rock (Yen, 1990, Zhang and Xiang, 2010, Lazar, 2007). The application can be either in a form of cyclic (single well simulation), microbial flooding recovery or selective plugging recovery (Lazar, 2007). The idea of microbial enhanced oil recovery was first proposed by Beckmann (1926) when he published results on the possibility to use microbial metabolic processes to improve the oil production rate. In the later parts of the 1940's experiments from Zobell (1947) further indicated the potential for microbial oil recovery from sand grains. The study highlighted the similarity between the compounds used to improve waterflood efficiency, in chemical and miscible EOR processes and the products of microbial fermentation of carbohydrates even though there was a setback due to hydrogen sulphide production.

Gases, solvents, surface active compounds, polymers, organic acids and biomass are all regular and predictable products of microbial metabolism similar to compounds used in chemical enhanced oil recovery (Sheehy, 1991). Microbial enhanced oil recovery in general has many advantages, such as economical, low toxicity, biodegradability and biocompatibility, selectivity and specificity (Desai and Banat, 1997). MEOR therefore, offers good alternative in improving the recovery of crude oil from reservoir utilizing microorganisms and their metabolic products.

From the classical works of Beckmann (1926) and ZoeBell (1947), it was a giant leap to the 1950s through 1980s with other scientists reporting advances made in MEOR (Updegraff and Wren, 1954; Davis and Updegraff, 1954; Kuznetsov, 1961; Kuznetov et al. 1962; Senyukov et al. 1970; Lazar, 1978, Ivanov et al. 1982, Zajic et al. 1983; Belyaev 1983; Bubela, 1983, Yarbrough and Coty, 1983, Grula et al. 1983; Donaldson and Grula, 1985). Further researches were carried out in the 1990s through 2000s with renewed significant interests (Lazar, 1991, Ivanov et al. 1993; Hitzman, and Sperl, 1994, McInerney and Sublette 1997; Bryant and Lockhart, 2002; Li et al. 2002; Maudgalya et al.2005). A parallel development was the rise in crude oil prices due to the petroleum crisis in the 1970s that boosted development of MEOR research and validated it to scientific enhanced oil recovery method (Lazar et al. 2007).

Hitzman (1988) published a review on MEOR field testing and also a review of many field applications of MEOR was presented by Bryant et al. (1989). Extensive body of scientific and technical understanding of the microbial enhanced oil recovery was presented by Donaldson et al. (1989). Additionally, significant

contributions to MEOR area were published in the proceedings of the international conference on MEOR, Donaldson (1991) and Lazar review papers (Lazar 1991; Lazar, 1998).

The continuum search for a cheaper and effective enhanced oil recovery method was a major driving force behind the development of microbial technique of enhanced oil recovery. The advances that were made in the 1950s through 2000s came in a large part, from a great deal of work looking at how microorganisms can benefit the recovery of oil from petroleum reservoirs. Many of the results from the laboratory studies were promising. The laboratory study of specific microorganism is done either for the surface production of various compounds or for the injection of cells into a reservoir for in situ production of metabolic compounds. These laboratory studies on MEOR have normally utilized core samples and columns containing the desired substrates. These substrates were employed to demonstrate the usefulness of biosurfactants in oil recovery from sandstone and carbonate. Similarly, core samples were used as model in the movement of microorganisms and nutrients through substrates to ascertain their usefulness after injection into oil reservoirs (Banat, 1995).

However, the results from field applications were mixed; from success to failure, because the biological, chemical and physical processes that occur in petroleum reservoirs where in situ metabolism occurs were not fully understood (Donaldson et al. 1991). As observed by Hitzman (1991), several reasons can be considered for the reported differences between laboratory results and field observation in MEOR studies. One of the important factors is the dynamic environment normally encountered in a reservoir which is difficult to duplicate or simulate in the laboratory with small cores and reactors. Physical and chemical changes also occur within the reservoir as a result of interactions of the multiplying microorganisms with the reservoir matrix that cannot be duplicated in the laboratory. Another major reason identified for the failure of field trials is insufficient consideration of the conditions which characterize petroleum reservoirs (Sheehy, 1991). He observed that the activity of bacteria in reservoirs depends on the physical and chemical conditions they encountered. These include pH, temperature, salinity, pressure, ionic strength, source of energy and nutrients. Moreover lack of adequate knowledge about growth of microorganisms in oil under anaerobic condition during the early days of MEOR was a major factor. It was not until recently that bacteria have been shown conclusively to metabolize hydrocarbons in oil under anaerobic environment (Kropp et al. 2000). However, it was suggested some of the perceived technical problems associated with MEOR applications can be overcome by carefully planning (Moses, 1991; Maudgalya, 2007).
In spite of some of the earlier setbacks, microbial enhanced oil recovery has developed rapidly over the past two or three decades around the world. From USA to Russia, Europe to China and Canada to Australia

China, several studies were carried out in different applications of MEOR, for example, Dienes and Yaranyi, 1973; Senyukov et al. 1970; Karaskiewicz, 1975; Lazar, 1978, Yarbrough and Coty, 1983; Hitzman, 1988; Wagner, 1991; Sheehy, 1991, 1992; Ivanov et al. 1993; Wang et al. 1993; He et al. 2000; Bryant and Lockhart, 2002; Li et al. 2002;). The first field trial was carried out in the Lisbon field, Union County, AR, in 1954 (Yarbrough and Coty, 1983). The field tests from many of these studies specified injection of mixed anaerobic or facultative anaerobic bacteria typically consisting of *Clostridium, Bacillus, Pseudomonas, Micrococcus, Mycobacterium, Arthrobacterium, Peptococcus* etc. with nutrients. Example of such nutrient is molasses, considered to be very cheap. The idea behind the selection of these microorganisms is based on their ability to generate high quantities of gases (e.g. CH_4, H_2, CO_2, and N_2), organic acids (e.g. butyric and acetic acids), solvents (e.g. acetone, butanol and ethanol), polymers (e.g. polysaccharides), biosurfactants and cell-biomass. Each mechanisms or a combination of these mechanisms could lead to increased oil recovery (McInerney et al. 2002).

Research carried out in 1970-2000 as illustrated in the study by Lazar et al. (2007) and more recently by Brown (2010), has established the basic nature and existence of indigenous microbiota in oil reservoirs, as well as reservoir characteristics being essential to a successful MEOR application. At the moment, research into MEOR is still continuing which can be said to be the fourth generation of studies. This is buoyed by the combined effects of increasing matured oilfields and increased oil prices and also the need to increase our understanding of MEOR processes; as many of the earlier studies identified the need to improve critical information on mechanisms, metabolic rates and required concentrations of microbial products. Some of the most recent works include those of Brown et al. 2002, Bryant and Lockhart, 2002, Maudgalya et al. 2005; Kowalewski et al. 2006, Kaster et al. 2009, Rudyk and Søgaard, 2011; Jimoh et al. 2011 and several other studies. All these were attempts to bridge the gap in laboratory success and the field applications of MEOR.

Study showed that among other things, there is an improvement in the availability of methods and analytical equipment. Also new strains of bacteria have been identified and isolated from deep seated reservoirs that have abilities to grow in extreme conditions of salinities and temperatures. Examples of such new strains of bacteria identified include thermoanerobic bacteria such as *Thermoanerobacter brockii* subsp. *lactiethylicus* strain 9801^T isolated from a deep subsurface French oil well at a depth of 2100 m where the temperature was 92 ^oC and optimum growth at temperatures between 55 and 60 ^oC (Cayol et al. 1995) and *Thermoanaerobacter tengcongensis* strain $MB4^T$ isolated from a Chinese hot spring capable of growth at temperatures between 50 and 80 ^oC (Xue et al.2001).

Moreover, the area of modeling of MEOR is improving. It was recognized that a mathematical model could be used to recognize the most important parameters and their practical relationships for the application of MEOR (Marshall, 2008). However, development of detailed mathematical models for MEOR is an extremely challenging task, not only as a consequence of the natural difficulty of the microbes, but also because of the diversity of physical and chemical variables that control bacteria activities in subsurface porous media. Modeling microbial has developed from the earlier work of Monod which modeled the bacteria growth (Monod 1949) to several mathematical models that were developed to stimulate MEOR processes. Examples include models for multidimensional flow of the multiphase fluid consisting of water and oil in porous media along with specific equations for adsorption and adsorption and diffusion of metabolites, microorganisms and nutrients (Chang et al. 1991; Islam, 1990), models for relative permeability changes (Al-Wahaibi et al. 2006; Nielson, et al. 2010) and models that incorporates salinity effects adsorption of microorganisms reduction of interfacial tension and wettability changes (Behesht et al. 2008).

In conclusion, there is absolute no question as to whether microorganisms have the capability of enhancing oil recovery by virtue of some of the products they can produce (Brown, 2010) but rather how to employ this ability in an economically, practical and scientifically valid manner, transferable from laboratory scale to large scale field applications. More research is required in this field and it is believe that by doing this, MEOR as part of the tertiary enhanced oil recovery methods could substantially increase the world's supply of oil.

2.2 Classification, mechanisms and constrains of MEOR

2.2. 1 Classification of MEOR

The objective of most microbial enhanced oil recovery is to reduce remaining oil in the reservoir however the implementation of the MEOR strategy can be different. Nevertheless, two major strategies are normally employed in MEOR. The first one is the bacteria injection normally referred to as 'traditional' MEOR method with nutrients and the second method involves simulation of indigenous bacteria through injection of nutrients. The application of MEOR technology can either be in the form of a cyclic (single well simulation), microbial flooding recovery or selective plugging recovery (Lazar, 2007; Zhang and Zeng, 2010).

In cyclic microbial recovery, microorganisms and nutrients are injected into production wells. The wells are shut-in for a period long enough to allow microbial growth and metabolites formation. This can be for a

number of days or weeks. Finally, the oil production phase begins and extends over a period of weeks or months. In cyclic microbial recovery, when production declines, another phase of injection is normally started. In this case, the depth of the area covered by bacteria would be limited by the injection rate and the kinetics of the microbial process (Bryant and Lockhart, 2002).

The second type of application is microbial flooding. In microbial flooding, the microbial growth is usually stimulated by adding nutrients to the injection water to encourage the proliferation of microorganisms indigenous to the formation. If the requisite microbial activity is not present, then microorganisms can be injected into the formation along with the nutrients. In some approaches injection into the formation is stopped to allow time for the in situ growth and metabolism to occur (Youssef et al. 2009). In other approaches, injection of brine continued after nutrient and/or cell injection. This option would most likely be less expensive as the growth would be stimulated in larger parts of the reservoir, particularly where the carbon source (residual oil) is located, which is usually the target of the enhanced oil recovery treatment (Kaster, 2012).

The microbial selective plugging encompasses microbial process to divert water into low permeability regions to block water channels deep in the reservoirs. With this type of treatment, nutrient preferentially flow into the high permeability regions, which then stimulates biomass and polymer production in these regions; both of which reduce the permeability of the rock (Raiders et al. 1985). In contrast, heavy oil modification is usually by microbial decomposition of long chain compounds within the formation.

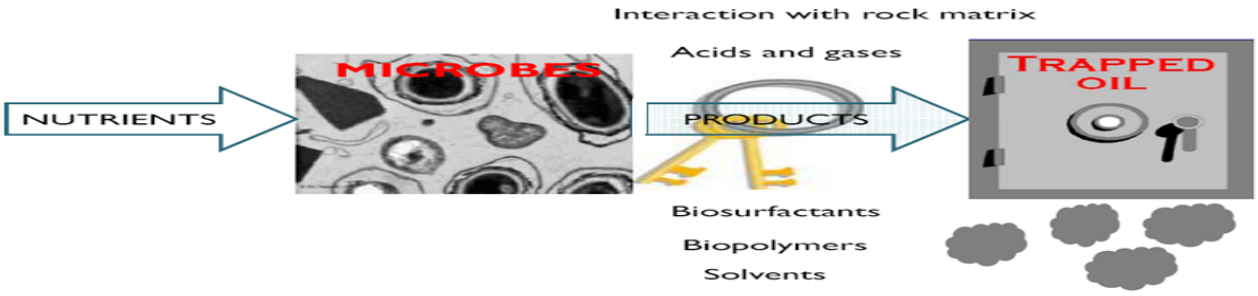


Figure 1: Basic process in microbial enhanced oil recovery schematic

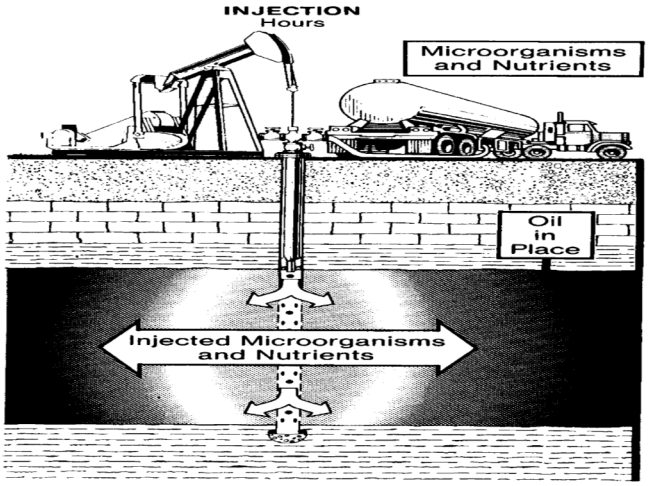


Figure 2: Cyclic microbial oil recovery (www.netl.doe.gov)

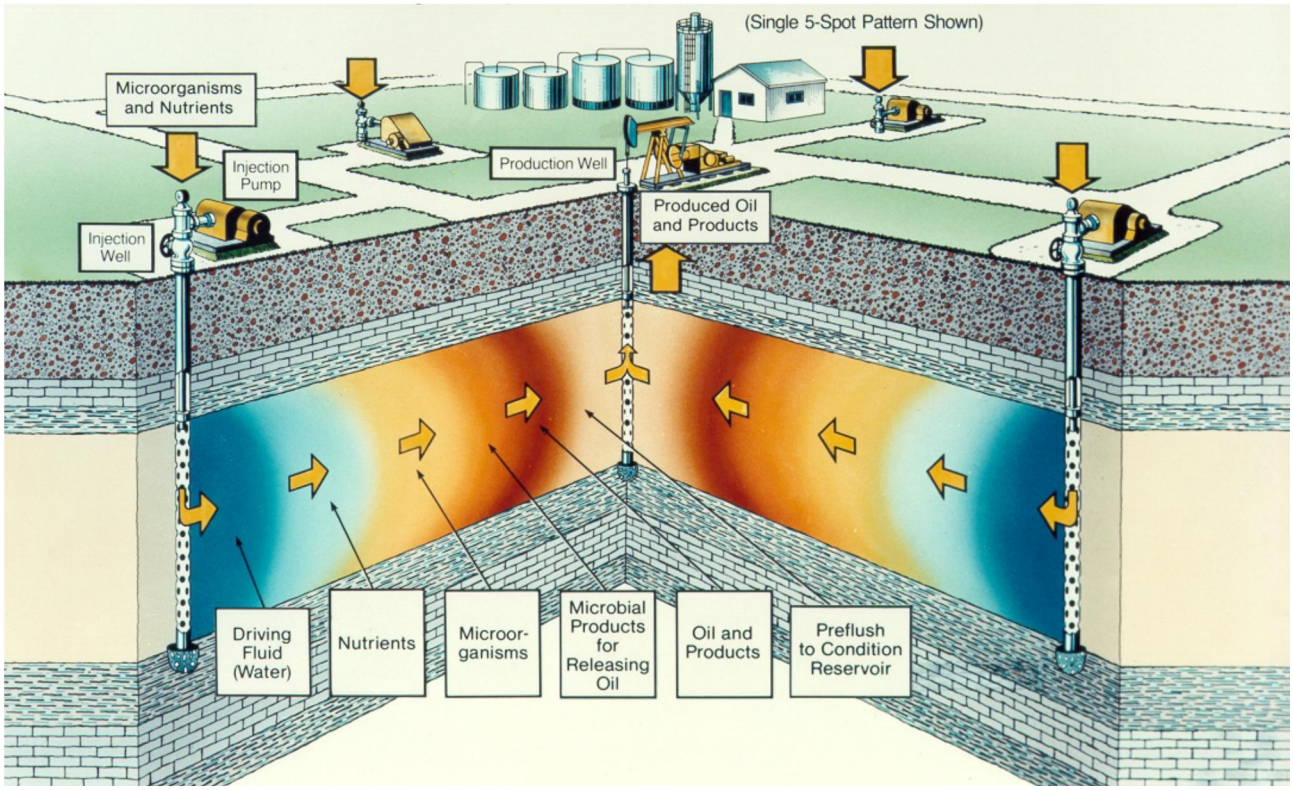


Figure 3: Microbial flooding recovery (www.netl.doe.gov)

2.2.2 The mechanisms of MEOR

Over the years attempt have been made to classify the main mechanisms involve in MEOR process. Theses process are identified based on the end products generated from bacteria metabolism. According to Janshekar (1985), the main mechanisms of MEOR include viscosity reduction, rock dissolution, permeability reduction etc. (Table 1). All these mechanisms are similar to those as being practiced in

chemical EOR. The main difference is that the required products are coming from bacteria metabolism. It is therefore expected that the MEOR mechanisms fulfills the basic law of thermodynamics. Review of literatures suggested that MEOR mechanisms can be different from bacteria to bacteria and are normally selected based on wells or reservoir requirement.

Table 1: Microbial products and their contribution to enhanced oil recovery (after Janshekar, 1985)

Bioproduct	Effect
Acids	Modification of reservoir rock
	Improvement of porosity and permeability
Biomass	Reaction with calcareous rocks and CO2 production
	Selective or non-selective plugging
	Emulsification through adherence to hydrocarbons
	Modification of solid surfaces
	Degradation and alteration of oil
	Reduction of viscosity and oil pour point
	Desulfurization of oil
Gases (CO_2, CH_4, H_2)	Reservoir re-pressurization
	Oil swelling
	Viscosity reduction
	Increase permeability due to solubilization of carbonate rocks
Solvents	Dissolving of oil
Surface-active agents	Lowering of interfacial tension
Polymers	Emulsification
	Mobility control
	Selective and non-selective plugging

It has been shown that microbial enhanced oil recovery techniques are generally applied to reservoirs where production rates have declined over time. The reasons behind the consideration of MEOR technologies (Hitzman, 1991) when evaluating reservoirs for residual oil recovery usually include:

(a). It offers multiple application possibilities

(b). Multiple oil recovery mechanisms available

(c). Treatment effectiveness increases with penetration and duplication

(d). Low startup capital and low operating costs

The MEOR methods are believed to be more constructive than other enhanced oil recovery based on the above perceived advantages and moreover, the microbes produce the necessary metabolites on place and the method is considered to be environmentally friendly and does not require large amount of energy.

2.2.3 MEOR constraints

On the other hand, a major reason for the failure of MEOR technology is insufficient consideration of the conditions which characterize petroleum reservoirs and the physiology of microorganisms which thrive in these conditions (Sheehy, 1991). The activities of microbes employed in MEOR process depends on the physical and chemical conditions they encounter in the reservoirs. These include salinity, temperature, pressure, pH, redox potential etc. although these reservoir conditions vary a great deal from one reservoir to another. All these factors which are mostly physical and environmental can affects the growth of bacteria, proliferation, metabolism and survival and limit their ability to produce desired quantities of metabolites needed for enhanced oil recovery. However it is of the general opinion that with proper planning most of these factors can be overcome. Some of the factors which are considered as limiting for successful application of MEOR are enumerated below.

Salinity

Sodium chloride makes up about 90% or more of the total dissolved solids found in reservoir brines, and therefore, tolerance of microorganisms to salt concentration is one of the most important characteristics needed for microorganisms used in MEOR. The effect to which salinity causes changes in bacterial growth and metabolism depends on the osmotic balance required for such growth since the solute concentration of the surrounding environment can affect cell growth. Grula et al. (1983) isolated Clostridia species capable of growth at 45 ^{o}C, but found that their ability to produce solvents and gases were reduced significantly at high sodium chloride concentrations (5% w/v).

General concentrations of oilfield brines can vary from 100 mg/l to over 300 g/l (Gran et al. 1992) and the salinity gradient can be different in the range of the same formation. Most bacteria overcome the osmotic stress by the accumulation of organic compatible solutes within the cytoplasm without the need for change of intracellular proteins. This method is called ‘organic osmolyte strategy’ (Roberts, 2005). The second

adaptation strategy is intracellular accumulation of high concentration of high concentration of K^+ (Oren, 2001).

Temperature

Temperature plays a significant role in bacteria metabolism. With increasing depth, the temperature increases. Therefore it is certain that bacteria growth and their metabolism will be affected as increasing temperature can exert negative effects on enzyme function by disruption of important cell activities. This molecular picture of the effects of temperature on enzyme function is generally accepted, but it is also to be observed that the temperatures at which these phenomena occur vary widely between organisms (Marshall, 2008). Depending on the temperature ranges for microorganisms survival, microbes can be classified according to their optimum temperature range as psychrophiles (< 25 o C), mesophiles (25-45 ^oC), and thermopiles (45-60 ^oC).

The depths at which most oil reservoirs are situated have temperature higher than 37 ^oC considered as optimum temperature for most bacteria. For example in the North Sea the temperature gradient is about 2.5 ^oC/100m (Vermooten et al 2004) therefore at a depth of 3000m, the temperature can reach about 90 ^oC.

Pressure

Pressure affects biological process in relation to accompanying volume changes however in many regions of the earth; the limiting boundary is probably set more by high temperatures than by high pressures (Marquis 1976; Marquis 1983a). The maximum depth for life in the deep earth has not been determined but for maximum recovery of oil, but in the range of 2000 to 3000 meters, the most applicable pressures for enhanced oil recovery in producing wells are some 20 to 30 MPa. High hydrostatic pressures in the range of several dozen MPa are generally assumed to be nonlethal but can exert adverse effects on the growth of organisms that are adapted to atmospheric pressure (Abe et al.1999, Bartlett, 2002).

The effect of pressure on microorganisms depends not only on the magnitude but also on the duration of pressure applied in combination with temperature, pH, oxygen supply and composition of the culture media (Abe, 2007). The effects of the pressure can be very complex and often difficult to interpret. For example, recent results indicated that lactic acid bacteria *Lactobacillus sanfranciscensis* growth at 50 MPa was 30 % less than at atmospheric pressure and that increase of temperature did not improve its piezotolerance (Molina-Höppner et al. 2003). In another study, it was shown that treatment of *E.coli* cells at a higher pressure of 75 MPa for 30 min does not readily cause any morphological changes (Kawarai et al.2004). The challenges are therefore to establish whether the physiological responses of bacteria cells to high pressure

are relevant to their growth and to identify the critical factors in cell viability and lethality under high pressure during microbial enhanced oil recovery.

pH

pH is one of the major environmental factors that affect microbial growth and is one of the most studies because of its importance in fundamental research. In general, the optimal pH for growth for microorganisms is between a pH of 4.0 and 9.0, but at very low pH, the metabolic activities of microorganisms can be affected. The detrimental effect of low pH on microbial growth is well documented (Brock, 1969) but the mechanisms involved are not well understood. Generally, a near-neutral intracellular pH is maintained in bacteria (Riebeling et al. 1975) but the intracellular pH can decrease considerably when the cell is subjected to an acidic environment.

As many enzymes are sensitive to pH, the growth inhibitions can be seen at low pH could be caused by a direct effect of the H ion on cellular components even though, such direct effects would not necessarily cause a decrease in the efficiency of growth (Russell and Dombrowski, 1980). pH values normally encountered in oil reservoirs may not pose a problem for the growth of organisms but pH gradients can affect the control of specific metabolic processes required for some MEOR processes (Jenneman and Clark 1992).

Pore size

Even though, the pores in rock can be connected in different ways, pore spaces less than 0.5 nm can place severe restrictions on the ability of most bacteria (most bacteria have dimensions of length approximating 0.5-10.0 um and widths of 0.5-2.0 um to be transported through the rock matrix especially for those bacteria that their sizes are comparable to the sizes of the rock pores (Jenneman and Clark 1992). Updegraff (1983) stated that pores must be at least twice the diameter of cocci or short bacilli for effective transport to occur. Fredrickson et al. (1997) also showed that the sizes of pores within the rock, or the pore throat diameter may be an important factor in regulating the observed microbial activity.

Pore-throat diameters of shale are on the average much smaller than those of sandstone (~0.2 mm for shale and up to 13 mm for sandstone (Krumholz, 2000) and the results of the study suggest that growth and metabolism of shale-bound organisms may be limited by slow diffusion of nutrients and/or by the inability of microbes to migrate easily through the narrow pores. Also, Zvyagintsev (1970) in an experiment with microbes stated that, placement of microbes in large capillaries (400 x 150 nm) increased the number of cells 7-10 times but in small capillaries, not only was an increase of cells observed but the size of the cells was reduced. In general, permeabilities of 75-100 mD are thought to be the lower limit for effective

microbial transport (Jenneman and Clark, 1992) but reports have indicated transportation of bacteria cells through cores of less than 75 mD (Hart et al. 1960, Kalish et al 1964).

Nutrients

Successful MEOR process will require the availability of essential nutrients in order for growth and metabolism to take place as it was recognized that there is a smooth relationship between growth rate and nutrient concentrations (Monod, 1949). Bacterial requirements for growth include sources of energy, mostly organic carbon (i.e. sugars and fatty acids) and mineral ions (e.g. iron and phosphorus). These nutrients are mostly transported in the aqueous phase. Fermentative bacteria use glucose, sucrose, or lactose containing nutrients.

The choice of nutrients is very important since the types of bioproducts that are also produced by different types of bacteria are dependent on the types and concentrations and components of the nutrients provided. Molasses which is a byproduct of sugar in general has been employed in many of the field applications as the carbon source because of its perceived low price and presence of essential minerals and vitamins. The use of molasses as a substrate was first proposed by Updegraff and Wren (1954). In addition, some microbes utilizes oil as carbon source which is excellent for heavy oil production because it can reduce the carbon chain of heavy oil and increase the quality (Cooper et al.1980, Moses, 1991). Under anaerobic conditions however the use of petroleum components as food is thought to be not effective at least within a time frame required for economic recovery. Even though growth can occur, the growth can be very slow and hardly detected for several months (Moses et al. 1983).

2.3 Microbe selection for MEOR

In view of the obstacles provided by the reservoir conditions the main question to be answered is how to find the right candidate of bacteria to fulfill the purpose of MEOR in terms of adequate production of required metabolites such as acids (*Clostridium* sp., *Enterobacter*) gases (*Enterobacter*, *Clostridium* sp.) solvents (*Clostridium acetobutylicum*, *Zymomomas mobile*) biomass (*Bacillus lichenifonnis, Xanthomonas campestris*) biosurfactants (*Acinetobacter calcoacticus, Arthrobacter paraffineus, Pseudomonas* sp.) and biopolymers (*Bacillus polymyxa, Brevibacterium viscogenes*) etc. that will enhance oil recovery. Finding the right bacteria is not a simple task; understanding of the physico-chemical parameters in the reservoir is the key in selection of bacteria for the MEOR process. This will determine the choice of bacteria that will produce bioproducts which can increase oil recovery and withstand the extreme reservoir conditions. In case of MEOR, successful field experiments mostly used anaerobic bacteria (Maudgalya, 2007) and it was suggested that four main sources from which bacteria species that are potential candidates for MEOR can

be isolated include formation waters, sediments from formation water purification plants, sludge from biogas operations and effluents from sugars (Lazar,1991).

Earlier studies on microbial enhanced oil recovery showed that both mixed culture and pure strain of bacteria have been used for microbial enhanced oil recovery. For example Hitzman (1983) used pure and mixed cultures of *Bacillus*, *Clostridium* and *Pseudomonas* in 2-4% molasses in USA. Wang et al. 1993, and 1995, used mixed enriched bacteria cultures of *Bacillus*, *Pseudomonas*, *Eurobacterium*, *Fusobacterium*, and *Bacteriodes* in a 4% residue sugar. Dostalek and Spurney (1957 and 1958) utilized injected sulphate-reducing *Desulfovibrio* and hydrocarbon-utilizing *Pseudomonas* bacteria with nutrients (generally molasses). Yaranyi (1968) documented use of mixed sewage sludge bacteria cultures predominantly *Clostridium*, *Pseudomonas*, *Desulfovibro*. Karaskiewicz (1975) also documented the use of mixed microbial cultures of *Pseudomonas*, *Escherichia*, *Arthrobacter*, *Mycobacterium*, *Micrococcus*, *Peptococcus*, *Bacillus*, and *Clostridium* grown in formation water and 4 % molasses in 18 field trials in Poland between 1960 and 1961. Further works that employed used of mixed cultures of bacteria include those of Wagner et al. (1993) that employed mixed cultures of thermophilic *Bacillus* and *Clostridium*, mixed cultures of hydrocarbon degrading bacteria in free corn syrup and mineral salts were used by Coates et al. 1993, Nelson and Schneider 1993, Jenneman et al. 1995 among others.

Those studies that employed the use of pure strain of bacteria include those of Wagner (1995) that used salt tolerant *Clostridium* to increase oil production in carbonate reservoir by in-situ gas and solvent production. Also, Grula et al. (1983) used isolated clostridia species that were able to produce solvents and gases. Furthermore, Senjucov et al. (1971), Ivanov et al. 1993, Nazina et al. 1994, Belyaev et al. (2004) and Jimoh et al. 2011, all used pure cultures of *Clostridium tyrobutyricum* in 2-6% molasses for different evaluation of MEOR processes.

From the above it is evident that both mixed cultures and pure strain have been selected in the past for MEOR purposes. There is a general opinion that mixed cultures were more efficient in releasing oil than pure strains, however, since the characteristics of reservoirs are different the choice of right candidate will be determined after much information about the reservoir ecology have been gathered.

2.4 Cases of MEOR Application

There are numerous examples of cases of MEOR applications in different oilfields across the globe. To list all these examples will be an enormous task; however some of the best known cases in the last 40 years as

adapted from Lazar 2007 are shown in the Table 2. The lists are many and currently there are other on-going MEOR projects in different parts of the world. In the North Sea, out of the 19 enhanced oil recovery projects carried out by 2006, only one is microbial enhanced oil recovery (Awan et al.2008) the other 18 projects have been or are gas enhanced oil recovery projects.

In general with an average of 35 -45 % recovery from the best currently available technology of the original-oil-in-place in an oil field coupled with an annual production declines of between 4%-15% in mature fields, many more oil companies and agencies are starting to open their minds to the possibility of using MEOR permanently. It is believed that the use of MEOR will continue to grow with time as understanding of the basic processes involved in MEOR become stronger.

Table 2: Cases of MEOR field trials (adapted from Lazar, 2007)

Country	Microbial systems	Nutrients	Incremental of oil production	References
Bulgaria	Indigenous oil-oxidixing bacteria from water injection and water formation	Water containing air + ammonium and phosphate ions	+	Groudeva et al. 1993
Canada	Pure culture of *Leuconostoc mesenteroides*	Dry sucrose + sugar beet molasses dissolved in water	-	Jack and Stehmeier 1988
Former East Germany	Mixed cultures of thermophilic:*Bacillus and Clostridium*	Molasses 2-4% with addition of nitrogen and phosphorus	+	Wagner et al. 1993
USA	Pure or mixed cultures of *Bacillus*, *Clostridium*, and *Pseudomonas*	Molasses 2-4%	+	Hitzman 1983, Grula et al. 1985, Bryant et al. 1993, Jenneman et al. 1995 , Dietrich et al. 1996
Russia	Pure culture of *Clostridium tyrobutyricum*	Molasses 2-6% with nitrogen and phosphorus salt addition	+	Senyucov et al. 1970, Ivanov et al. 1993, Wagner et al. 1995
China	Mixed enriched bacterial cultures of *Bacillus*, *Pseudomonas*, *Bacteroides* etc.	Molasses 4-6% Crude oil 5% Residue sugar 4% + Xanthan 3%	+	Wang et al. 1993, Zhenggao et al. 2000
Romania	Adapted mixed enriched cultures; *Clostridium, Bacillus, Pseudomonas*	Molasses 2-4%	+	Lazar and Constantinescu, 1985, Lazar et al. 1991,1998

References

Abe, F. 2007: Exploration of the effects of high hydrostatic pressure on microbial growth, physiology and survival: perspectives from piezophysiology. Bioscience, Biotechnology and Biochemistry, 71 (10): 2347-2357.

Abe, F., Kato, C., and Horikoshi, K. 1999: Pressure-regulated metabolism in microorganisms. Trends Microbiol, 7, 447–453.

Al-Wahaibi, Y.M., Gratttoni, C.A., and Muggeridge, A.H. 2006: Drainage and imbibition relative permeabilities at near miscible condition. J.Petroleum Sci. Eng., 53:239-253.

Awan, A.R., Teigland, R., and Kleppe, J. 2008. A Survey of North Sea enhanced-oil-recovery projects initiated during the years 1975 to 2005. SPE Res Eval & Eng 11 (3): 497-512.

Banat, I.M., 1995: Biosurfactants production and possible uses in microbial enhanced oil recovery and oil pollution remediation: a review. Biores. Technol., Vol. 51, pp. 1-12.

Bartlett, D.H., 2002: Effect of pressure on in vivo microbial processes. Biochimica Biophysica Acta-Protein Structure and Molecular Enzymology 1596 (1-2):367-381.

Beckmann J.W., 1926: The action of bacteria on mineral oil. Ind. Eng. Chem. News, 4:3

Behesht, M., Roostaazad, R., Farhadpour F., and Pishvaei, M.R. 2008: Model development for MEOR process in conventional non-fractured reservoirs and investigation of physic-chemical parameter effects. Chem. Eng. Tech., 7: 953-963.

Belyaev, S.S., 1983: The dynamics of development of aerobic and anaerobic bacteria during an oil-bearing stratum to enhance oil recovery. In: T.F Yen (Chairman), Symposium on Biological Pressures Related to Petroleum Recovery. Div. Pet. Chem., Am. Chem. Soc., Seattle, Wash., March 20-25, pp. 810-812.

Belyaev, S.S., Borzenkov, I.A., Nazina,T.N., Rozanova, E.P., Glumov, I.F., and Ibatullin, R.R. 2004: Use of microorganisms in the biotechnology for enhancement of oil recovery. Microbiol. (Maik Nauka Interperiodica). 73: 590-598.

Brock, T.D. 1969: Microbial growth under extreme conditions. Sym. Soc. Gen. Microbiol. 19: 15-41.

Brown L.R., 2010: Microbial enhanced oil recovery. Curr. Opinion Microbiol. 13: 316-320

Brown, L.R., Vadie A.A., and Stephens, J.O., 2002: Slowing production decline and extending the economic life of an oil field: New MEOR, technology, Soc. Pet. Eng. Reservoir Eval. Eng. 5, 33-41.

Bryant R.S, Burchfield, T., Chase, K., Bertus, K., and Stepp, A., 1989: Optimization of oil mobilization, transport of microbes and metabolites, and effects of additives. In: SPE 19686 presented in 64th Annual Technical Conference and Exhibition of the Society of Petroleum Engineers held in San Antonio, TX, pp. 567-578.

Bryant R.S., Stepp, A.K., Bertus, K.M., Burchfield, T.E., and Dennis, M., 1993: Microbial enhanced waterflooding field pilots. Dev. Pet. Sci. 39. 289-306.

Bryant, S.L., and Lockhart, T.P., 2002: Reservoir engineering analysis of microbial enhanced oil recovery. Soc. Pet. Eng. Reservoir Eval. Eng. 5, 365-374.

Bubela, B., 1983: Combined effect of temperature and other environmental stresses on microbiologically enhanced oil recovery. In: E.C. Donaldson and J.B. Clarks (Eds.), Proceedings, 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS, Springfield, Va., 219 pp.

Cayol L, Ollivier B, Patel B.K.C, Ravot G, Magot M., Ageron, E., Grimont, P.A., and Garcia J.L., 1995: Description of Thermoanaerobacter brockii subsp. lactiethylicus subsp. nov., Isolated from a Deep Subsurface French Oil Well, a Proposal To Reclassify Thermoanaerobacter finnii as Thermoanaerobacter brockii subsp. finnii comb. nov., and an Emended Description of Thermoanaerobacter brockii. Int. Journal of Systematic and Evolutionary Microbiology 45: 783-789.

Chang M.M., Chung, F., Bryant, R., Gao, H., and Burchfield T. 1991: Modeling and laboratory investigation of microbial transport phenomena in porous media. In: SPE 22845 presented at 66th Annual Technical Conference and exhibition of SPE in Dallas Texas.

Coates J.D., Chisholm, J.L., Knapp, R.M., McInerney, M.J., Menzie, D.E., and Bhupathiraju, V.K., 1993: Microbially enhanced oil recovery field pilot, Payne, County, Oklahoma, Developments in Petroleum Sci. 39: 197-205.

Cooper D.G, Zajic, J.E., Gerson, D.F., and Manninen, K.I. 1980: Isolation and identification of biosurfactants produced during anaerobic growth of Clostridium pasteurianum. J.Ferm. Tech. 58:83-86.

Davis, J.B., and Updegraff D.M, 1954: Microbiology in the Petroleum industry. Bacteriol. Rev. 18.215-238.

Desai J.D. and Banat, I.M., 1997: Microbial production of surfactant and their commercial potential. Microbiol. Mol. Biol. Rev. Vol. 61, 1: 47-64

Dienes, M., and Jaranyi, I., 1973: Increase of oil recovery by introducing anaerobic bacteria into the formation, Demjen field, Hungary. Koolaj es Foldgaz 106, 205-208.

Dietrich F.L., Brown, F.G., Zhou, Z.H., and Maure, M.A., 1996: Microbial EOR Technology Advancement: Case Studies of Succesful Projects. SPE 36746. Proceedings of the SPE Annual Technical Conference and Exhibition. Society of Petroleum Eng. Richardson, Texas.

Donaldson E.C. (Ed.), 1991: Microbial enhancement of oil recovery- recent advances. Development in Petroleum Science 31, 530 pp.

Donaldson E.C., Chiligarian, G.V. and Yen, T.F. (Eds.), 1989: Microbial enhanced oil recovery. Developments in Petroleum Science, 21. Amsterdam: Elsevier. 227pp.

Donaldson E.C., and Grula, E.A., 1985: There are bugs in my oil well. CHEMTECH, 602-604.

Dostalek et al.1957: Effects of micro.ovrddot. organisms on petroleum hydrocarbons. Ceskoslov. Mikrobiol.

Dostalek M., and Spurny, M., 1958: Bacterial release of oil: A preliminary trials in an oil deposit. Fol. Biol. (Praha) 4.

Fredrickson, J.K., McKinley, J.P., Bjornstad, B.N., Long,P.E., Ringelberg,D.B., White, D.C., Krumholz, L.R., Suflita, J.M., Colwell, F.S., Lehman, R.M., Phelps T.J. and Onstott, T.C. 1997: Pore-size constraints on the activity and survival of subsurface bacteria in a late cretaceous shale-sandstone sequence, northwestern New Mexico. Geomicrobiology Journal, 14(3): 183-202.

Gran, K., Bjorlykke,K., and Aagaard, P., 1992: Fluid Sainity and Dynamics in the North Sea and Haltenbanken basins derived from well logs. Geological Society, London, Special Publications; 1992; v. 65; p. 327-338

Groudeva V.I., Ivanova, I.A., Groudev, S.N., and Uzunov, G.C., 1993: Enhanced oil recovery by stimulating the activity of the indigenous microflora of oil reservoirs. Biohydrometal. Technol. Proc. Int. Biohydrometalurgical Symp. 2.349-356.

Grula E.A., Rusell, H.H., Bryant, D., Kenaga, M., and Hart, M., 1983: Isolation and screening of Clostridia for possible use in microbially enhanced oil recovery. In: E.C. Donaldson and J.B. Clark (Eds.), Proceedings, 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS, Springfield, Va., pp. 43-47.

Hart, R.T., Fekete, T., and Flock, D.L. 1960: The plugging effect of bacteria in sandstone systems. Can. Min. Metal. Bull. July: 495-501.

He, Z., She, Y., Xiang, T., Xue, F., Mei, B., Li, Y., Ju, B., Mei, H., and Yen T.F., 2000: MEOR pilot sees encouraging results in Chinese oil field. J.Oil Gas 98: 46-52.

Hitzman, D.O., 1983: Petroleum microbiology and the history of its role in EOR. In: E.C. Donaldson and J.B. Clarks (Eds.), Proceedings, 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS, Springfield, VA. 162-218.

Hitzman D.O., 1988: Review of microbial enhanced oil recovery field trials. Proc. Symposium on Application of Microorganisms to Petroleum Technology, Bartlesville, OK, August 12-13, 1987. Burchfiled, F.E. and Bryant, R.S. (Eds.).

Hitzman, D.O.,1991: Microbial enhanced oil recovery: The time is now. In: Microbial Enhancement of Oil Recovery- Recent Advances. Development in Petroleum Science, 31, E.C. Donaldson (Eds.), Amsterdam: Elsevier, pp.11-20.

Hitzman, D.O. and Sperl, G.T. 1994: New microbial technology for enhanced oil recovery and sulfide prevention and reduction. SPE/DOE 27752. Proceedings of the 9th Sympossium on Improved Oil Recovery. Society of Petroleum Engineers, Richardson, Texas.

Islam, M. 1990: Mathematical modeling of microbial enhanced oil recovery. In: 65th Annual Technical Conference and Exhibition of the Society of Petroleum Engineers, September 23-26, New Orleans, LA.

Ivanov, M.V., Belyaev, S.S., Laurinavichus, K.S., Obraztova, A.Y., and Gorlatov, S.N., 1982: Microbiologiya 4: 336.

Ivanov M.V., Belyaev, S.S., Borzenkov, I.A., Glumov, I.F., and Ibatulin, P.B., 1993: Additional oil production during field trials in Russia. In: Microbial Enhancement of Oil Recovery-Recent Advances, Premuzic, E., and Woodhead, A. (Eds.). Amsterdam: Elsevier, pp.373-381.

Jack T.R., and Steheier, G.L., 1988: Selective plugging in watered out oil reservoirs. In: Proceedings of the Symposium on Applications of Microorganisms to Petroleum Technology. T.E. Burchfield and R.S. Bryant (Eds.), National Technical Information Service, Springfield, VA, pp.VII I-VII 13.

Janshekar, H. 1985: Microbial enhanced oil recovery processes. In: J.E. zajic and E.C. Donaldson (Eds.), Microbes and Oil Recovery. Bioresources Publications, El Paso, Texas, pp.54-84.

Jenneman, G.E. and Clark J.B: 1992: The effect of in-situ pore pressure on MEOR processes. SPE 24203. SPE/DOE Enhanced Oil Recovery Symposium, 22-24 April 1992, Tulsa, Oklahoma, 15pp.

Jenneman, G.E., Geverrtz, D., Davey, M.E., Clark, J.B., Wood, W.A., Stevens, J.C., and Tankersley, C., 1995: Development and application of microbial selective plugging process. In: Proceedings 5th International Conference on Microbial Enhanced Oil Recovery and Related Problems for Solving Environmental Problems (CONF-9509173). R.S. Bryant and K.L. Sublette (Eds.), National Technical Information Service, Springfield, VA, pp.7-26.

Jimoh, I.A, Rudyk, S.N., and Søgaard E.G., 2011: Microbial fluid rock interactions in chalk samples and salinity factor in divalent Ca^{2+} ions release for microbial enhanced oil recovery purposes, Chemical Engineering Transactions 24, 889-894.

Kalish P.J., Stewart, J.A., Rogers, W.F., and Bennett, E.O., 1964: The effects of bacteria on sandstone permeability. J. Petroleum Technology 16:805-814.

Karaskiewicz, J. 1975: Studies on increasing petroleum oil recovery from Carpathian deposits using bacteria. Nafta (Petroleum) 21, 144-149.

Kaster, K. M., Bonaunet, K., Berland, H., Kjeilen-Eilertsen, G., & Brakstad, O. G. 2009: Characterisation of culture-independent and -dependent microbial communities in a high-temperature offshore chalk petroleum reservoir. Antonie van Leeuwenhoek, 96, 423–439

Kaster, K.M., Hiorth, A., Eilertsen, G.K., Boccadoro, K., Lohne, A., Berland, H., Stavland, A., and Brakstad, O.G., 2012: Mechanisms Involved in Microbially Enhanced Oil Recovery, Transport in Porous Media, 91: 1, 59-79.

Kawarai, T., Wachi, M., Ogino, H., Furukawa, S., Suzuki, K.,Ogihara, H., and Yamasaki, M. 2004: SulA-independent filamentation of *E*.coli during growth after release from high hydrostatic pressure treatment. Applied Microbiology and Biotechnology, 64 (2): 255-262.

Kowalewski, E., Rueslatten, I., Steen, K.H., Bødtker, G., and Torsaeter O., 2006: Microbial improved oil recovery-bacterial induced wettability and interfacial tension effects on oil production. J. Pet. Sci. Eng. 52, 275-286.

Kropp, K., Davidova I.A., Suflita J.M. 2000: Anaerobic oxidation of n-dodecane by an addition reaction in a sulfate reducing bacteria enrichment culture. Appl. Environmetal Biotech. 66:5393-5398.

Krumholz, L.R. 2000: Microbial communities in deep surface. Hydrogeology Journal 8 (1): 4-10.

Kuznetsov, S.I. (Editor) 1961: Geologic activity of microorganisms (translated from Russian by Consultants Bureau, New York, 1962). Tr. Inst. Mikrobiol. Akad. Nauk SSSR, IX, 112 pp.

Kuznetov, S.I., Ivanov, M.V., and Lyalikova, N.N., 1962: Intoduction to Geological Microbiology (translated from Russian by P.T. Broneer and C.H. Oppenheimer). Introduction to Geological Microbiology, McGraw-Hill, New York, N.Y., 252 pp.

Lazar I., 1978: Microbiological methods in secondary oil recovery. European Symposium on Enhanced Oil Recovery, Edinburgh, July 5-7, Institute of Offshore Engineering and Herriot-Watt University (Eds.), pp. 279-287.

Lazar, I., 1991: MEOR field trials carried out over the world during the past 35 years. In: Microbial Enhancement of Oil Recovery-Recent Advances, Donaldson, E.C. (Ed.), Amsterdam, Elsevier Science, pp. 485-530.

Lazar, I. 1998: International MEOR applications for marginal wells. Pakistan J. Hydrocarbon Res. 10. 11-30.

Lazar, I. and Constantinescu, P. 1985: Field trials results of MEOR. Microbes and Oil Recovery, 1: 122-131.

Lazar, I. Petrisor, I.G., and Yen, T.F., 2007: Microbial enhanced oil recovery, Petroleum Science and Technology, 25: 11, 1353-1366.

Li, Q., Kang, C., Wang, H., Liu, C., and Zhang, C. 2002: Application of microbial enhanced oil recovery technique to the Daqing oilfield. Biochem. Eng. J. 11: 197-199.

Marquis, R.E. 1976: High pressure microbial physiology. Adv. Microbial Physiol. 14: 159-241.

Marquis, R.E., 1983a: Barotolerance and microbial enhancement of oil recovery. In: J.E. Zajic, D.C. Cooper, T.R. Jack and N.Kosaric (Eds.). Microbial Enhanced Oil Recovery. PennWell Publishing Co., Tulsa, Oklahoma, pp.8-13.

Marshall, S.L. 2008: Fundamental aspects of microbial enhanced oil recovery: A literature survey, CSIRO Land and Water Floreat, Western Australia, pp.1-42.

Maudgalya S., Knapp, R.M., McInerney, M.J., 2007: Microbial enhanced oil recovery technologies: A review of the past, present and future, SPE 106978

Maudgalya, S., McInerney, M.J., Knapp, R.M., Nagle, D.P., and Folmsbee, M.M. 2005: Tertiary oil recovery with microbial biosurfactant treatment at low permeability Berea sandstone cores. SPE 94213. Proceedings of Society of Petroleum Engineers Production and Operations Symposium. Society of Petroleum Engineers, Richardson, Texas.

McInerney, M.J. and Sublette, K.L. 1997: Petroleum microbiology: Biofouling, souring and improved oil recovery. In: Manual Environmental Microbiology. C.J. Hurst, G.R. Knudsen, M.J. McInerney, L.D. Stetzenbach and M.V. Walter (Eds). ASM:Washington D.C. pp. 600-607.

McInerney, M.J., Maudgalya, S., Nagle, D.P and Knapp, R.M. 2002: Critical assessment of the use of microorganisms for oil recovery. Recent Res. Dev. Microbiol., 6:269-284.

Molina-Höppner, A., Sato, T., Kato, C., Gänzle, M.G. and Vogel, R.F: 2003: Effect of pressure on cell morphology and cell division of lactic acid bacteria. Extremophiles 7: 511-516.

Monod, J., 1949: The Growth of Bacterial Cultures. Annual Review of Microbiology, v. 3, p. 371.

Moses, V. 1991: MEOR in the filed: why so little? Microbial Enhancement of Oil Recovery. Recent Advances. Development in Petroleum Science, 31, E.C. Donaldson (Eds.), Amsterdam: Elsevier, pp.21-28.

Moses, V., Brown, M., Burton, C.C., Gralla, D.S., and Cornellius, C. 1993: Microbial hydraulic acid fracturing. Developments in Petroleum Science 39: 207-229.

Nazina, T. N., Ivanova, A. E., Mityushina, L. L. & Belyaev, S. S., 1994: Analysis of microbial community from water flooded oil field by chromatography-mass pectrometry. Mikrobiologiya 63: 876-882.

Nelson L. and Schneider D.R.,1993: Six years of paraffin control and enhanced oil recovery with the microbial products, Para-Bac. Developments in Petroleum Sci. 39:355-362.

Nielson, S.M., Shapiro, A.A., Michelsen, M.L., and Stenby, E.H. 2010. 1D simulations for microbial enhanced oil recovery with metabolite partitioning. Transp. Porous Media. 85: 785-802.

Oren A. 2001: The bioenergetics basis for the metabolic diversity at increasing salt concentrations: an implications for the functioning of salt lake ecosystem. Hydrobiologia 466, pp. 61-72.

Raiders, R.A., Freeman, D.C., Jenneman, G.E., Knapp, R.M., McInerney, M.J. and Menzie, D.E. 1985: The use of microorganisms to increase the recovery of oil from cores. SPE 14336, presented at 60th Annual Technical Conference and Exhibition of SPE in Las Vegas, NV. Sept. 22-25.

Riebling, V., Thauer, R.K., and Jungermann, K. 1975: The internal-alkaline pH gradient, sensitive to uncoupler and ATPase inhibitor in growing *Clostridium pasteurianum*. Eur. J. Biochem. 55: 445-453.

Roberts, M.F. 2005: Organic compatible solutes of halotolerant and halophilic microorganisms. Saline Syst., 1, pp. 5.

Rudyk S.N., and Søgaard E. G, 2011: Microbial Enhanced Oil Recovery In: Applied Microbiology and Molecular Biology in Oilfield Systems, Eds. Whitby C. and Skovhus T.L., Springer, Dordrecht, 179-187.

Russel, J.B. and Dombrowski, D.B. 1980: Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Applied and Environmental Microbiology 39 (3): 604-610.

Senyukov, V.M., Yulbarisov, M.E., Taldykina, N.N and Shissherina, P.E. 1970: Microbial method of treating petroleum deposit containing highly mineralized stratal waters. Mikrobiologiya 39: 705-710.

Sheehy, A.J., 1991: Microbial physiology and enhancement of oil recovery- recent advances. In: Development in Petroleum Science, 31, Donaldson, E.C., (Eds.). Amsterdam: Elsevier, pp. 37-44.

Updegraff, D.M. 1983: Plugging and penetration of petroleum reservoir rock by microorganism. In: E.C. Donaldson and J.B. Clark (Eds). Proceeding 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS Springfield, Va.,pp. 80-85

Updegraff D.M., and Wren, B.G., 1954: The release of oil from petroleum bearing materials by sulfate reducing bacteria. Appl. Microbiol. 2: 307-322.

Vermooten, J.S.A., Verweij, J.M., Simmelink, H.J. 2004: Quality control, correction and analysis oftemperature borehole data in offshore Netherlands. A. Quality control and correction of temperatureborehole data; B. Analysis and interpretation of corrected temperatures. Netherlands Institute of AppliedGeoscience TNO - National Geological Survey. Internal TNO-report NITG 04-043-B0506.

Wagner, M., 1991: Microbial enhancement of oil recovery from carbonate reservoirs with complex formation characteristics. Dev. Pet. Sci. 31, 387-398.

Wagner M., Lunherhausen D., Nowak, U., and Ziran, B., 1993: Microbially improved oil recovery from carbonate. In: BIohydrometalurgical Technologies, Vol. II, Torma, A.E., Appel, M.L., and Brierley, C.I. (Eds.). Wallande, Pennysylvania: A Publication of TMS, Minerals-Metals-Materials, 695-710.

Wang, X.Y, Xue, Y., and Xie, S. 1993: Characteristics of enriched cultures and their application to MEOR field tests. Development in Petroleum Science 39, 335-348.

Wang X.Y., Xue, Y., Dai, G., Zhao, L., Wang, Z.S., Wang, J.L., Sun, T.H., and Li, X.J., 1995: Application of bio-huff and puff technology at Jilin oil field. In: The 5th International Conference on Microbial Enhanced Oil Recovery and Related Problems for Solving Environmental Problems (CONF-9509173). R.S Bryant and K.L. Sublette (Eds.), National Technical Information Service, Springfiled, VA, pp.15-128.

www.netl.doe.gov viewed on 17-06-2011

Xue Y., Xu, Y., Liu, Y., Ma, Y., and Zhou, P., 2001: Thermoanaerobacter tengcongensis sp. nov., a novel anaerobic saccharolytic thermophilic bacterium isolated from a hot spring in Tengcong, China, Int. J. Syst. Evol. Microbiol. 51, 1335-1341

Yaranyi, I., 1968: Bezamolo a nagylengyel tezegeben elvegzett koolaj mikrobiologiai kiserletkrol. M. All. Faldany Intezet Evi Jelentese A., Evval, pp. 423-426.

Yarbrough H.F. and Coty V.F., 1983: Microbially enhanced oil recovery from the Upper Cretaceous Nacatoch Formation, Union County, Arkanas. In: E.C. Donaldson and J.B Clark (Eds.), Proceedings, 1982 International Conference on Microbial Enhancement of Oil Recovery. NTIS, Springfield, Va., pp.149-153.

Yen T. 1990: Microbial enhanced oil recovery-principle and practice. Boca Raton FL: CRC Press Inc.

Youssef, N. H., Elshahed, M. S., and McInerney, M. J. 2009: Microbial Processes in Oil Fields: Culprits, Problems, and Opportunities. In Laskin A. I., Sariaslani,, S. and Gadd, G. M., editors: Adv. Appl. Microbiol. 66: 141-251. Burlington: Academic Press

Zajic, J.E., Copper, C.D., Jack, T.R., and Kosaroc, N., 1983: Microbial Enhanced Oil Recovery. Tusla, OK: Penn Well Books.

Zhang, X., and Xiang, T.,2010: Review of microbial enhanced oil recovery technology and development in China. International Journal of Petroleum Science and Technology, 4:1, pp.61-80.

Zhengguo, H., Yuehui, S., Tiugsheng, X., Feng, X., Brown, M., Yong, L., Banginq, J., Hai, M., and Yen, T.F.2000: MEOR pilot sees encouraging results in Chinese oil field. Oil and Gas J. 98:46-52.

Zobell, C.E., 1947: Bacterial release of oil from oil-bearing materials, Part I and II. World Oil, 126 (13): 36-47 (I); 35-41 (II).

2 Papers I-VIII

As already mentioned, this thesis is presented as combination of different articles. The research was conducted within the field of microbial enhanced oil recovery drawing on several aspects of microbiology in the deep subsurface and different processes to form the basis to describe the microbial enhanced oil recovery in detail. The subjects covered by the articles intend to provide fundamental understanding of the specific problem investigated by laboratory experiments using different techniques. The papers are listed from Paper 1-VIII in the proceeding pages.

Paper 1

Dissolution of carbon dioxide in aqueous sodium chloride solutions and its relationships to salinity, pH and electrical conductivity properties

I. A. Jimoh[A], E. G. Søgaard[A,B] and Svetlana N. Rudyk[A]

[A]Department of Biotechnology, Chemistry and Environment, Section of Chemical Engineering,
Aalborg University, Niels Bohr Vej 8 Esbjerg, Denmark.
[B]Corresponding author. Email: egs@bio.aau.dk

Abstract: In this study, dissolution of carbon dioxide in aqueous sodium chloride was investigated by FT-IR spectroscopy simple modeling. The result from FT-IR spectrometry showed that the concentration of dissolved carbon dioxide is directly related to the salinity of the aqueous sodium chloride solution. Concomitant alterations in dissolution profile showed that at different salinity and constant temperature, the concentration of dissolved carbon dioxide in aqueous sodium chloride solutions decreased from 0.0554 - 0.0085 moles/L over the salinity range of 0 - 130 g/L while pH of the was reduced by about 2.0 units over the same salinity range. The relative variation of pH at different salinity after dissolution of carbon dioxide can be represented by a polynomial function which indicated a kind of salting out effect after 70 g/L. Model prediction between theoretical and experimental pH showed an average difference of 0.13 units. At a constant salinity of 30 g/L, the concentration of dissolved carbon dioxide decreases from 0.0463 - 0.0129 moles/L when temperature was increased from 25 ^{o}C to 45 ^{o}C. Additional results showed that dissolution of carbon dioxide in aqueous sodium chloride has no significant effect on the electrical conductivity of the solution.

Keywords: Salinity; dissolved CO_2; infrared spectroscopy; conductivity; pH, activity coefficients.

Introduction

Greenhouse gases such as carbon dioxide play an important role in efforts to address global climate change due to their high global warming potentials and the availability of cost-effective emission reduction opportunities. There has been increasing interest on the perspective of long term CO_2 storage, and carbon dioxide dissolution into brine is one of the long term carbon dioxide storage mechanisms available. Saline aquifers appear to have great potential and geological properties for long term storage of carbon dioxide. However, it requires different data sets such as chemical, physical and thermodynamics parameters to carry out successful simulations of CO_2 injection in aquifers of depleted oil reservoirs. Simple laboratory experiments on carbon dioxide dissolution in brine and other parameters can increase our knowledge to predict and understand the mechanisms of reaction. Sodium chloride solution was selected because the highest percentage of salt composition in saline aquifers consists of sodium chloride.

Many works have been previously reported in the literature on carbon dioxide in brine. For example, the density of aqueous solutions with dissolved CO_2 was calculated in experimental investigation by Parkinson and Nevers [12]. The first set of experimental data of density of CO_2 solution at a lower CO_2 concentration (less than 1.0% in mass fraction) at temperature of 273.25-284.15 K and pressure of 5-12.5MPa was also obtained in detail study by Ohsumi et al. [11]. Spycher and Pruess [16] measured the solubility of carbon dioxide in aqueous solutions of sodium chloride at temperature of 40 to 160 ^oC in up to 6 mol/kg salt solutions and total pressure of 10 MPa and deduced that the carbon dioxide molality is 0.42 mol/kg in pure water whereas it is 0.2 or 0.15 mol/kg in a 4 or 6 mol/kg solution of sodium chloride. An average difference of 3% for solubility and less than 0.05% for density between experimental and theoretical values was found for measured solubility of liquid CO_2 using synthetic seawater at temperatures from 278 K to 293 K and pressures from 6.44 MPa to 29.49 MPa and densities of the corresponding aqueous solutions [19]. An improved model has been applied to calculate the CO_2 solubility in pure water and aqueous NaCl solutions [6]. The study investigated a temperature range of 273 to 533 K and pressure of 0 to 2000 bar and showed that comparison of the model predictions with experimental data was within or close to experimental uncertainty, that was about 7% in CO_2 solubility. In terms of viscosity, it was shown that the measured the viscosity of CO_2 - saturated brines increased with increasing mole fraction of dissolved CO_2 [3]. Moreover, other recent studies showed that brine conductivity with dissolved CO_2 is also of interest in the context of electromagnetic monitoring and logging techniques [7]. The result of their work presented the effect of dissolved carbon dioxide on electrical conductivity and viscosity of aqueous sodium chloride solutions of varying salinity (20, 80, and 160) g/L and concluded that the variations of conductivity and viscosity as a function of temperature up to 100 ^oC are not modified by the presence of CO_2.

From the above, it is evident that a lot of work has been carried out regarding the solubility of carbon dioxide in aqueous solutions at different salinity range, pressure and temperature. It is not the object of this paper to describe the basics of solubility of CO_2 in aqueous sodium chloride solutions as details has been described elsewhere [14, 16]. However, there is a need to understand how specific parameters such as pH and electrical conductivity vary during quick saturation of brine with carbon dioxide.

The aim of this investigation is therefore to study a quick saturation of brine with carbon dioxide and relationship that exists between these parameters and dissolved carbon dioxide in solutions over salinity range 0-130 g/L. Even though a lot of work has been done regarding solubility of carbon dioxide in brine, few have mentioned what happened during quick saturation of brine with carbon dioxide and how it relates to pH changes and electrical conductivity. It is therefore essential to determine fully the effect of dissolved CO_2 on these parameters because the injection of CO_2 into saline aquifers involves multiphase flow in porous media and mass-transfer (dissolution) at the CO_2-brine interface which requires multi-dimensional solutions and requires additional knowledge to widen our understanding of this phenomenon especially during enhanced oil recovery by CO_2 injection.

Theoretical background

In order to check if the pH values obtained after the dissolution of carbon dioxide in aqueous sodium chloride solution is expected, the thermodynamics of the dissociation was calculated using the relationship below. The approximation we employed is that carbon is mostly present as hydrogencarbonate or carbon dioxide coming from carbon dioxide dissolution.

$$K_a = \frac{aH^+.aHCO_3^-}{(aCO_2)+(aH_2CO_3)} \quad (1)$$

K_a is the thermodynamics dissociation constants and a is the activity for each species in the reaction. However the amount of H_2CO_3 is negligible and hence the expression can be re-arranged to:

$$K_a = \frac{aH^+.aHCO_3^-}{(aCO_2)} \quad (2)$$

Since we can assume $a(H^+) \approx a(HCO_3^-)$,

$$a(H^+) = (K_a aCO_2)^{1/2}$$

and therefore

$$pH = (\frac{1}{2} pK_a - \frac{1}{2} \log aCO_2) \quad (3)$$

pK_a is acid dissociation constant and value of 6.35[1] for carbonic acid was used in the calculation. aCO_2 is the activity of CO_2 added to aqueous sodium chloride at different salinities. The pK_a value used is independent of salinity since this has been taken care of by the activity coefficient.

Activities of dissolved species can be written in terms of mole concentrations by use of an activity coefficient γ

For example, the CO_2 activity can be written as shown in equation 4 [15].

$$a_{CO_2} = \gamma * m_{CO_2} \tag{4}$$

where m_{CO_2} is the concentration of CO_2 in molality and γ is the activity coefficient.

The activity coefficient can be represented as a function of ionic strength of aqueous solution by Debye Hückel relationship as shown in equation 5[9]. However because equation 5 is only suitable for solutions of low ionic strength we have used the extended Debye Hückel equation as shown in equation 6 below.

$$\log(\gamma_{\pm}) = \frac{A|z_+ z_-| I^{1/2}}{1 + B\alpha I^{1/2}} \tag{5}$$

A and B are constants with value of 0.509 and 3.29 at 25 ^{o}C. I is the ionic strength in concentration units of mol/L, z is the charge number of an ion while α is related to the size of the ion.

$$\log(\gamma) = \frac{0.51 z^2 \sqrt{I}}{1 + (\alpha \sqrt{I/305})} \tag{6}$$

where γ is the activity coefficient of an ion charge $\pm z$ and size α (picometers) in an aqueous solution of ionic strength I at 25 ^{o}C.

At molality greater than 1 mol kg^{-1} the extended Debye Hückel also fails, thus Pitzer's equation which is a viral expansion of excess Gibbs energy that is valid up to molality ≤ 6 mol kg^{-1} can be used [13]. Pitzer's equation for calculating the mean ionic activity coefficient, $\gamma_{\pm}$ for 1:1 electrolyte is given by:

$$\ln \gamma\pm = f^{y\pm} + m\beta^{y\pm} + m^2 C^{y\pm} \tag{7}$$

where

$$f^{\gamma\pm} = -A^{\phi}\left\{\left(\frac{m^{1/2}}{1 + bm^{1/2}}\right) + (2/b)\ln(1 + bm^{1/2})\right\}$$

$$\beta^{\gamma\pm} = 2\beta^{(0)} + \frac{2\beta^{(1)}}{\alpha^2 m}\left\{\left(1 + \alpha m^{1/2} - \frac{\alpha^2 m}{2}\right)\right\} e^{-\alpha m^{1/2}}$$

$$C^{\gamma\pm} = 3/2C^{\phi}$$

M is the molality and equals the ionic strength, $f^{\gamma\pm}, \beta^{\gamma\pm}, C^{\gamma\pm}$ are virial coefficients, while $\beta^{o}, \beta^{1}, \alpha$ and b are coefficients related to the virial coefficients and ionic strength[13]. The activity coefficients used in this study were derived from Pitzer's equations above.

Experimental methods

Aqueous sodium chloride solution of different salinity was prepared by accurately weighing specific amount of NaCl and dissolve in 1000 mL of deionised water (w/v % as molalities). The composition of the sodium chloride salt used for the experiment is shown in Table 1 while Table 2 presents the composition of the seawater used for comparison. Magnetic stirrer was employed to completely dissolve the salt to have a homogenized solution. The next part of the experiment was to measure pH and conductivity of the prepared solutions at different temperature. For carbon dioxide injection, 80 mL of a NaCl solution was transferred to a 100 mL reactor of the SFE -CO_2 apparatus (Fig. 1), for injection with carbon dioxide at temperature of 25 ^{o}C and pressure of 100 bar (10MPa). The same procedure was repeated for all the samples at different salinity. The combined uncertainties of the set point pressure and temperature were 0.2 MPa and 1.0 ^{o}C. The injection time was 20 min at a flow rate of approximately 20 mL.min^{-1} at a pressure of 10 MPa (± 0.1MPa). Water and carbon dioxide form two phase mixtures immediately after injections which become single liquid phase when slowly raising pressure to set point. Constant pressure was maintained to equilibrate the system. For measurements a waiting period of time of about 1 h was observed after depressurizing the mixture to 1 bar. Since the gas phase formed at the top inside the reactor will keep the dissolved gas in solution and prevent escape of CO_2 it is can be assumed that the amount of CO_2 loss is negligible and equilibrium is reached.

1 mL of liquid sample at different salinity (containing dissolved carbon dioxide) was pippeted for spectral analysis with FT-IR. (The measurements were performed after depressurizing the mixture to 1 bar and at temperature of 25 ^{o}C). The spectroscopic measurements were performed using model FTIR spectrometer (Nicolet Avatar 370, Thermo Electron, USA). Each spectrum was recorded in the region of 4000-650 cm^{-1} by an average of 32 scans at a resolution of 4 cm^{-1}. For measurement of carbon dioxide with FTIR, it is necessary to purge the FTIR to obtain a good background value because of the sensitivity of FTIR to carbon dioxide in air. The method of peak fitting was used to determine the peak. The peak area at 2343 cm^{-2} measured to a baseline drawn between 2337.4 and 2347.9 as determined was used to establish the calibration peak. Carbon dioxide standards containing 1.16 and 2.14 g/l of CO_2 were used for calibration of the spectra. This method for quantification of concentration of CO_2 with FT-IR spectrometer has the

advantage of requiring few calibration samples. Estimation of the percentage error in the measurements was calculated to be 3.85%.

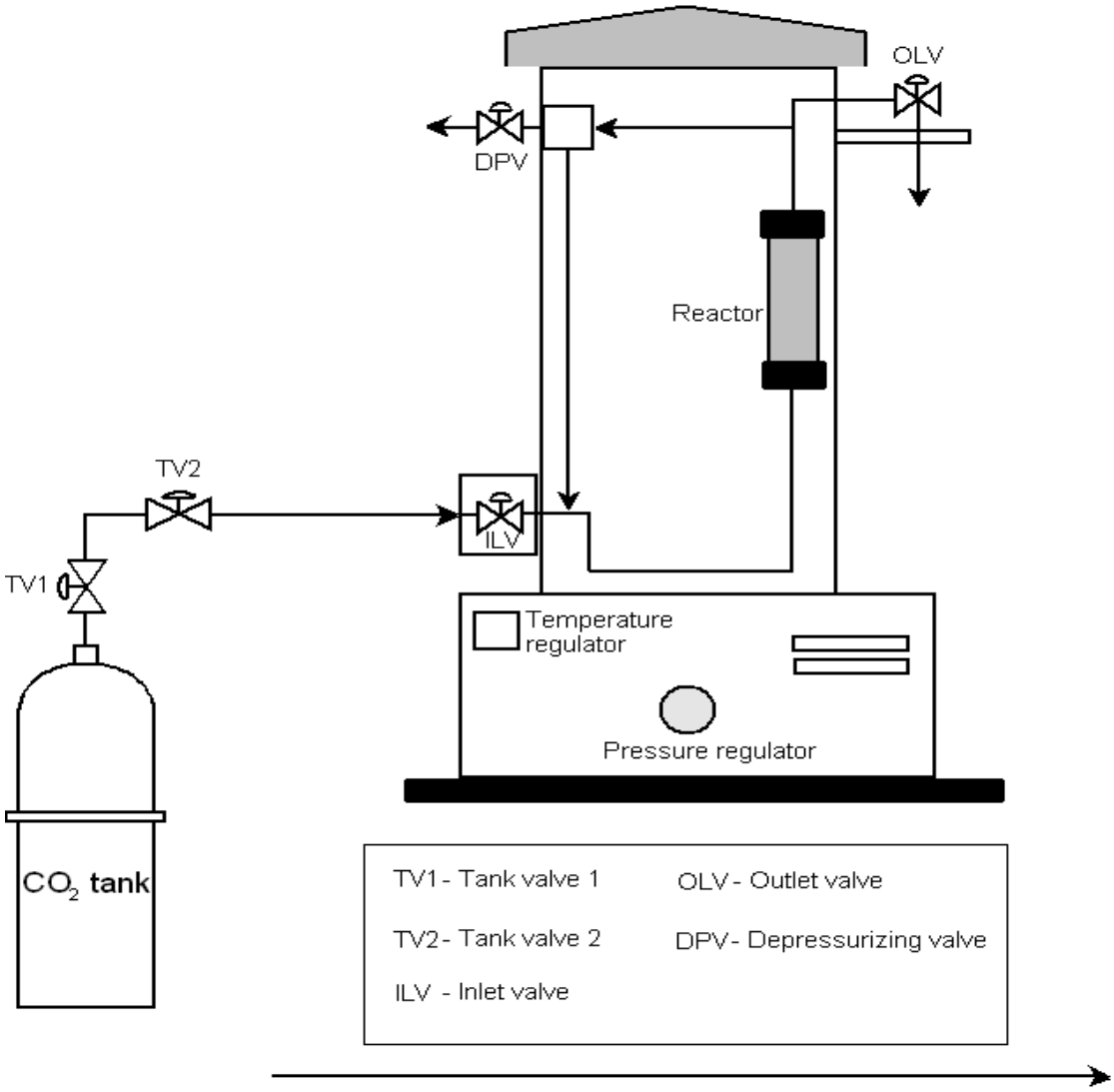


Fig. 1. Schematic diagram of supercritical reactor *Spe-ed SFE*

pH and electrical conductivity of the prepared solutions were measured before and after injection of carbon dioxide (same condition above). This was achieved by use of pH meter (Model: PHM 210, Radiometer Analytical) and conductivity meter (Model: CDM 210, Radiometer Analytical); the two instruments were calibrated prior to taking measurements. The pH meter was calibrated. The electrode was rinsed with distilled water from a wash bottle into an empty beaker before (in dry condition) immersing it into new solution. This was repeated every time electrode was moved from one solution to another in order to minimize contamination. Similarly, the calibration for electrical conductivity meter was carried out using a standard calibration method.

The activity coefficient for CO_2 in NaCl was also calculated using Pitzer's equation given in equation 7. The parameters used in the Pitzer's equation were taken from Campbell and Bhatnagar [5] and are shown in Table 3.

Table 1. Composition of the sodium chloride salt use for the experiment

Salt	Composition %					
	NaCl	Ca	Mg	K	SO_4	Moisture
Sodium chloride	99.980	0.001	0.001	0.006	0.009	0.001

Table 2. Composition of the seawater use for the experiment

Sample	Composition %					
	NaCl	Ca	Mg	K	Br	SO_4
Seawater	94.18	0.013	0.036	0.025	0.0032	2.00

Table 3. Parameters for NaCl used in equation 7

Temp, ^oC	$\beta^{(o)}$	$\beta^{(1)}$	$C^{(\phi)}$	α	b
25	0.0388	0.6522	0.0039	2	1.2

Results and discussion

The effects of CO_2 dissolution on the relationship of the measured parameters; pH-conductivity at different salinities and temperature 25 ^oC were investigated. Fig. 2 shows the obtained spectra at a higher frame scale with zero line for all the spectra obtained for range of salinity (0-130 g/L). From the figure, it can be observed that the absorbance is directly related to concentration which again is related to the salinity of the solution.

The result for the concentration of dissolved carbon dioxide in aqueous sodium chloride solutions is presented in Fig. 3. From the plot of the results, it is indicated that the concentration of CO_2 decreases with increased salinity i.e. from 0.055 - 0.0085 moles/L for salinity range of 0-130 g/L. This is because the amount of gas dissolved decreases because more water molecules are immobilized by the salt ions suggesting a kind of salting out effect [8, 14, 17]. This means that the equilibrium is shifted toward the left

where we can assume that the species present represents the sum of both H_2CO_3 itself and dissolved CO_2 as shown in equation 8.

$$CO_{(g)} \Leftrightarrow CO_{2(aq)} + H_2O_{(l)} \Leftrightarrow H_2CO_{3(aq)} \Leftrightarrow H^+_{(aq)} + HCO^-_{3(aq)} \quad (8)$$

Therefore we can deduce the following simple relationship for measured dissolved CO_2 and salinity (equation 9) with a R^2 value of 0.95.

$$[CO_2] = -0.0003S + 0.056 \quad (9)$$

where S is the salinity of the aqueous solution.

This equation showed that salinity play a major role in the amount of carbon dioxide and the species of CO_2 available for chemical reaction when carbon dioxide is dissolved in water and can therefore be inferred to be a significant factor in controlling the amount of dissolved CO_2 present in aqueous salt solutions.

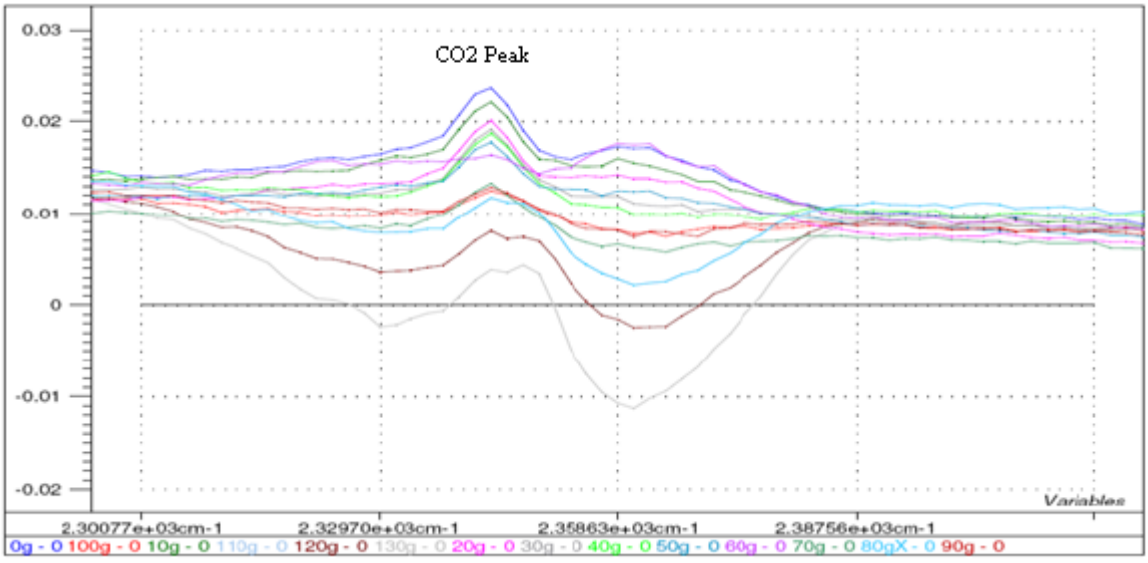


Fig. 2. FT-IR spectra of the sodium chloride solution with peak area for dissolved CO_2

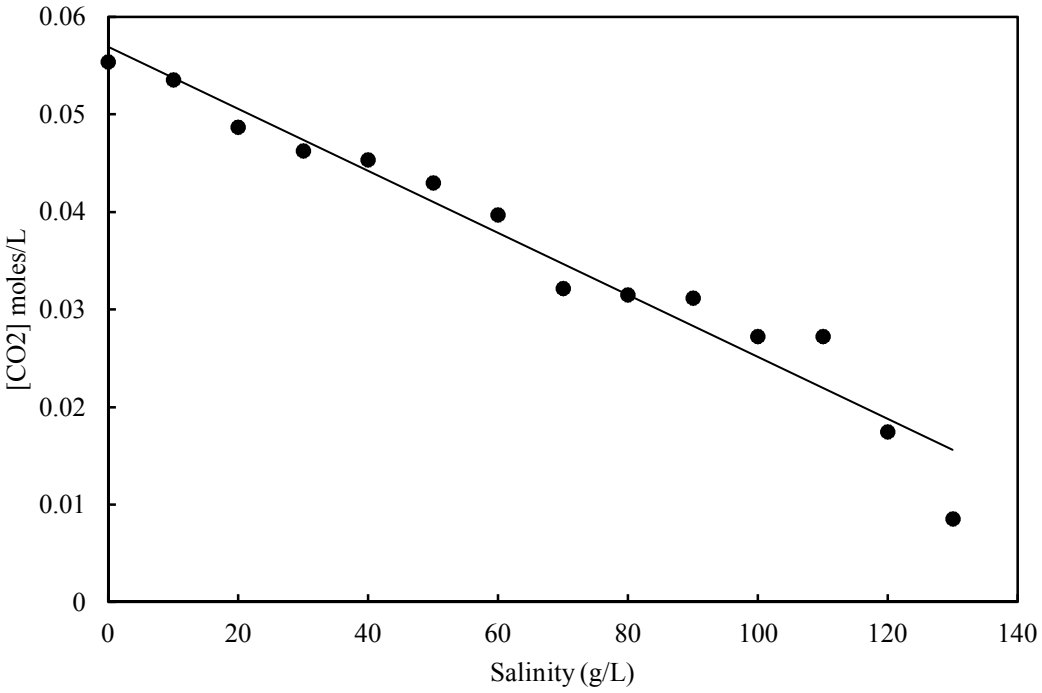


Fig. 3. Concentration of dissolved CO_2 at different salinity

Measurements were taken of pH and electrical conductivity to see the variation in their relationships after injecting the sodium chloride solutions with carbon dioxide. Fig. 4 shows that the measured pH of the aqueous solutions (0-130 g/L) before the injection of carbon dioxide was 5.13-6.25, however the values of the measured pH was 3.37-3.98 after injection due dissolved CO_2 indicating that there is a pH difference of 0.61 between salinity 0-130 g/L after carbon dioxide dissolution. The specie of CO_2 that will exist when dissolved in water varies in proportion based on water temperature, salinity and pressure [2]. In this case, it evident that carbonic acid (H_2CO_3 + CO_2) concentration is greater at low salinity and decreases with increasing salinity as indicated by value of pH 3.37-3.98 measured for 0-130 g/L. It is hard to find experimental data under the same condition from the literature for comparison; however data from Barner et al. [4] showed a final pH value of 3.35 for 1 Molar of NaCl solution at pressure of 10 MPa and temperature of 27 ^{o}C after dissolution of CO_2. In our experiment the result indicated that the final pH of 1 Molar NaCl solution at 10 MPa and 25 ^{o}C when extrapolated from the graph will be 3.6. The calculated concentration of hydrogencarbonate (Fig. 5) decreased with increasing salinity with a less pronounced change over the range of salinity measured.

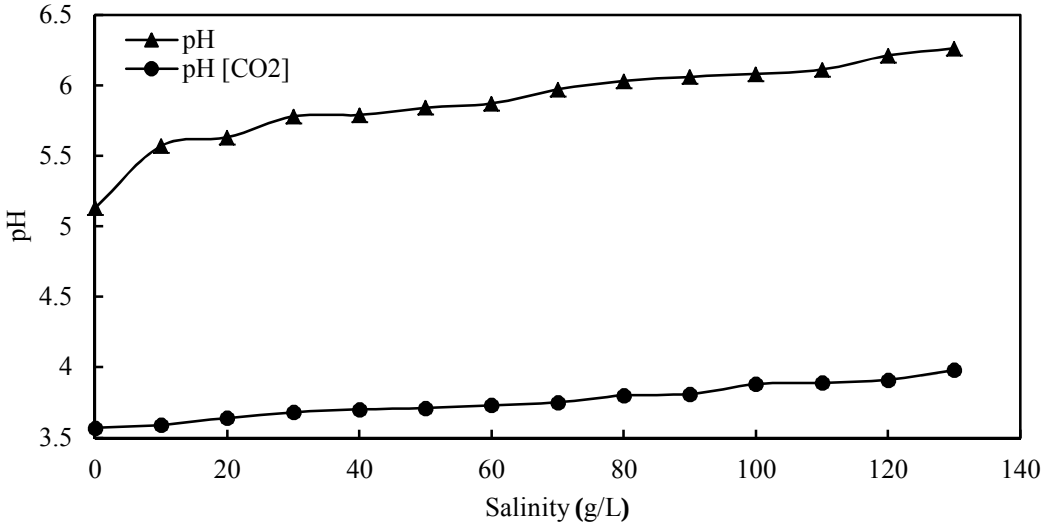


Fig. 4. pH before and after injection of carbon dioxide

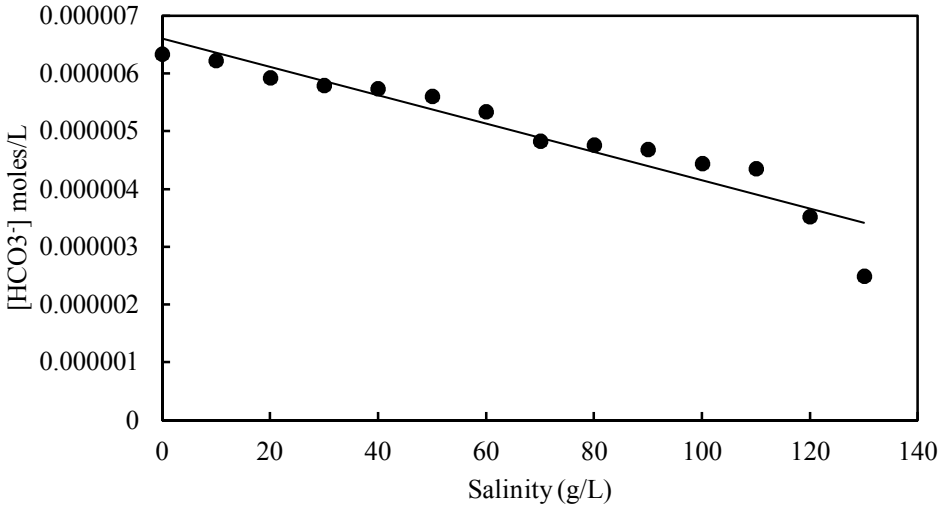


Fig. 5. Concentration of hydrogencarbonate at different salinity after dissolution of carbon dioxide

The relative variation in pH and electrical conductivity measurement before and after injection of carbon dioxide to aqueous solutions at different salinity was calculated and plotted against the salinity of the medium (Fig. 6). A polynomial relationship was observed between the relative variation of pH and salinity after dissolution of carbon dioxide that reaches maximum value at 70 g/L and started falling as the salinity increases (equation 10). The relationship indicated a kind of salting out effect after salinity 70 g/L, which tries to neutralize hydrogencarbonate.

$$\frac{\Delta pH}{pH} = -0.00001\ S^2 + 0.0025\ S + 0.48 \quad (10)$$

where S is the salinity in g/L.

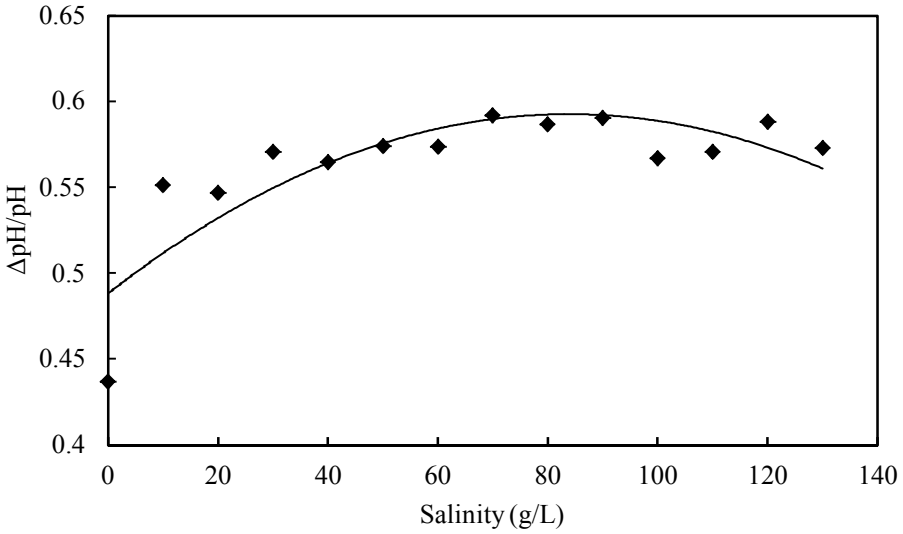


Fig. 6. Relative variation of pH with salinity after carbon dioxide injection

In order to check if the pH values obtained after the dissolution of carbon dioxide in aqueous sodium chloride solution is expected, the experimental pH was compared with theoretical pH. The theoretical pH was calculated using equation 3. The value of activity coefficient at each salinity (measured as molality) used was derived from Pitzer's equation given in equation 7. The result obtained is presented in Fig. 6. The plot shows that at 25 ^{o}C deviation exists between the two even though the trends were similar. The theoretical and experimental pH differ by average of 0.17 units at salinity < 70 g/L and by an average of 0.10 units at salinity > 70 g/L suggesting a closer agreement at high salinity. Even though, there is an observed deviation from experimental pH that can be due to uncertainty in activity coefficients calculation (i.e. uncertainty in the magnitude of the ionic strength correction). The theoretical pH derived from Pitzer's activity coefficients can be used to approximate the changes in pH over the ionic strength range in this study.

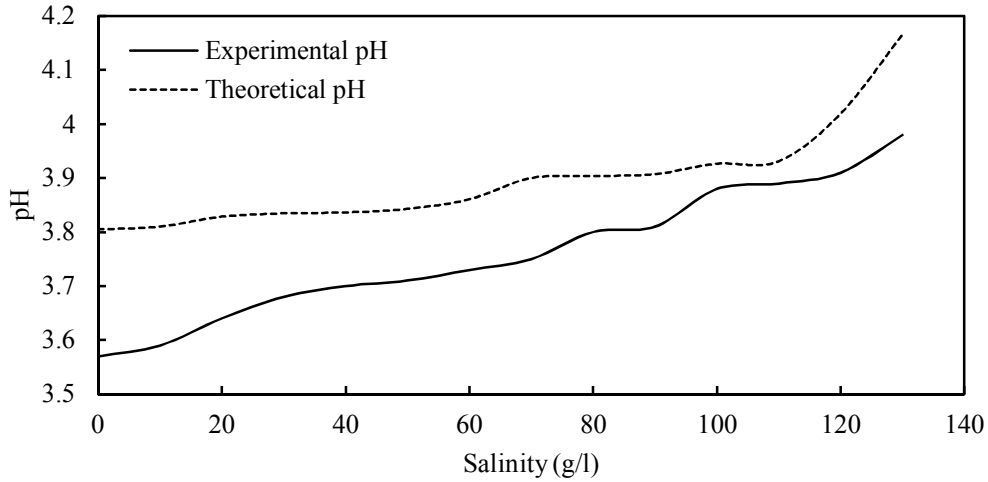


Fig. 7. Comparison of theoretical pH derived and experimental pH after dissolution of carbon dioxide in aqueous sodium chloride solutions

Fig. 8 shows the results for electrical conductivity relationship with salinity. The electrical conductivity is relatively unchanged before and after injection of carbon dioxide. The dissolved CO_2 probably do not affect the degree of ionization significantly, or since conductivity is directly related to salinity, the ions from the dissolved salts control the conductivity, thus the negligible effect of dissolved CO_2 on the conductivity of the solution. This observation confirms the earlier findings of dissolved CO_2 and electrical conductivity relationship in aqueous sodium chloride solution reported by Fleury and Deschamps [7].

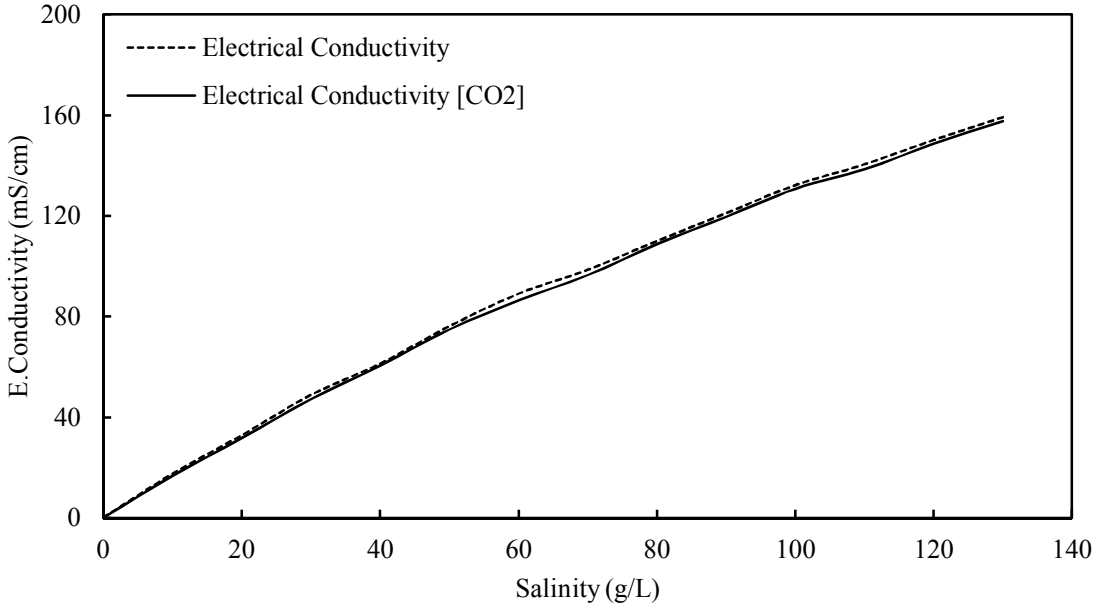


Fig. 8. Conductivity and salinity relationship before and after injection of carbon dioxide

The variation of carbon dioxide solubility with temperature at constant salinity is shown in Fig. 9. Salinity 30 g/L was selected because it is close to the salinity of seawater. It is evident from the plot that the concentration of dissolved carbon dioxide decreases with increasing temperature. The dissolution of CO_2 decreases with increasing temperature this suggests that less and less carbon dioxide is in aqueous solution at constant salinity when temperature was increased from 25 ^oC to 45 ^oC. Temperature is known to affect the solubility of CO_2 in brine [10, 18]. The relationship between the amount of dissolved CO_2 and temperature is given by equation 11 with a R^2 value of 0.97.

$$[CO_2] = -0.002T + 0.09 \quad (11)$$

Finally, the result of the dissolved CO_2 in aqueous sodium chloride at a specific salinity was compared with that dissolved in seawater; the composition of the seawater used is given in Table 2. The concentration of dissolved CO_2 in seawater of salinity 22 g/L was found to be around 23.07 mg/L. This amount is equivalent to about 0.00055 moles/L with measured pH of 7.80 at a temperature of 25 ^oC and represents the total dissolved carbon dioxide. At 1 atm the theoretical concentration of dissolved CO_2 in seawater is about 0.00882 moles/L at 25 ^oC. The gases dissolved in sea water are known to be in constant equilibrium with the atmosphere but their relative concentrations depend on each gas solubility, which depends also on salinity and temperature. The value is less than the value obtained when compared to extrapolated aqueous solution of 22 g/L injected CO_2 at 10 MPa in our experiment (0.05 moles/L) probably because pressure was 100 times greater and the total dissolved inorganic carbon load of seawater varies over time as dissolved CO_2 is continuously consumed by the production of carbonate and organic carbon sediments [4].

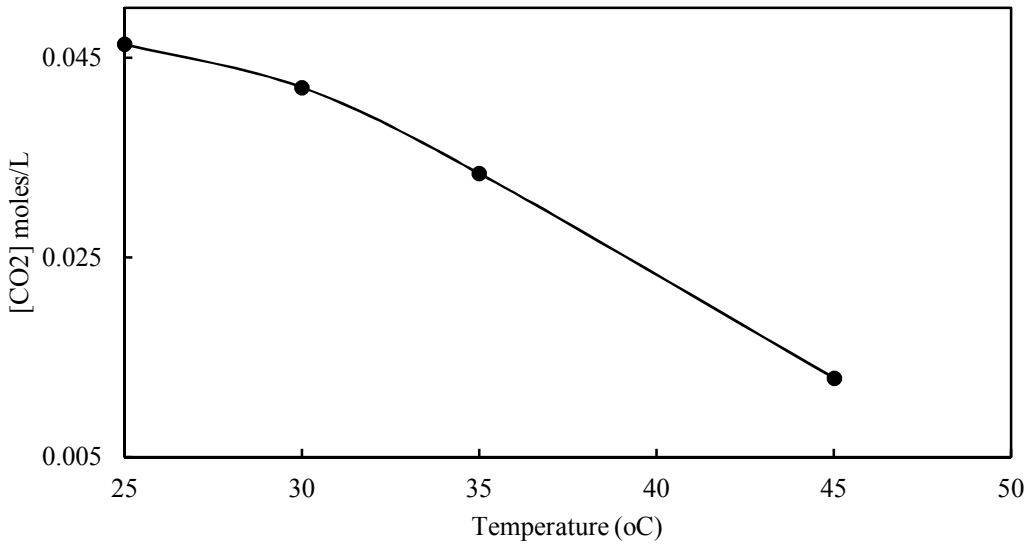


Fig. 9. Carbon dioxide concentration as function of temperature at salinity 30 g/L

Conclusion

Laboratory investigation of dissolution of CO_2 in aqueous sodium chloride solutions and effect on pH and electrical conductivity was investigated. The experiments were performed with salinity range of 0-130 g/L that fall within the range of many oil reservoirs. It was found that concentration of dissolved carbon dioxide decreased with increasing salinity. Also presence of CO_2 alters pH of aqueous sodium chloride solutions considerably and there was agreement between the experimental pH and the theoretical pH calculated using the activity coefficients derived with Pitzer's equation but with a small deviation. Additionally, the relationships between the relative variation in pH and salinity with dissolved CO_2 can be represented by a simple polynomial function that reached a maximum at 70 g/L before decreasing at higher salinities which suggested a kind of salting out effect. However there was no appreciable effect on the electrical conductivity of the solution after dissolution of CO_2 in brine. This confirms previous observation because the dissolved CO_2 probably do not affect the degree of ionization significantly since the dissolved salts control the conductivity of the aqueous solution.

References

[1] P.W. Atkins, Physical Chemistry Fifth Edition. Oxford University Press, **1994**.

[2] S. Bachu, K. Micheal, J.J. Adams, Effect on in-situ conditions on aquifer capacity for CO_2 sequestration in solution, Second Annual Conference on sequestration: developing and validating the technology base to reduce carbon intensity, Alexandria VA, **2003**.

[3] S. Bando, F. Takemura, M. Nishio, E. Hihara, M. Akai, Viscosity of aqueous NaCl solutions with dissolved CO_2 at (30 to 60) ^oC and (10 to 20) MPa. J. Chem. Eng. Data, **2004**, 49, 1328.

[4] R.A. Berner , A. C. Lasaga, R. M. Garrels, The carbonate-silicate geochemical cycle and its effect on atmospheric carbon dioxide over the past 100 million years. Am. J. Sci., **1983**, 283, 641.

[5] A.N. Campbell, O.N. Bhatnagar, Osmotic and activity coefficients in water from 150 to 250 ^oC. J. Chem. Eng. Data, **1984**, 29, 166.

[6] Z. Duan, R. Sun, An improved model calculating CO2 solubility in pure water and aqueous NaCl solutions from 257 to 533 K and 0 to 2000 bar. Chemical Geology, **2003** 193, 257.

[7] M. Fleury, H. Deschamps, Electrical conductivity and viscosity of aqueous NaCl solutions with dissolved CO_2. J. Chem. Eng. Data **2008**, 53, 2505.

[8] M. Georgenyi, J. Dewulf, H.V. Langenhove, K. Heberger, Aqueous salting out effect of inorganic cations and anions on non-electrolytes. Chemosphere, **2006**, 65 (5)

[9] E. Hückel, Theorie Konzentrierterer wässriger lösungen starker electrolyte. Physikalische Zeitschrift, **1925**, 26, 93-147.

[10] C. Lu, W. Han, S. Lee, D. Thorne, R. Esser, B. McPherson, Effects of density and mutual solubility of CO_2-brine system on CO_2 storage in geological formations. American Geophysical Union, Fall Meeting **2008**, abstract no. H23D-0998.

[11] T. Ohsumi, N. Nakashiki, K. Shitashima, K. Hirama, Density change of water due to dissolution of carbon dioxide and near field behaviour of CO_2 from a source on deep sea floor. Energy Convers. Management **1992**, 33, 685.

[12] W.J. Parkinson, N.D. Nevers, Partial molal volume of carbon dioxide in water solution. Ind. Eng. Chem. Fundam., **1969**, 8, 709.

[13] K.S Pitzer, and G. Mayorga, Thermodynamics of electrolytes, II: activity and osmotic coefficients with one or both ions univalent. J. Phys. Chem. **1973**, 77 (19): 2300.

[14] S. Portier, C. Rochelle, Modeling CO2 solubility in pure water and NaCl-ytpe waters ` from 0 to 300 ^oC and from 1 to 300 bar. Chemical Geology, **2005**, 217, (3-4), 187.

[15] R.M. Pytkowicz, Activity coefficients of bicarbonates and carbonates in seawater. Limnology and Oceanography, **1975**, 20 (6).

[16] B. Rumpf, H. Nicolaisen, C. Ocal, G. Maurer, G., Solubility of carbon dioxide in aqueous solutions of sodium chloride: experimental results and correlation. J. Solution Chemistry, **1994**, 23 (3), 431.

[17] H.T. Schaef, B.P. McGrail, Direct measurments of pH and dissolved CO_2 in CO_2-H20 brine mixtures to supercritical conditions. AAPG annual meeting, Dallas Texas, **2004**.

[18] N. Spycher, K. Pruess, CO2-H2O mixtures in the geological sequestration of CO_2, II: partitioning in chloride brines at 12-100 ^oC and up to 600 bar. Geochimica et Cosmochimica Acta, **2005**, 69 (13).

[19] H. Teng, A. Yamasaki, M.K. Chun, Solubility of liquid CO2 in water at temperatures from 278 K to 293 K and pressures from 6.44 MPa to 29.49 MPa and densities of the corresponding aqueous solutions. J. Chem. Thermodyn., **1997**, 29, 1301.

Paper II

Laboratory investigation of pH, electrical conductivity, and temperature relationships in aqueous high salinity sodium chloride solutions

Ismaila A. Jimoh, Erik G. Søgaard* and Svetlana N. Rudyk Section of Chemical Engineering, Aalborg University, Esbjerg Institute of Technology, Denmark. E-mail: egs@bio.aau.dk; Telephone: +4599407622

*Correspondence author

Abstract

Basic knowledge of the relationships between environmental and physical parameters such as pH, electrical conductivity, salinity and temperature are essentials to successful applications of many industrial and natural processes. In this study the dissolution of sodium chloride in water has been treated physico-chemically and the relationship between pH, electrical conductivity, salinity and temperature in aqueous sodium chloride were investigated. The results showed that the pH of aqueous sodium chloride solutions increases with increasing salinity. Within the salinity range of 0-130 g/L, measured pH of the aqueous sodium chloride solution can be increased by one unit with a gradient of 0.005/ $g.L^{-1}$. This effect on pH was due to salt effect on activity coefficient which also implies non ideality for aqueous solution of varying salinity. The average gradient for increase in electrical conductivity with increase salinity at a constant temperature was approximately 0.009 $mS.cm^{-1}.gL^{-1}$. Comparison of theoretical and measured data showed a deviation of 0.48 and 5.7 for pH and electrical conductivity respectively. Additionally, regression analysis indicated that simple empirical relationships can quantify the observed relationships between the variables of pH and electrical conductivity and independent factors of salinity and temperature. These simple models can be useful in many different applications.

Keywords: Desalination process, salinity, pH, electrical conductivity, temperature, relationships

1. Introduction

An accurate understanding of parameters such as pH, temperature, salinity and electrical conductivity is fundamental to many applications especially water and environmental monitoring. In desalination process, the end result is clean and useful water, there are several aspects of the process which need to be carefully controlled and monitored so as not to negatively impact on the environment. For example, in reverse osmosis desalination process, various important parameters such as pH and conductivity must be controlled [1]. Also, the solid-water interface plays an important role in biology, environment, and desalination technology since the acidity or alkalinity of the surrounding aqueous medium measured by pH is significant because it controls the extent of ionization, enzyme function and oxidation potential [2, 13]. It is expected that all these knowledge can provide an insight to identify the factors that constrain use of such applications in subsurface environments, thereby providing a set of criteria by which the suitable models can be assessed because many variables can influence seawater reverse osmosis design [15].

Moreover, electrical conductivity is widely used in monitoring the mixing of fresh water and saline water, separating stream hydrographs, and geophysical mapping of contaminated ground water [9]. The amount of dissolved salt can affect the quality of water and also have critical influence on the aquatic life. Additionally, the performance characteristics of fluid system used in analyses to arrive at the optimum system designs in desalination plant depends on the feedwater salinities that has a strong relationship with conductivity [15]. Earlier work dated back to 1934 when Thomas et al. [24] measured the electrical conductivity of sea water. Calles and Calles 1990 [5] worked on method for temperature correction of electrical conductivity data based on regression analysis of the actual temperature/conductivity relationship of a number of water samples from natural streams showed that the generally used formulae for temperature correction give corrected results that deviate considerably from the values determined by actual measurements. The error increases with decreasing water temperature and may result in data that deviate by as much as 20 per cent from the true values. Patience et al. [19] reported that the limiting equivalent conductance A_o of NaCl increases linearly with decreasing density from 0.75 to 0.3 g-cm^{-1} and also with increasing temperature from 100 to 350 °C, and Vollmer et al. [25] develop a new algorithm to calculate salinity from in-situ conductivity measurements. Hayasi [9], working with natural waters with vastly different compositions and salinities showed that electrical conductivity - temperature relation of all water samples can be well represented by the linear equation with a common compensation factor in a temperature range of 0-30 ^{o}C.

There are not so many studies dedicated to pH variation in aqueous sodium chloride even though there is large volume of work related to influence of pH on different chemical parameters. Illustrations of work

related to pH investigation in aqueous salt solutions include those of Bada [4] who worked on the pK_a of weak acid as a function of temperature and ionic strength using a pH meter. There result indicated this technique can be used to give good estimates of temperature variation of the pKa of most weak acids however due to the liquid junction potential problems involved in the use of the pH meter, the result should not be considered as nearly as accurate as those determined from electromotive cells measurements. Marcus [17], also determine pH in highly saline waters and recommended that the pH of highly saline waters should be measured with a cell of type (l), i.e. with a glass electrode responsive to hydrogen ions and a reference electrode (such as calomel) incorporating a salt bridge (3.5 mol/L KC1) that provides a liquid junction.

The work of Malinowski [16], showed the methods for calculating the pH of aqueous solutions of salts of monoprotic acids and bases and found out that the simultaneous-equation method has the capability to solve a wide variety of complicated chemical equilibrium problems including the pH of aqueous solutions of salt. All these studies pointed to importance of these parameters especially in the field of water and environmental sciences. However, most of the earlier works cover low salinity range, study each parameter differently, or did not study the relationships that existed between pH and electrical conductivity and salinity together over the range of salinities presented in this study.

It is the object of this investigation to study the relationship between pH, conductivity, temperature in aqueous high salinity solutions of sodium chloride (0-130 g/L) and different range of temperature (13- 45 ^oC). The salinity range was selected because it covered the range of salinity for different seawaters that can be exploited in desalination processes.

1.1 Theory

The dissociation of salt in water can be written as shown in equation 1[4].

$$HA \rightleftharpoons H^+ + A^- \qquad (1)$$

The dissociation constant for eqn. (1) is given by

$$K_a = \frac{a_{H^+} a_{A^-}}{a_{HA}} = \frac{[H^+][A^-]}{[HA]} \frac{\gamma H^+ \gamma A^-}{\gamma HA} \qquad (2)$$

where a is the activity of the species H^+, A^- and HA in aqueous solution and γ is the respective activitiy coefficients and the bracket refers to molar concentrations.

The pH can be rewritten from equation 2 as

$$pKa = pH - \log\frac{\left[A^-\right]}{HA} - \log\frac{\gamma A^-}{\gamma HA} = pKa - \log\frac{\gamma A^-}{\gamma HA} \quad (3)$$

When $\left[A^-\right] = \left[HA\right]$, $pKa = pH$ and $pKa = pH - \log \gamma A^- / \gamma HA$

In ionic solutions, the interactions between ions are very strong and thus the approximation that solute activities can be replaced by their molalities does not hold any longer. The activity a is related to the molality m by the equation shown below [3]:

$$a = \gamma * \frac{m}{m^{\varnothing}} \quad (4)$$

where is $m^{\varnothing}$ is the molality of standard state hypothetical solution, the activity coefficient γ depends on the composition, molality and temperature of the solution. As the solution approaches idealities at low molalities, the activity coefficient tends towards 1:

As shown by Debye-Hückel theory [7] activity coefficient can be used explain the departures from ideality in ionic solutions as a result of long range and strength of the Columbic interaction between ions [20]. At very low concentrations, the activity coefficient can be calculated from the Debye-Hückel limiting law [3], to see how it varies with salinity.

$$\log \gamma \pm = \left|z_+ + z_-\right| A\left(I / m^{\theta}\right)^{1/2} \quad (5)$$

where A=0.509 for and aqueous solution at 25 ^{o}C, m^{θ} is the molality, z_+ and z_- are the positive and negative charge numbers and I is the ionic strength of the solution. The ionic strength I of the solution is given by:

$$I = \frac{1}{2}\sum_i z_i^2 m_i \quad (6)$$

where z_i is the charge number of an ion (positive for cations and negative for anions) and m_i is the molality. When the ionic strength of the solution is too high for the limiting law to be valid, the activity coefficient may be estimated from the extended Debye-Hückel law [3]:

$$\log \gamma_{\pm} = -\frac{A\left|z_{+}\, z_{-}\right|(I/m^{\varnothing})^{1/2}}{1+B(I/m^{\varnothing})^{1/2}} \quad (7)$$

where B is a dimensionless constant. Other parameters in the equation have been defined above.

At molality greater than 1 mol kg^{-1} the extended also fails, thus Pitzer's equation that is valid up to molality ≤ 6 mol kg^{-1} can be used. Pitzer's equations [21] for the activity coefficient of the electrolyte in a solution of molality m and ionic strength I is given by

$$\ln \gamma \pm = f^{y\pm} + m\beta^{y\pm} + m^{2}C^{y\pm} \quad (8)$$

m is the molality and equals the ionic strength, $f^{\gamma\pm}, \beta^{\gamma\pm}, C^{\gamma\pm}$ are virial coefficients. The activity coefficient used in this study was derived from experimental values of Rumpf et al. [22] that was based on the Pitzer's equation described above.

2. Materials and methods

2.1 Sample preparation

The first stage involves preparation of solutions of sodium chloride (99.9 0% NaCl) with different salinity. The method adopted was accurately weighing specific amount of sodium chloride i.e. 10, 20, 30, 40 g etc. using a mass balance and dissolve in 1000 mL of deionised water (w/v% as molalities) from Denmark. Magnetic stirrer was employed to completely dissolve the salt to have a homogenized solution. The specific temperatures were achieved by placing the sample flask in water bath until a constant value was reached for each level of temperature. For lower temperatures below room temperature of 25 ^{o}C, ice cubes were added to the water bath and regulated to ± 0.10 ^{o}C. Measurement for higher temperature was carried out with the aid of water bath with an in-built electric heater also regulated to ± 0.10 ^{o}C. Potential influence of dissolved CO_2 in the water samples was reduced to minimal by boiling off the dissolved gas in the demineralized water used for the experiment before preparing the aqueous solutions for the experiment in glass where thus atmospheric CO_2 does not equilibrate with the solutions. The calculated standard deviation between two set of measurements of pH and electrical conductivity for the range of salinity and temperature measured are 0.023 and 0.026 respectively. The first set of demineralized water used is referred to as water A (pH = 5.49 at 0 g/L and temperature 25 ^{o}C) while water B (pH = 6.13 at temperature 25 ^{o}C) was a distilled water used for comparison of measured parameters.

2.2 Calibration and measurements

The next part of the experiment was to measure pH and electrical conductivity of the prepared aqueous solutions at different temperature.

2.3 pH measurement

This was achieved by use of pH meter (Model: PHM 210) and conductivity meter (Model: CDM 210); the two instruments were calibrated prior to taking measurements. Calibration of pH meter was done by putting the electrode into pH 4.0 and pH 7.00 buffer solutions. The electrode was rinsed with distilled water from a wash bottle into an empty beaker before immersing it into new solution. This was done every time electrode is moved from one solution to another in order to minimize contamination. pH meter was automated for temperature correction and the electrode and solution was allowed to equilibrate thermally before measurements were taken. The experiment was repeated two times and the average was found.

2.4 Electrical conductivity measurement

The calibration for electrical conductivity meter was done with solution of known salinity. The measuring bottle was rinsed with demineralized water. 100 mL of the calibration solution was put into the measuring bottle and the conductivity meter was put into the solution. Time was allowed for the solution to adjust to the temperature. The conductivity meter was adjusted until the display reads the same salinity as the calibration solution. For the electrical conductivity, linear temperature compensation for electrical conductivity is normally assumed. The linear temperature compensation assumed that the temperature coefficient of variation α has the same value for all measurements of temperature [12]. Generally over a limited temperature range, the way temperature affect conductivity can be modelled linearly using the equation of Sorensen and Glass [23]:

$$EC_t = EC_{25}[1 + a(t - 25)] \qquad (9)$$

where EC_t is electrical conductivity at temperature t (^{o}C), EC_{25} is electrical conductivity at 25^{o}C, and a ($^{o}C^{-1}$) is a temperatures compensation factor and can be taken as approximately 2% of electrical conductivity per 1 ^{o}C of temperature [9].

2.5 Derivation of activity coefficient values

The mean activity coefficient of each solution was derived from experimental results of Rumpf et al.[22] for mean ionic activity coefficient for NaCl at 25 ^{o}C and the plot of the experimental result of where the values of activity coefficient used in this study was extrapolated is shown in Figure 1. This method of extrapolation offers a quick way of deriving activity coefficient for high salinity aqueous solutions because the parameters of equations for Pitzer's equations for specific conditions are hard to come by and also Pitzer's equation can explains variation in ionic activity for aqueous sodium chloride for molality $\leq 6.0 molkg^{-1}$ which adequately covers the molality range for our experiment.

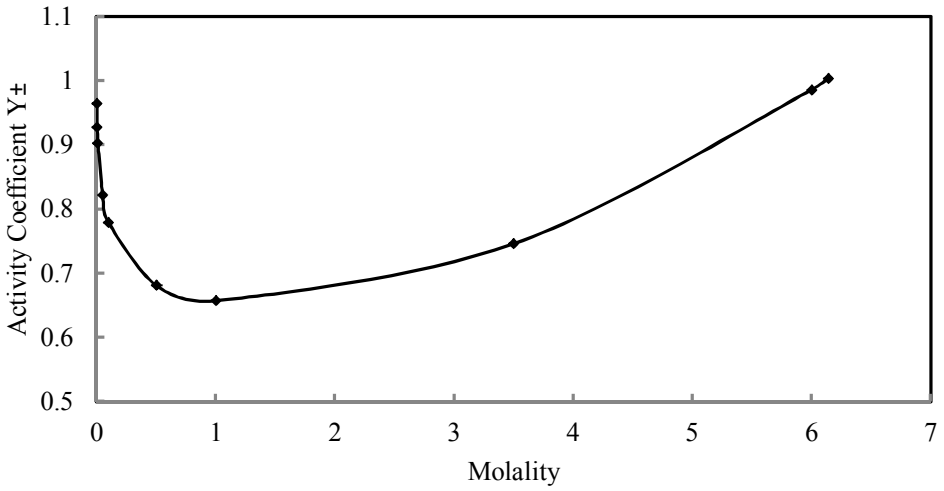


Fig. 1. Plot for extrapolation of activity coefficients values used in this study

2.6 Data analysis

Data collected were subjected to statistical analysis using Statgraphics Plus®. Multifactor Anova was used to test differences while general linear model (GLM) was used to establish the relationships between different parameters.

3. Results

The derived values for mean ionic activity coefficient for 0-130 g/L salinity in our experiment are given in Table 1. Tables 2 and 3 showed the results of the measured parameters.

Table 1. Mean ionic activity coefficient for NaCl at 25 ^{o}C as derived from Rumpf et al.[20] experimental result.

Salinity g/l)	Molality mol/kg	Y±
0	0.00	1.000
10	0.171	0.735
20	0.342	0.706
30	0.513	0.685
40	0.684	0.678
50	0.856	0.663
60	1.027	0.650
70	1.198	0.660
80	1.369	0.670
90	1.540	0.675
100	1.711	0.680
110	1.882	0.685
120	2.053	0.690
130	2.225	0.700

Table 2. pH measurement at different salinity and temperature.

Salinity (g/L)	pH/Temperature °C								
	13 °C	18 °C	23 °C	25 °C	28 °C	33 °C	38 °C	43 °C	45 °C
0	5.84	5.72	5.51	5.49	5.31	5.22	5.10	4.99	4.89
10	6.31	6.18	5.96	5.93	5.77	5.67	5.56	5.46	5.35
20	6.34	6.22	6.01	5.97	5.81	5.72	5.60	5.49	5.40
30	6.48	6.36	6.16	6.12	5.95	5.87	5.75	5.64	5.54
40	6.51	6.39	6.18	6.14	5.98	5.88	5.77	5.66	5.56
50	6.55	6.43	6.22	6.18	6.01	5.93	5.81	5.70	5.60
60	6.57	6.45	6.25	6.20	6.04	5.95	5.83	5.73	5.63
70	6.67	6.56	6.36	6.31	6.15	6.06	5.94	5.82	5.73
80	6.74	6.62	6.41	6.36	6.21	6.12	6.00	5.90	5.79
90	6.76	6.64	6.44	6.39	6.23	6.14	6.02	5.91	5.81
100	6.79	6.67	6.46	6.42	6.26	6.17	6.05	5.94	5.84
110	6.85	6.73	6.52	6.46	6.31	6.23	6.11	6.00	5.90
120	6.92	6.80	6.59	6.50	6.38	6.30	6.18	6.07	5.97
130	6.96	6.84	6.64	6.55	6.42	6.34	6.23	6.10	6.01

Table 3. Electrical conductivity measurements at different salinity and temperature

Salinity (g/L)	E. Conductivity (mS/cm)/Temperature (oC)								
	13°C	18 °C	23 °C	25 °C	28 °C	33 °C	38 °C	43 °C	45 °C
0	0.06	0.07	0.08	0.09	0.09	0.10	0.11	0.12	0.13
10	12.84	14.53	16.92	17.60	18.59	19.94	21.47	23.32	23.99
20	24.02	27,14	31.63	32.87	34.77	37.28	40.35	43.60	44.87
30	35.79	40.50	47.11	48.99	51.81	55.57	59.81	64.99	66.89
40	44.76	50.65	58.90	61.63	66.76	69.50	74.59	81.28	83.83
50	55.48.	62.79	73.10	75.21	78.33	82.14	87.18	94.74	100.67
60	64.37	70.84	79.67	82.07	86.18	89.94	93.31	101.88	112.27
70	72.09	79.52	84.79	86.58	92.29	96.86	102.68	112.82	121.61
80	80.56	86.18	95.85	98.08	104.60	110.04	117.93	124.19	131.30
90	88.54	95.19	106.48	109.13	116.17	122.47	130.66	140.78	146.43
100	96.72	106.12	117.20	120.20	127.42	135.48	144.08	154.45	161.62
110	102.60	115.05	125.15	128.50	136.52	144.30	155.40	161.30	172.72
120	109.50	120.92	134.40	138.17	146.45	150.00	166.33	173.82	185.13
130	116.43	128.73	143.15	147.27	155.80	165.77	177.95	185.41	198.56

From the results of the experiments for water A, it was observed that there exist significant relationships between the measured parameters; these results are presented below. The output shows the results of fitting a linear model to describe the relationship between pH and salinity (Figure 2). The result showed that pH increases with salinity at constant temperature almost as a linear function. The mean difference in pH between 0 g/L and 130 g/L of salinities for range of temperatures is approximately 1.0 for all temperatures.

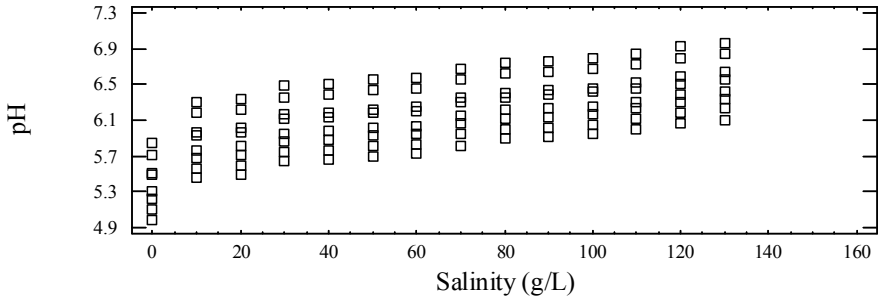


Fig. 2. Variation of pH with salinity

The gradient of the increase of pH with salinity at different temperature was also calculated, the gradient ranged between 0.0055 and 0.0063 /g.L^{-1} with a mean gradient of 0.005/ g.L^{-1} for the range of salinity and temperature measured for this study. The equation of the fitted model between pH and salinity is shown in equation 10.

$$pH=5.72+0.0065*Salinity \quad (10)$$

Figure 3 shows the variation of pH with temperature. A negative correlation was detected between pH and temperature as pH values decreased with increased temperature. The average difference in pH for each salinity level when temperature was increased from 13- 43 ^{o}C was 0.94. The relationship between pH and temperature is represented by equation 11.

$$pH =6.95-0.029*Temperature \quad (11)$$

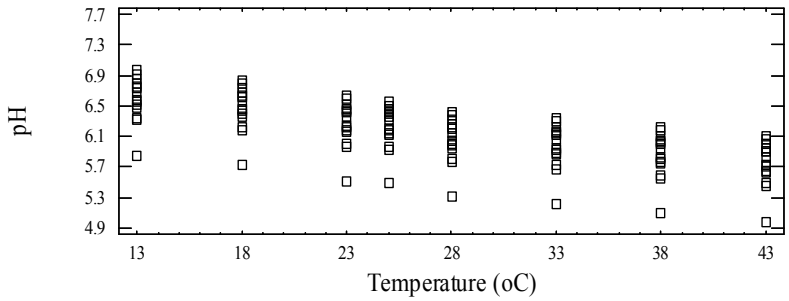


Fig. 3. Variation of pH with temperature

The correlation coefficient equals -0.69 which indicated a moderately strong negative relationship between pH and temperature.

In Figure 4 the plot of the relationship between electrical conductivity and salinity is shown. From the plot it is indicated that electrical conductivity as expected increases as salinity increases for different temperature. The variation of the solution electrical conductivity is linked to the variation in ionic strength. The rate of increase at salinity range of 70-130 g/L appears to be bigger when compared to salinity range of 0- 60 g/L. The correlation coefficient was 0.95 that suggest relatively strong relationships between the two variables. The linear model that described the relationship is given in equation 12.

$$E.Conductivity = 11.37 + 1.12*Salinity \quad (12)$$

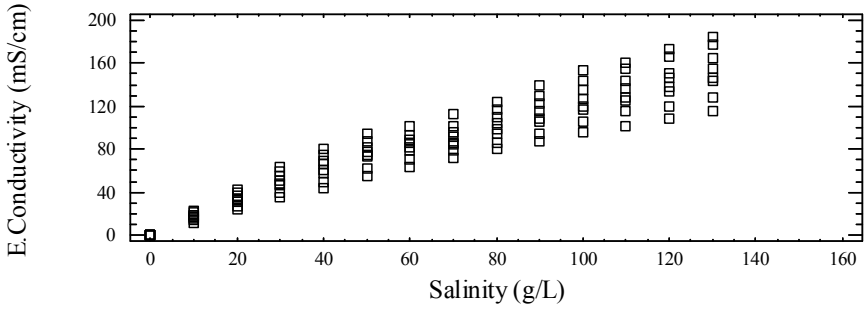


Fig. 4. Variation of electrical conductivity with salinity

In Figure 5, the plot for conductivity against temperature is shown at different salinity. The correlation coefficient equals 0.260 that suggested a relatively weak relationship between the variables and proposed that electrical conductivity was influenced by salinity rather than temperature of the medium. Additionally,

at salinities greater than 80 g/L, the curves seems to converge, an indication that increment in conductivity values with temperature is not as high as observed with lower salinities.

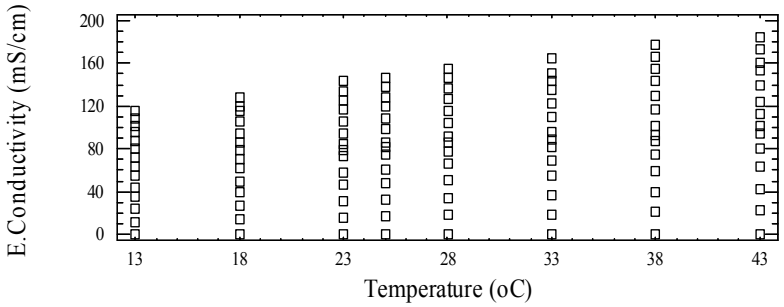


Fig. 5. Variation of electrical conductivity with temperature at different salinity

If the changes in pH and conductivity were related (Figure 6), the result showed that there was a positive correlation between the two parameters which however was a weak relationship. As pH increases the electrical conductivity showed a little increase as well. However at a value of electrical conductivity greater than 80 mS/cm, the lines of pH started converging and no appreciable increase in electrical conductivity was observed.

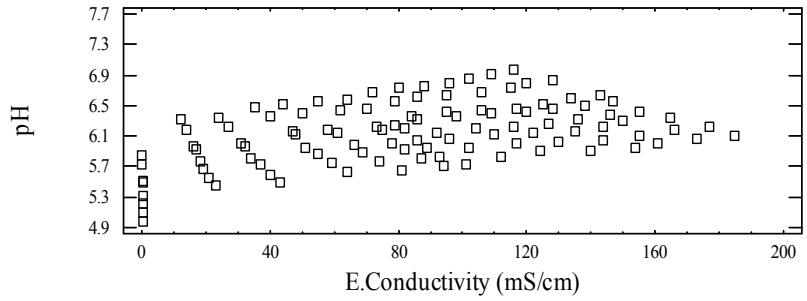


Fig. 6. Plot of pH and electrical conductivity relationship

A multifactor analysis of variance (ANOVA) was used to test the relationship between the measured variables. The results of this regression analysis with pH and conductivity as dependent variables and salinity and temperature as quantitative factors are shown in Tables 4 and 5. The output shows the results of fitting a multiple linear regression model to describe the relationship between pH and two independent variables of salinity and temperature. The equation of the fitted model was estimated as shown in equation 13.

$$pH = 6.52235 + 0.00655249*Salinity - 0.0289974*Temp \quad (13)$$

Similarly for electrical conductivity (EC), the result of the multiple linear regressions with salinity and temperature as independent variables showed that the equation of the fitted model can be represented by equation 14.

$$E.Conductivity = -40.2958 + 1.29025*Salinity + 1.7251*Temp \quad (14)$$

The two results of the multifactor regression suggested the significant relationships between the variables (pH and electrical conductivity) and independent factors of salinity and temperature based on the values of R^2 (0.94 and 0.99) and the P- values (<0.01) as shown in Tables 4 and 5.

Table 4. Analysis of variance for pH

Source	Sum of squares	Df	Mean square	F-ratio	P-value
Model	16.2042	2	8.10212	650.38	0.0000
Residual	1.35786	109	0.0124575		
Total (Corr.)	17.5621	111			

Type III sum of squares

Source	Sum of squares	Df	Mean square	F-ratio	P-value
Salinity	7.82005	1	7.82005	627.74	0.0000
Temp	8.3842	1	8.3842	673.03	0.0000
Residual	1.35786	109	0.0124		
Total (corrected)	17.5621	111			

R-squared-92.26%
Mean absolute error-0.076%

Table 5. Analysis of variance for electrical conductivity.

Source	Sum of squares	Df	Mean square	F-ratio	P-value
Model	246215	2	123107	1844.58	0.0000
Residual	7274.67	109	66.7401		
Total (Corr.)	253490	111			

Type III sum of squares

Source	Sum of squares	Df	Mean square	F-ratio	P-value
Salinity	229048	1	229048	3431.94	0.0000
Temp	17167.1	1	17167.1	257.22	0.0000
Residual	7274.67	109	66.7401		
Total (corrected)	253490	111			

R-squared- 97.13%
Mean absolute error - 6.01%

The deviation between the measured pH and theoretical pH calculated with equation 3 was checked at 25 ^{o}C and similarly for measured conductivity and the theoretical electrical conductivity for the measured temperatures using equation 9. The data showed that the trend of both theoretical and measured pH were the same however with a mean deviation of 0.48 between the two values (Figure 7).

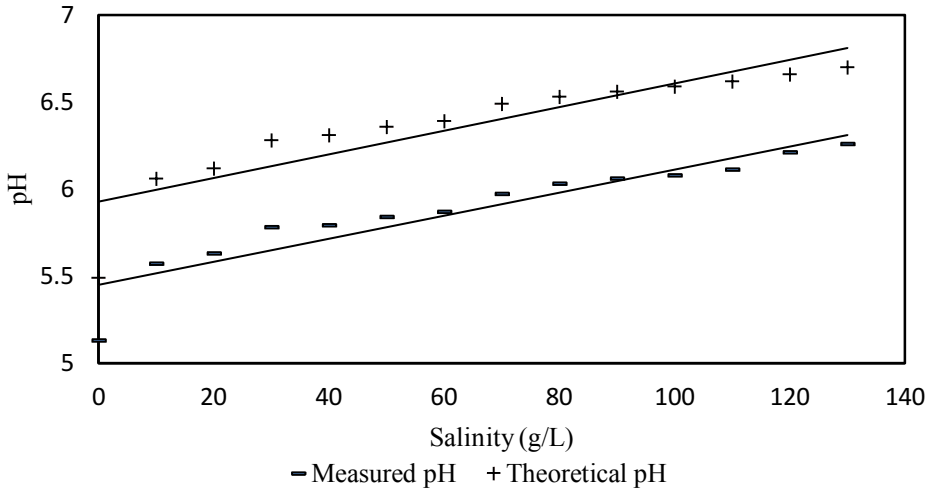


Fig. 7. Comparison of measured pH and theoretical pH at 25 ^{o}C

For electrical conductivity as shown in Figure 8, it was found that the mean deviation between the two set of data was approximately 5.7.

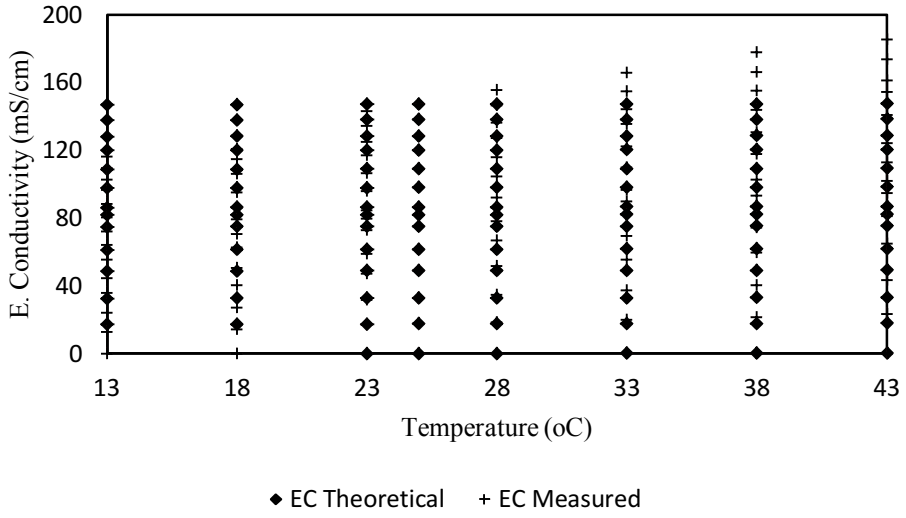


Fig. 8. Comparison of measured and theoretical electrical conductivity

The relationships were evaluated further by making surface plots based on general linear models and these were used to compare the variation of the measured variables in water A and B. The results indicated that for pH variation with predictive factors of salinity and temperature, there was a significant difference between water A and water B as can be seen from Figure 9 and 10. The slope of the pH line was falling for water A while it was increasing for water B as both salinity and temperature increase.

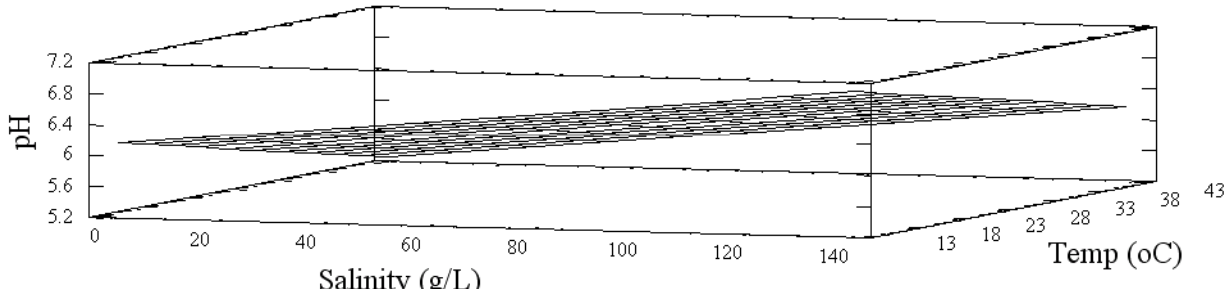


Fig. 9. Surface plot for pH relationship with salinity and temperature (Water A)

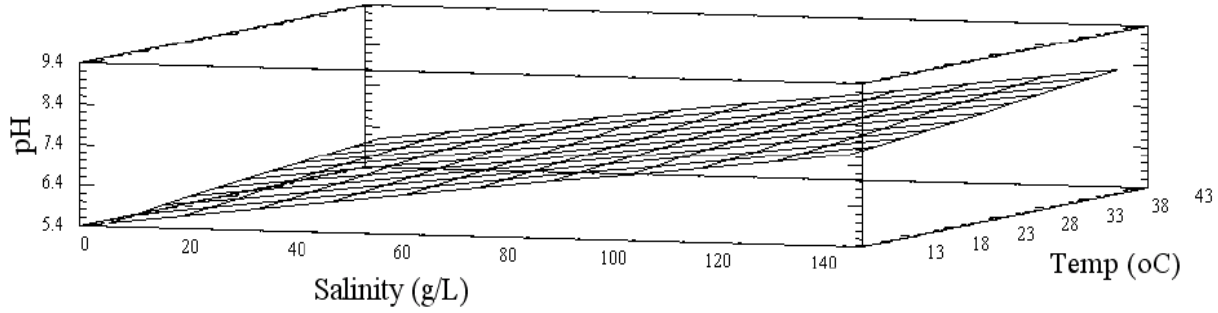


Fig. 10. Surface plot for pH relationship with salinity and temperature (Water B)

However for electrical conductivity, (Figure 11 and 12) the surface plots look similar even though they have different magnitudes.

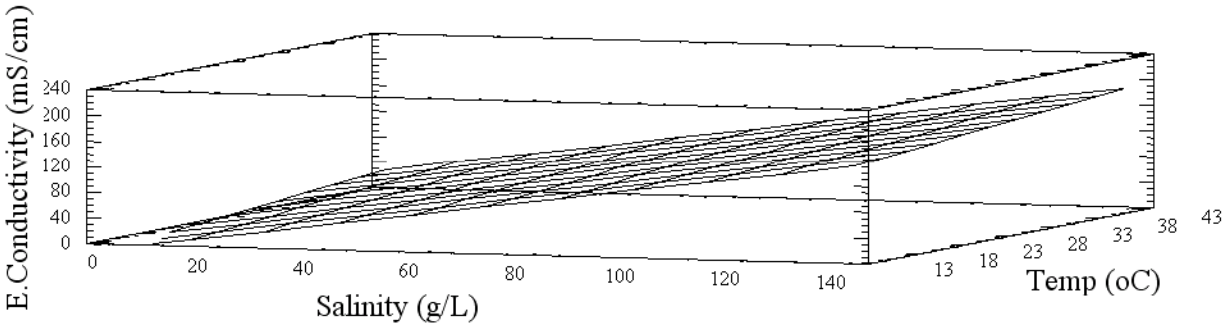


Fig. 11. Surface plot for electrical conductivity with salinity and temperature (Water A).

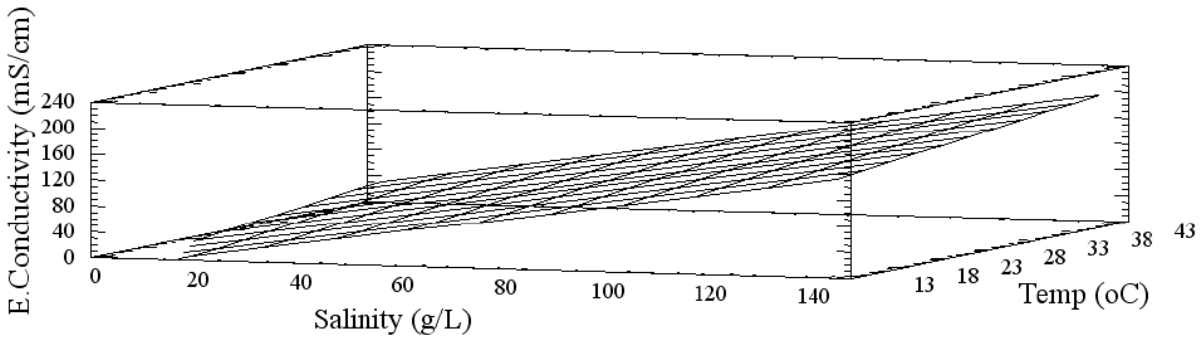


Fig. 12. Surface plot for electrical conductivity with salinity and temperature (Water B).

4. Discussion

The investigated water at 0 g/L of salinity has a low pH which characterizes it as slightly acidic, so it is not neutral. Experimental results showed that pH of the aqueous sodium chloride solution increased slightly with increasing salinity, even though it is normally believed that pH will remain constant when sodium chloride dissolves in water because of its electrical neutrality [6, 26]. From the average pH of about 5.2 for 0 g/L (starting pH for all the solutions of water sample A) at measured temperatures, the pH gradually increased to an average of 6.3 for 130 g/L salinity. This represents a change of about one unit over the measured salinity range and translates approximately to a gradient of 0.005/ $g.L^{-1}$. pH is normally represented by logarithm scale; a difference of one pH unit is equivalent to approximately ten fold difference in hydrogen ion concentration. The change in pH of the solution can be interpreted to be due to ionic strength of the aqueous sodium chloride solution that changes with salinity. The ionic strength is not constant for the range of salinity measured, when calculated, it has a gradient of approximately 0.019. This means that the ionic strength in 130 g/L solution is about 12 times higher than solution of 10 g/L. The ionic

strength normally reflects the concentration of free ions [18], and since there is a strong interaction between the ions, this is likely to be responsible for the non-ideality behaviour in the aqueous solutions and thus the observed relationship between pH and salinity with increase concentration of salt. The dissociation of water and sodium chloride is shown in equation 15 and 16 below:

$$H_2O \Leftrightarrow H^+ + OH^- \quad (15)$$

$$NaCl \rightarrow Na^+ + Cl^- \quad (16)$$

The solutions have been boiled before using for the experiments, it is assumed this would remove (or at least highly reduced) any dissolved CO_2, and thus the main effect on pH change is coming from salinity. In this case the system is controlled by OH^- and H^+ dissociation and not by dissolved CO_2. Lack of pH data for aqueous solutions of different salinity makes it difficult to fully compare the results with other studies, but it is probably certain that this pH increases with increase salinity is tenable for water with low to medium pH values even though chemically speaking sodium chloride salt is neutral.

In contrast, water with dissolved carbon dioxide showed opposite relationship for pH with increasing salinity. pH decreases with increasing salinity when carbon dioxide is dissolved in aqueous solution of sodium chloride. The result of measurements of pH in concentrated brines by Hinds et al.[10] with dissolved carbon dioxide showed a decrease of about 1 from starting pH of 4 to final pH of about 3 for NaCl solutions of concentration of 0 - 250000 ppm (0-250 g/L) at 25 ^oC.

When the values of mean activity coefficient of each solution was used to derived the theoretical pH, the result (Figure 7) showed that the theoretical values are slightly larger than the experimental values with a magnitude of between 0.36-0.52, but the observed trends of increasing pH with increasing salinity still holds. Attempt to calculate the mean activity coefficient from the equation shown below to compare with derived values from Rumpf et al. [22] yield a constant value of 1 for measured salinity range in this study.

$$pH = -\log \gamma \left[H^+ \right] \quad (17)$$

pH is known to be temperature dependent, this is in agreement with Le Chatelier's principle [3] in this case it decreases with temperature. The reason was because K_w gets larger as temperature increases. This increases H^+ and therefore pH decreases. Besides, pH increases slightly with conductivity as seen from the plots at constant temperature, probably because the electrical conductivity is directly related to salinity of the aqueous solutions.

In addition, multiple regression analysis confirmed that there was significant relationship between pH and the predictive factor of salinity and temperature. The salinity was positively correlated with pH while temperature was negatively correlated. For pH as a function of salinity and temperature the R-Squared value indicates that the model as fitted explains 93% of the variability in pH. Since the P-value was less than 0.01, there is a statistically significant relationship between the variables. However, there was no interaction between salinity and temperature.

For an electrolyte solution such as aqueous sodium chloride solution, with increased salinity, there are more ions in solution, and the conductivity increases as well The conductivity depends on the number of ions in the solution and therefore on the degree of ionization of the electrolyte [11, 14, 19]. We can therefore expect that the variation of the solution electrical conductivity is related to the variation in ionic strength. For 0 g/L the ionic strength was approximately 0.0 mol L^{-1} while for 130 g/L the ionic strength was approximately 2.23 mol L^{-1}. The average gradient for increase in electrical conductivity with increase salinity of 10 g/L at a constant temperature approximates 0.009 $mS.cm^{-1}.gL^{-1}$. This value calculated was based on simple empirical model at constant temperature and might not be equivalent to the effective correction computed from physical models using equation of state with salinity as a variable. However simple empirical relationships are useful when used with automatic data logging systems having limited programming capability or with portable EC meters with built in temperature compensation [9] during many chemical processes.

Further results from multifactor regression showed that there was significant relationship between electrical conductivity and factors of salinity and temperature. However the most significant influence came from salinity. The P-value was less than 0.01 and the model was able explain the 96% of the variability in electrical conductivity. No significant interaction between salinity and temperature occurred in this experiment.

The difference between measured values and the theoretically derived pH and electrical conductivity were estimated. In the case of pH the difference was 0.47 while for electrical conductivity mean difference of 5.7 was estimated. The difference is not very much larger than the typical uncertainty arising from electrical conductivity measurements but significantly higher as temperature increases. This confirms the earlier findings that the error of this equation will increase significantly at higher temperature [11].

Comparison of water A with another set of water B indicated significant difference in the variation of pH with factors of salinity and temperature. For water A, pH increased as function of salinity but decreased when temperature increased. For water sample B, pH increased when both salinity and temperature

increases. It may be argued that species present in either sample A or B affected the variation of pH as a function of temperature. Therefore it evident that dissolved particles can influence the trend of observed relationship between pH and temperature. With salinity the pattern of increased pH was constant in both water samples.

5. Conclusion

Sodium chloride solutions are mostly neutral solution and might at first be expected to vary little in terms of their pH value. However, this study showed that significant differences are found between the pH of aqueous sodium chloride of different salinities using different samples of water. The reason for the direct relationship between pH and salinity was due to different ionic strength at different salinity that can be interpreted as deviation from ideality. It is indicated that there is a significant relationship between pH, electrical conductivity (measured variables) and salinity and temperature (independent factors) for aqueous sodium chloride and that simple empirical formulas are adequate to model the relationships. The calculated electrical conductivity values showed a significant deviation from the experimental values at 13-45 ^{o}C which is the range of temperature in this experiment but are this is still within the range of acceptable limit. The model equations obtained were found to be adequate to describe the relationships between the measured variables (pH and electrical conductivity) and the independent factors of salinity and temperature. Through the analysis, salinity was found to be the major significant factor in the relationships.

References

1. Alatiqi IM, Ghabris AH, Ebrahim S, 1989. System identification and control of reverse osmosis desalination. Desalination 75: 119-140.

2. Allison JB, Anderson JA, Cole WH, 1973. The method of electrical conductivity applied to studies on bacterial metabolism. The Journal of Bacteriology, 33: 645-646.

3. Atkins PW, 1978. Physical Chemistry, 5th ed.: Oxford University Press, Walton Street, Oxford.

4. Bada JL, 1969. The pKa of a weak acid as a function of temperature and ionic strength. Chem. Educ., 46, 689-690.

5. Calles B, Calles UM, 1990. Temperature correction of electrical conductivity values. Earth Surface Processes and Landforms, 15(7) : 673-678.

6. Chawla G, Drummond G, 2008. Water, strong ions, and weak ions. Oxford Journals, V8 (3): 108-112

7. Debye P, Hückel E, 1923. The theory of electrolytes I. Lowering of freezing point and related phenomena. Physikalische Zeitschrift 24: 185–206.

8. Dickerson RE, 1969. Molecular Thermodynamics, W.A Benjamin Inc., New York, p 376.

9. Hayashi M, 2004. Temperature-electrical conductivity relation of water for environmental monitoring and geophysical data inversion. Journal of Environmental Monitoring and Assessment, 96: 1-3.

10. Hinds G, Cooling P, Turnbull A, 2009. Measurements of pH in concentrated bines. Corrosion, 65 (10), 635-638.

11. Hsu SY, 2003. Effect of water flow rate, salt concentration and water temperature on efficiency of electrolyzed oxidizing water generator. Journal of Food Engineering, 60**,** (4): 469–473.

12. John JB, Ashton C, 2007. The effect of temperature on conductivity measurement TSP-Issue 3.

13. Kallay N, Drzymala J, Cop A, 2006. Electrical charge at ice-aqueous electrolyte interface. Encyclopedia of Surface and Colloid Science August.

14. Karl ES, Bradshaw A, 1956. A conductivity bridge for measurement of the salinity of water. ICES Journal Mar. Sci. 22: 9-10.

15. Klinko KA, Light WG, Cummings CM, 1985. Factors influencing optimum seawater reverse osmosis system designs. Desalination 54: 3-18.

16. Malinowski ER, 1990. Method of calculating the pH of aqueous solutions of salt of monoprotic acids and bases. J. Chem. Educ. 67 (6) 502-504.

17. Marcus Y, 1989. Determination of pH in highly saline waters. Pure and Appl.Chem., 61, 1133

18. Moore RD, Richards G, Story A, 2008. Electrical conductivity as an indicator of water chemistry and hydrologic process. Streamline Watershed Management Bulletin Vol. 11/No.2

19. Patience CO, Donald AP, Robert EM, 1994. Electrical conductivity measurements of aqueous sodium chloride solutions to 600 ^{o}C and 300 MPa Journal of Solution Chemistry, 23(9): 997-1018.

20. Philip HR, 1987. Electrochemistry (International Edition), Prentice-Hall: Englewood Cliffs, New Jersey, p 112 (1987).

21. Pitzer KS, 1973. Thermodynamics of Electrolytes I: Theoretical basis and general equations. J. Phys. Chem. 77, 268-277

22. Rumpf B, Nicolaisen H, Ocal C, Maurer G, 1993. Solubility of carbon dioxide in aqueous solutions of sodium chloride: Experimental results and correlations. Journal of Solution Chem., 23 (3): 431-448.

23. Sorensen, J.A. and Glass, G.E., 1987: Ion and temperature dependence of electrical conductance for natural waters. Analyt. Chem. 59, p.1594-1597

24. Thomas B.D., Thompson, T.G., Utterback, C.L.1934, The Electrical Conductivity of Sea Water ICES J. Mar. Sci., 9: 28-34

25. Vollmer, M.K. Weiss, R.F., Williams, R.T., Falkner, K.K., Qiu, X., Ralph, E.A. Romanovsky V.V. 2002, Physical and chemical properties of the waters of saline lakes and their importance for deep water renewal: Lake Issyk-Kul, Kyrgyzstan. Geochimica et Cosmochimica Acta 66: (24) 4235-4246.

26. Wyllie, M.R.J. Southwick, P.F, 1954, An experimental investigation of the S.P and resistivity phenomena in dirty sands. Journal of Pet. Tech. Vol. 6 (2) 44.

Paper III

Microbial enhanced oil recovery-laboratory experiments with salinity adapted strain of *Clostridium tyrobutyricum*

Jimoh IA, Søgaard EG, and Rudyk SN, Section for Chemical Engineering, Aalborg University Esbjerg, Denmark.

Telephone: +4599407622, E-mail: egs@bio.aau.dk

Abstract

A salinity adapted strain of *Clostridium tyrobutyricum* (DSMZ 663) labeled as 90F was investigated for metabolites production, potential to improve oil recovery and adaptation to high temperature using laboratory scale experiments with molasses based nutrient. The strain showed substantial production of gases, bio-acids, and biofilm at salinities of up to 100 g/L. The amount of gas produced was 100-1200 mL per 20 mL of molasses over a period of 120 h with a composition of about 80 % carbon dioxide. Over the same period of time the concentration of acetic and butyric acids produced during the microbial fermentation of molasses changed almost linearly (acetic acid increased while butyric acid decreased) with average yield of 3.5 g/L and 0.2 g/L, respectively. Biofilms consisting of polysaccharides with maximum thickness of 0.525 cm at 70 g/L were formed in the oil water interfaces at salinity of 40-130 g/L over 40 days. Gas production of 500 and 260 mL after 120 h per 20 mL of molasses from two different formation waters of salinities 38 g/L and 110 g/L from the North Sea signified possible application in some part of the Danish fields. In microbial oil recovery experiments at salinity 90 g/L the strain gave 38 % and 25 % additional oil recovery in sandstone and carbonate packed columns respectively. The increased oil recovery was attributed to microbial growth and metabolic production. Additional result specified that the maximum achieved adaptation temperature in our tests was 45 ^{o}C compared to 37 ^{o}C of optimal temperature. Results of FT-IR spectroscopy clearly distinguished the strains adapted to salinity and temperature from that of pure culture and thus probably their improved abilities for metabolic production at high salinity and temperature levels.

Keywords: Adaptation, microbial enhanced oil recovery, *Clostridium tyrobutyricum*, metabolites, oil reservoir, fermentation.

Introduction

Microbial enhanced oil recovery (MEOR) is the method of using microorganisms to improve recovery of oil from the reservoirs after secondary oil recovery (Bryant and Lockhart 2002; McInerney et al. 2005; Lazar et al. 2007). This technique is based on in situ growth and metabolism of selected microorganism in the reservoir rock with certain nutrients. The microorganism produce gases and/or chemicals in the formation such as polymer, acids, gases, surfactants and biomass Production of these metabolites in the formation changes fluid and rock properties of the reservoir and improves the sweep efficiency. These changes respectively, increase the oil production from the reservoir. The MEOR application can be either in a form of single well simulation, microbial flooding recovery or selective plugging (Zhang and Xiang, 2010). The idea of MEOR was first proposed by Beckmann (1926) when he published results on the possibility to use microbial metabolites to improve the oil production rate. Additional work by Zoebell, 1947 showed that these metabolites are analogue to the chemicals used in chemical enhanced oil recovery and are expected to perform the job of residual oil recovery

The mechanisms by which MEOR process operate can be quite complex and may involve multiple biochemical process steps (Janshekar1985; Donaldson et al.1989; McInerney et al. 2005; Lazar et al. 2007). These mechanisms can be different from bacteria to bacteria and are normally selected based on reservoir requirement. In general, the mechanisms normally include selective plugging, rock dissolution, emulsification, viscosity reduction, permeability modification and wettability alteration.

The physiological properties of a bacterium that would be useful in MEOR should be such that it will grow abundantly and vigorously under the physico-chemical conditions of the reservoir such as high salinity, temperature and pressure (Grula et al. 1991). It is therefore important that adequate steps are taken to finding the right microorganism that will proliferate under these conditions. One of the available options is the pre-adaptation of bacteria to reservoir conditions. Proper adaptation of microbes before injection will not only improve the ability of bacteria cells to withstand any physiological and environmental stress but also ensure the production of desired metabolites that can be controlled and monitored. As observed in Almehaideb and Zekri 2002, adaptation time is a critical factor on the overall process recovery efficiency, and should be determined in the laboratory for every proposed microbial flooding system. A method of adaptation (Liu et al.2006) through a pH-controlled continuous culture with 70 g/L NaCl as the selective criterion showed that when compared to the parent strain, adapted strain could grow well on the medium containing 70 g/L NaCl. This was measured by the growth rate and pyruvate yield that was 32.5% and 33.9% (in 150 g/L glucose as carbon source) compared to the parent strain. In another trial, laboratory experiments with halophilic culture adapted to subsurface conditions of 55 ^{o}C and 150 g/L using oilfield

carbonate rocks showed successful enhancement of oil recovery from an average of 50 tons per day to 150 tons per day after treatment with bacteria (Wagner, 1991).

Bacteria species grow at particular minimum, optimum and maximum salinity and temperatures. At extreme salinity and temperature conditions metabolism of organisms can be affected. Many organisms therefore over the years have evolved special mechanisms to adapt and survive at different salinity and temperature conditions. Strategies for adaptation to high salt concentrations involve the accumulation of organic compatible solutes that include amino acids, sugars, polyols, betaines and ectoines within the cytoplasm without the need for change of intracellular proteins and intracellular accumulation of high concentration of K^+ (Oren 2001; Roberts 2005). Adaptation strategies by bacteria to environmental temperature changes include alteration of membrane lipid composition. The details on mechanisms for temperature adaptation can be found (Rusell 1984; Edward et al. 2010; van de Vossenberg et al. 1999). The object of this study is not to investigate strategies of bacteria for adaptation to extreme salinity and temperature conditions but on how to improve the metabolites production needed for enhanced oil recovery during the adaptation process.

There are extensive literatures on MEOR studies, however, many of these have focused on pure or isolated culture of bacteria, data on strains that have been adapted to high salinity and temperature and their microbial enhanced oil recovery potentials are rarely investigated. Therefore, in this study, we have investigated an adapted strain of *Clostridium tyrobutyricum* labeled as 90F. The detail of adaptation process can be found in earlier work (Rudyk and Søgaard, 2011). Strains of *Clostridium tyrobutyricum* have been previously applied in MEOR studies (Wagner 1985; Wagner and Lungerhausen 1995) because of their ability to produce metabolites which are useful in microbial enhanced oil recovery process. However, it is widely recognized that a limiting factor in the growth and metabolic activity of *Clostridium* species in situ is salinity in the water phase of the reservoir environment (Russell and Dombrowski, 1980). The main objective of this study is to investigate the growth and metabolic products capability of this adapted strain and the potential to enhance oil recovery at elevated salinity. It is expected that the knowledge gain from this study can give useful information in successful implementation of MEOR technology for enhanced oil recovery. The main parameters considered for this study are acid and gas production, biofilm production and the capability of the adapted strain to improve recovery in sand and carbonate packed columns. The growth and gas production ability of this strain at elevated temperatures was also investigated.

Materials and method

Cultures and medium

Fresh inoculums of the salinity adapted strain of *Clostridium tyrobutyricum* (DSMZ 663) labeled as 90F stored in 1 mL ampullas and frozen at -80 ^{o} C for 12 months were defreezed and grown on reinforced clostridia medium (RCM) in a 1000 mL fermentation bottle that was previously sterilized. The medium salinity was adjusted to 90 g/L by dissolving specific amount of sodium chloride. This was followed by injection of 1 mL inoculum, completed with addition of resazurine to ensure anaerobic condition. The medium was incubated at 37 ^{o}C. The pH of the medium was 6.70 at 25 ^{o}C. Bacterial growth in the medium was indicated by optical density (D_{650} = 1.8). The optical density measurement during growth was taken using a Cary 50 Conc. UV-Visible Spectroprotometer. The Bürker cell counting chamber was used for cell density estimations with an average bacterial population of 1 x 10^6 cells/mL.

Solution preparation

To prepare aqueous solutions for bacteria growth, specific amount of sodium chloride measured in g was dissolved in 500 mL demineralized water to make solutions of the different required salinities in 1000 mL fermentation bottles previously purged with nitrogen. This was followed by addition of 20 mL of molasses as source of nutrient. The media was sterilized (by autoclaving at 121 ^{o}C for 15 min) before subsequent addition of inoculum followed by addition of resazurine to ensure anaerobic condition.

Fermentation experiments

A salinity adapted strain 90F was used in this experiment to determine the gas and acid production abilities. Solutions of different salinities were prepared as described above. The measured salinities were 0, 10, 30, 40 50, 60, 70 80, 90 and 100 g/L. All the fermentation bottles and their content were sterilized and inoculated with 1 mL of 48 h grown culture (1 x 10^6 cells/mL). The whole set up completed with water displacement facilities for gas measurement and sample withdrawal was incubated at 37 ^{o}C for 120 h using a water bath system (model : MGW LAUDA M20). The water bath was covered with polystyrene beads and black polythene to prevent heat loss and any photo oxidation during the fermentation process.

Fermentation experiment was also carried out with two different formation waters, Gorm and Harald formation waters from the North Sea. The salinity of the formation waters was determined by conductivity measurement at 25 ^{o}C using conductivity meter (Model: CDM 210, Radiometer Analytical). The equivalent salinities for Gorm and Harald formation waters were approximately 38 g/L and 110 g/L, respectively.

Product analysis

The cumulative gas production was calculated as total sum of gas produced during the period of 120 h. Liquid samples were taken out from the fermentation bottles every 24 h. pH measurement was carried out using a pH meter Radiometer Analytical (Model: PHM 210). The pH measured during at intervals of 24 h during the fermentation process was to be compared with initial pH condition of each medium.

Gas analysis was carried out with a gas chromatography Clarus 500 PerkinElmer equipped with Elite-Q PLOT (30 m by 0.53 inner diameters). This method was completed by an external standard clause. The sample amount was 1.0 mL with a dilution factor of 1.0. Each sample was run for 3 cycles.

Fluid samples taken after 120 h fermentation process were analyzed for acids with a Varian 430 series gas chromatograph, operating at 120 ^{o}C. The column is equipped with a glass column (Varian 25 m by 0.32 mm internal diameter) and helium as the carrier gas. Injections were made with an autosampler in the on-column mode. The specific acids measured were acetic acid and butyric acid. All products measurements were average of two experiments.

Preparation of biomass

Cells of strain 90F cultivated in 500 mL aqueous solution under standard anaerobic condition at 37 ^{o}C were allowed to grow over a period of 120 h at medium salinity of 90 g/L. 100 mL of sample was carefully withdrawn from the fermentation bottle at every 24 h for cell harvesting. Cells were harvested by centrifugation at 10,000 x g for 10 min using a centrifuge model (Heraeus -Biofuge *Primo*). The cell pellets were dried in the oven at a temperature of 25 ^{o}C and the mass was determined using a weighing balance. The biomass yield was expressed as g/L.

Oil-water interface activity

The activity of *Clostridium tyrobutyricum* 90F at the oil-water interface was monitored using the strain using the adapted method of Hiorth et al. (2010). In each cylindrical jar (Sherf Prazision, Germany) aqueous solution of sodium chloride (80 mL) with measured salinity of 40-130 g/L, enriched with molasses (5% v/v) and crude oil (5% v/v) was inoculated with 1 mL bacteria solution. The whole set up was kept in an incubator and the growth of biofilm at the oil-water interface was monitored as a function of height every tenth day for 40 days.

Oil recovery using packed columns

A cylindrical column of volume 440 cm^3 modified after Suthar et al. 2008 was constructed with a sieve (100μm) and cap at both ends of the column. The column consisted of 1. 2 mm holes in the centre for

insertion of tubes for injection and outflow of fluid. Rubber rings were used for packing to prevent leakage in the column. Approximately 450 g sand of porosity 32 % was packed into the column and evacuated by passing nitrogen through it to remove oxygen. The column was then saturated with aqueous sodium chloride solution (90 g/L) to mimic reservoir brine using a syringe at normal atmospheric pressure. The volume of brine injected into the packed column during the saturation was measured to determine pore volume (PV) of the column (1 pore volume =170 mL). Approximately, 3 pore volumes of the brine were passed through the column to ensure its saturation. Saturation of the sand pack with oil was carried out similarly using light oil with viscosity of 7 cP by help of a syringe until residual brine saturation was reached. The column initial oil saturation (S_{oi}) was 120 mL calculated by measuring the volume of the brine displaced. The column was again flooded with brine until residual oil saturation (S_{or}) of 33 mL calculated by measuring the volume of oil displaced. The packed column was then inoculated with 0.2 pore volume inoculums (consisting of 48 h cells *Clostridia tyrobutyricum* 90F) supplemented with 0.4 pore volume of nutrient consisting of reinforced clostridia media (RCM). The shut in period was 7 days and incubation temperature of 37 ^{o}C. After the shut in period of 7 days, the column was flooded with water by the same method used to saturate the column. Discharges from the column were collected in a graduated cylindrical jar. 50 ml of toluene was added to separate the displaced oil from the discharged water and carefully pipetted to calculate the amount of oil recovered. Two sets of experiments were performed to check the oil recovery efficiency. A similar experiment was carried out but with granular chalks with average porosity of 40% packed into the cylindrical column, and following the same procedure as described above for the sand. The control experiment consisted of an oil saturated sand column which was not inoculated with nutrient and bacteria. The main purpose of this control experiment was to monitor the amount of oil that can be recovered by brine after residual oil saturation was achieved when incubated under the same condition as the main experiment.

Temperature adaptation

In this experiment two strains of *Clostridium tyrobutyricum* were used. The pure strain *Clostridium tyrobutyricum* (DSMZ 663) and the salinity adapted strain 90F. This adapted strain could grow and produced gas at salinity 0 -100 g/L compared to the pure strain that can only grow and produced gas between salinity 0 - 40 g/L. A method similar to Hugh-Leifson technique (Hugh and Leifson, 1953) was used. The bacteria were placed in large test tubes containing 20 mL of aqueous solutions. For strain 90F, the aqueous solutions were adjusted to 90 g/L salinity by adding NaCl to the medium. Each test tube was then added 5 mL of molasses and 1 mL of 48 h inoculums of each strain cultivated on reinforced clostridia media (RCM). 10 test tubes were prepared for pure strain (DSMZ 663) and 10 test tubes for the adapted

strain (90F). Anaerobic condition was maintained by first purging the test tubes with nitrogen gas, addition of resazurine and the sealing tubes with wax after the solution and inoculum had been put in place. The tubes were later secured by caps. The tubes were placed in a water bath that was covered with beads to maintain a constant temperature, prevent evaporation and loss of heat to the surrounding. The range of temperatures in the experiment was 40-48 ^{o}C. These bacteria were normally grown at 37 ^{o}C considered as the optimum temperature for bacteria growth. The method of repeated sub-culturing of the bacteria was employed in the presence of successive increase of the temperature condition. Adaptation was indicated by growth and production of gas that displaced the wax plug. The time in h and height of wax in mm displaced were measured. The height of the displaced wax in the test tube corresponds to the amount of gas produced. The next step of the experiment was to move the bacteria from temperature condition of 40 ^{o}C to a new temperature level of 43 ^{o}C, and correspondingly to 45 ^{o}C and 48 ^{o}C using the same procedure.

FT-IR spectroscopy

In order to compare and quantify any changes in cell content during adaptation, cells were harvested from grown cultures of the three different strains; the pure culture *Clostridium tyrobutyricum* (DSMZ 663), salinity adapted strain 90F, and the temperature adapted strain 90F-45. Cultures of each strain were grown in 500 mL aqueous solutions under standard anaerobic condition and cells were harvested after centrifugation at 10,000 x g for 10 min. The samples collected after open-air drying was used for FT-IR spectroscopy. The infrared spectra of each strain were recorded using the FT-IR spectra. All the FT-IR spectroscopy measurements were carried out with Avatar 370 FT-IR Thermo Nicolet at a resolution of 4 cm^{-1} and 32 scans.

Results and discussion

Salinity adapted strain of *Clostridium tyrobutyricum* (DSMZ 663) denoted as 90F was studied for its possible implementation for microbial enhanced oil recovery in the North Sea oil fields. Its properties were tested in a number of experiments that includes cell growth and biomass production. Changes of acid and gas production rates were also investigated at different stages of the adaptation procedure. The effect of chalk added as a buffer was investigated by comparing the pH alteration in microbial solutions in salinity range of 30-100 g/L with and without chalk. The composition of the acid was measured at salinity of between 30-100 g/L. Biofilm production and its composition were also determined.

Additionally, ability of the strain to produce gas in formation waters produced from Gorm and Harald fields in the North Sea and displacement of oil from chalk and sand columns were tested. At the last stage, the

strain was tested for adaptation to higher temperature and the obtained strain denoted as 90F-45 was investigated for gas production. FT-IR analysis was carried out in order to find out whether spectroscopy is able to distinguish strains of pure culture from adapted.

Cell growth and biomass

The fermentation data (Figure 1) showed that the specific growth rate at salinity 90 g/L is about 0.064 h^{-1} for cells grown on molasses based nutrient. The strain has a particular lag phase of about 15 -20 h. Long lag phase is often associated with metabolic burden on cells caused by possible high concentration of salt. In our case, this is rather due to the low amount of inoculum used (1 mL) in the fermentation since this strain was already adapted to high salinity. The cell biomass yield showed an almost linear increase and reached a maximum value at about 100 h and that corresponds to an OD of approximately 1.70 before decreasing probably indicating the depletion of nutrient afterwards or starting from this point. The average biomass yield was on the average, approximately 2.3 g/L of cell dry weight / g of substrate for cells grown in the molasses medium.

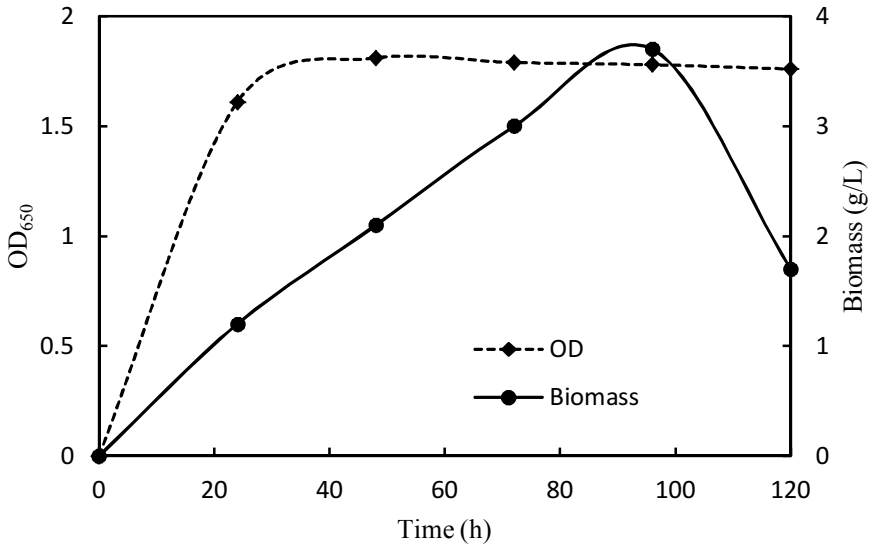


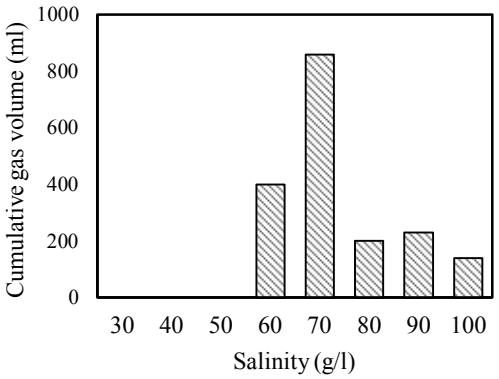
Figure 1: Biomass production and growth rate at different time

In comparison to other strains, experiments showed a biomass yield of 0.05 to 0.13 g/L of cell dry weight/g of glucose at salinity 50 g/L for both anaerobic and aerobic strain *Bacillus lincheniformis* strain JF-2 (Lin et al. 1991). In another experiment, biomass yield of 0.5-0.8 g/L of cell dry weight/g of substrate at salinity of 2 g/L was recorded for *Clostridium butyricum* isolated from anaerobic sewage sludge (Liu et al. 2006). Additionally, biomass yield of 1.73 g/L of cell dry weight/g of substrate at salinity of < 1 g/L was measured

for *Bacillus claussi* (Aparna et al. 2012). This confirmed that our strain showed active growth at high salinity.

Gas production

Gas production has often been mentioned as an important mechanism for microbial enhanced oil recovery (Brown 1992; Singh et al. 2007). The initial cultivations of 90F adapted strain in nutrient source showed that it produced gas from salinity 60-100 g/L as shown in Figure 2 with highest gas production occurring at 70g/L and the lowest at 90 g/L an indication of the influence of high salt concentration on gas production. Indeed, microscopic observations revealed that the cells of *Clostridium tyrobutyricum* which had been grown in higher salinity media were no longer rod shaped, but instead assumed a spherical to agglomeration of cells (Jimoh et al. 2012b). This physiological change in relation to increased salt concentration of the media affects the metabolic activities of the cells as shown by the initial gas production profile (Figure 2). However with continuous and repeated cultivation over time, the ability and pattern of gas production has changed, this strain can now produce gas over a wider range of salinity (30-100 g/L) and correspondingly higher for the same range of salinity as shown in Figure 3. The strain 90F produced from initial pure strain, *Clostridium tyrobutyricum* (DSMZ 663) has shown promise of gas production till salinity 100 g/L by utilizing molasses as carbon source. The composition of the gas was about 83 % carbon dioxide, and 15 % hydrogen. This is comparable to earlier results published (Jimoh et al. 2012a, Wagner and Lungerhausen 1995) and also 70% carbon dioxide and 10% hydrogen reported with Clostridia species (Wagner 1991. However, the reported volume of gas produced varies in literatures. Wagner reported gas generation of 300 mL per g of molasses for *Clostridia* species at salinity greater than 75 g/L after 24 h (Wagner, 1991). The current gas production profile of this strain showed gas volumes of 100-1200 mL in salinity range of 30-100 g/L; however at lower salinity (0-20 g/L) gas production can exceed 2000 mL per 20 mL of molasses. From our previous study, we showed that the alteration of dissolved CO_2 concentration with salinity correlates with the bulk volumes of the produced gas and that change can occur to carbonate rock properties during microbial process from the effect of gas and acid production (Jimoh et al. 2011; Jimoh et al. 2012a).



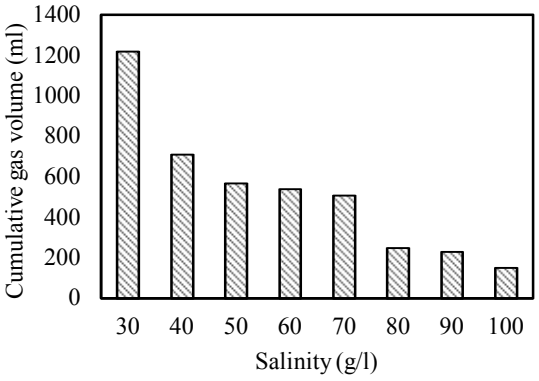


Figure 2: Initial gas production profile of 90F Figure 3: Current gas production profile of 90F

Acid production

Clostridium sp. has been used in a number of studies due to their abilities to produce acids, gases and solvents (Grula et al. 1983; Wagner, 1991; Behlulgil and Mehmetoglu, 2002). From carbohydrates sources, the volume of acids produced can reach 0.0034 moles of acids per kilogram of molasses (Gray et al. 2008). The main acids measured in this experiment were acetic and butyric acids. The profile of the final concentration of the acids produced after 120 h is shown in Figure 4.

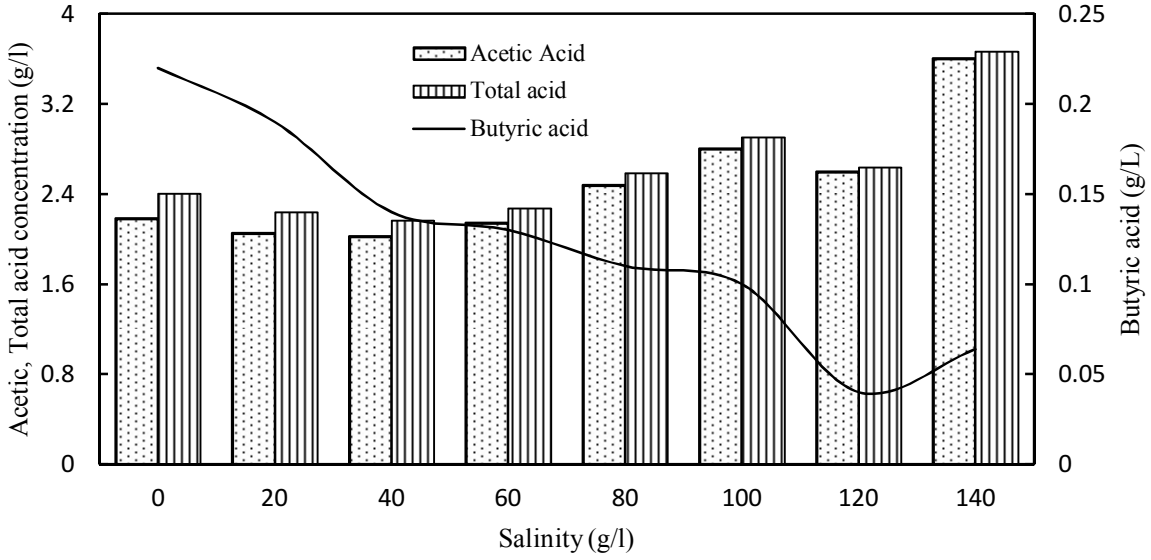


Figure 4: Acid production profile from molasses by salinity adapted strain 90F

The highest concentration of acetic acid of about 3.5 g/L was measured at highest salinity of 140 g/L that corresponds to a final acetic acid yield of 0.175 g/g of substrate while the highest concentration of butyric acid was approximately 0.2 g/L or an equivalent of 0.01 g/g of substrate. The average acetic/butyric acid

ratio (g/g) involving all salinities was approximately 18. The total yield of acetic acid and butyric acid per g of molasses was approximately 0.176 g/g of substrate. The total yield of the acid yield generally increases with salinity and the total acid relationship with salinity is given by a polynomial function as shown in equation 1. The increase in acidity correlated well with increase in salinity with a R^2 value of 0.85.

$$Total\ acid = 0.047S^2 - 0.264S + 2.599 \quad (1)$$

If acid production is correlated with salinity and pH as shown in Figure 5, the result indicates an increasing yield of acetic acid with increasing salinity and pH while the butyric acid has almost a reverse profile; a decreasing yield with increasing salinity and pH. Correlation of acetic acid with salinity gave a R^2 value of 0.71 while correlation with butyric acid showed R^2 value of 0.92. The equation that represents the relationship of the acetic acid with salinity is given by:

$$Acetic\ acid = 0.1829S + 1.63 \quad (2)$$

The corresponding equation for butyric acid relationship with salinity is given by:

$$Butyric\ acid = -0.0236S + 0.23 \quad (3)$$

The S in the equations (1-3) denotes salinity of the medium.

From equations 2 and 3, it showed that acetic acid correlates positively with salinity while the correlation was negative for butyric acid. Therefore it can be suggested that production of butyric acid decreases as salinity rises while production of acetic acid reversely increases with increasing salinity. The point of intersection between the two acids corresponds to salinity 70 g/L and pH of about 6.0. One of the adaptations of this strain could be that its activity changes to more acetic production at higher salinity. Acetic acid is a stronger acid than butyric acid if we compared the pKa values; acetic acid: pKa 4.76; butyric acid: pKa 4.82 (Martin et al. 1983). This suggests that the strain can better handle the situation with increasing pH due to increasing salinity.

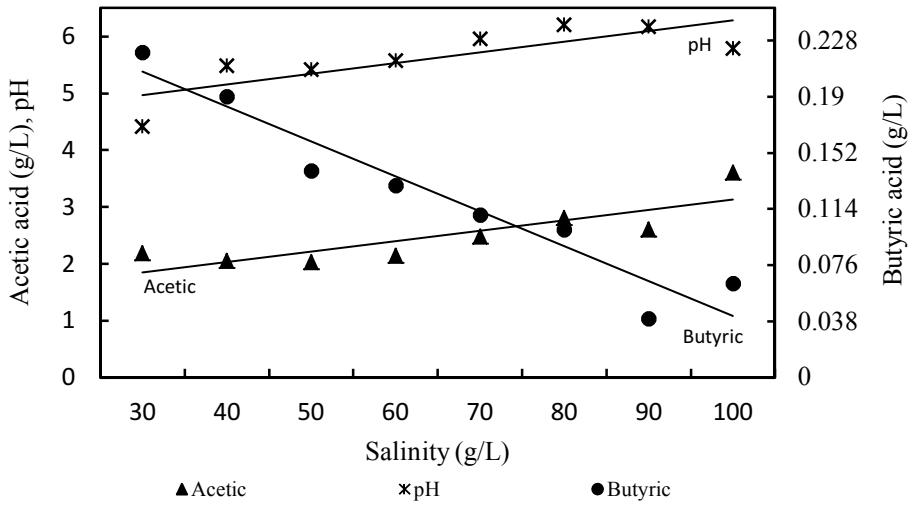


Figure 5: Acetic and butyric acid relationship with pH and salinity

Data on organic acids produced by similar strains indicated that the amount and types of organic acids produced can vary between 3 and 45 g/L depending on the quantity of inoculum, medium, volume of substrate used and the end product desired. For different strains of *Clostridium tyrobutyricum,* Liu et al. reported a value of 9.7-42 g/L, Jiang et al. reported a value of 26.2 g/L for butyrate in a fibrous bed reactor using cane molasses while Wagner and Lungerhausen reported organic acid production of 11.4 g/L (Wagner and Lungerhausen, 1995; Liu et al. 2006; Jiang et al. 2009).

Variation of pH

pH measurements during the gas and acid production is shown in Figure 6. It can be observed that acidity in microbial experiments increased significantly in all the media. The values were variable depending on the salinity of the medium; however we observed a fast decrease in pH after 24 h for all media. In the case of media without chalk, for salinity between 30-60 g/L , pH continue to drop after 24 h while for salinity range of 70-100 g/L, pH were relatively stable. The lowest pH of 5.23 after 120 hours corresponds to salinity 30 g/L while the highest pH of 5.84 corresponds to 120 g/L. For media saturated with chalk, the 30-60 g/L were similar to the trends observed in media without chalk; however the reverse was the case for salinity range of 70-100 g/L where the pH was increasing towards the end. The media with chalk has lowest pH of 5.6 at salinity 30 g/l while the highest pH after 120 h was 6.28 at salinity 100 g/L.

The decrease in pH in both cases was due to production of acids and gases during fermentation of molasses medium. However the presence of chalk samples in some of the media presented was able to neutralise the effect of acids, hence the increasing pH observed in the media with chalk (70-100 g/L). The average separation in measured pH with and without chalk for salinities between 30-60 g/L corresponds to a pH

unit of 0.16 while for salinities between 70-100 g/L the measured average separation corresponds to a pH unit of 0.18. This showed separation of the pH values into two groups of low and high salinity with a common boundary at 70 g/L that matches the intersection of acetic and butyric acid (Figure 5) and further confirms the earlier observation made in similar study (Jimoh et al 2011).

Oil-water interface activity

The activity at the oil-water interface was monitored at salinity range of 40-130 g/L by the thickness of biofilm growth measured from the bottom of oil-water interface which was approximately the same in each container. The structure of the dried biofilm was also examined with FT-IR spectroscopy. Figure 7 shows the variation in biofilm thickness with salinity at different time. The highest thickness was observed at salinity 70 g/L for each period, while no growth of biofilm was observed at salinity of 110 -130 g/L that might be as a result of inhibition by high salinity of these media for the strain of bacteria used. All the curves approached zero amount of biofilm at 110 g/L irrespective of number of days; this probably suggests that the salinity limit for formation of biofilm for this adapted strain of bacteria around is approximately 100 g/L.

The microscopic images of the harvested biofilm shows that they were composed of immobile, irreversibly attached live bacteria in clusters and some whitish fibrous material at varying degrees of density throughout the slides as shown in Figure 8a. Such observation has earlier been reported by Rachman et al. (1997). Furthermore the spectra analysis using the FT-IR revealed the structure shown in Figure 8b. There is a broad absorption band between 3200 and 3400 cm^{-1} which is suggestive of O-H content, a C-H stretch between 2950 and 2860 cm^{-1}, a strong band at 1630 cm^{-1} which specified the presence of >C=O group, an absorption at 1403 cm^{-1} indicating the presence of C-N bond and a band at 1074 cm^{-1} indicating the presence of ether group (C-O-C) probably from carbohydrates. The structure of the biofilm as shown by the FTIR is suggested a polysaccharide. The biofilm formation can probably increase the adherence of the cells to oil-water interface, how they can affect crude oil biodegradation still remains obscure. However, biofilms can act as a surfactant that can be able to bring small oil drops out in the water phase.

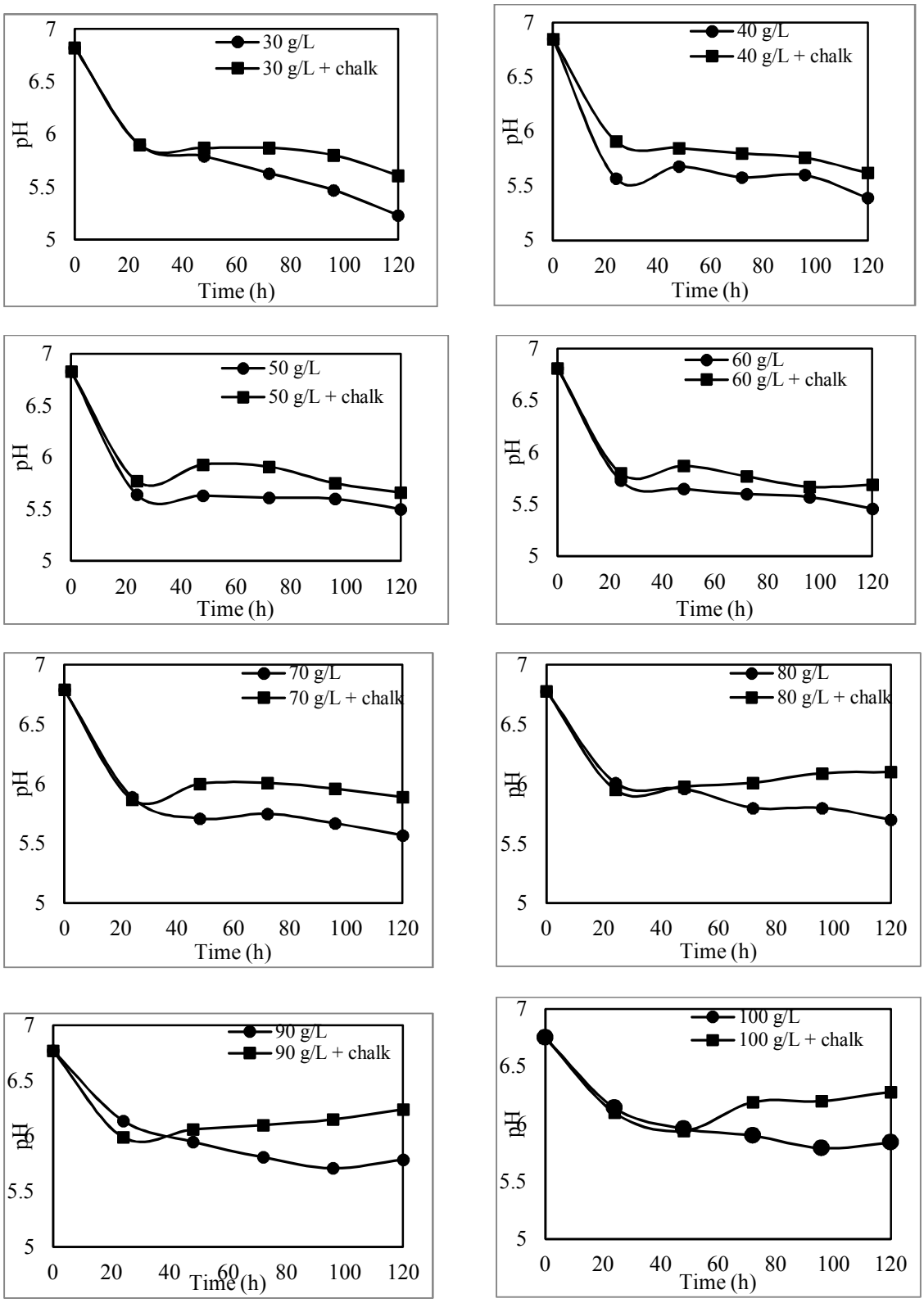


Figure 6: Comparison of the pH profile at different salinity for media with chalk and without chalk

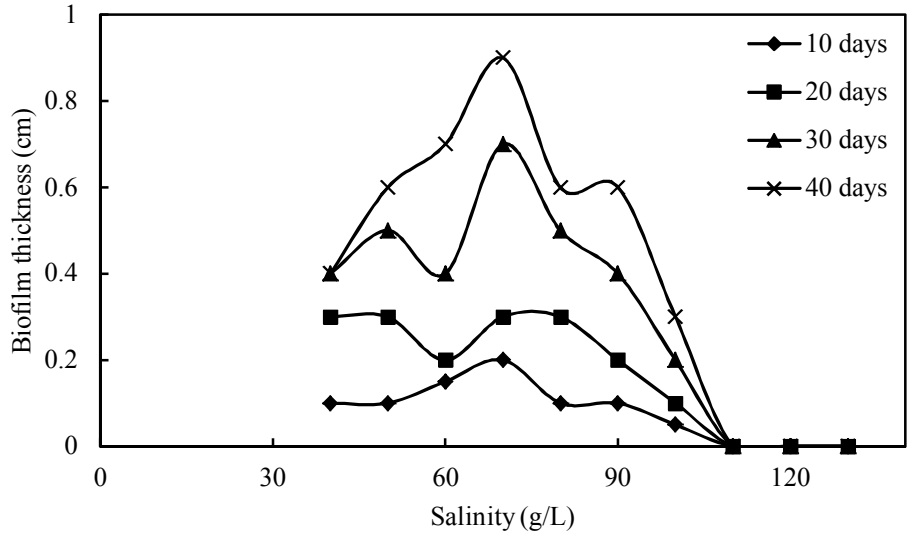


Figure 7: Biofilm formation at different salinity

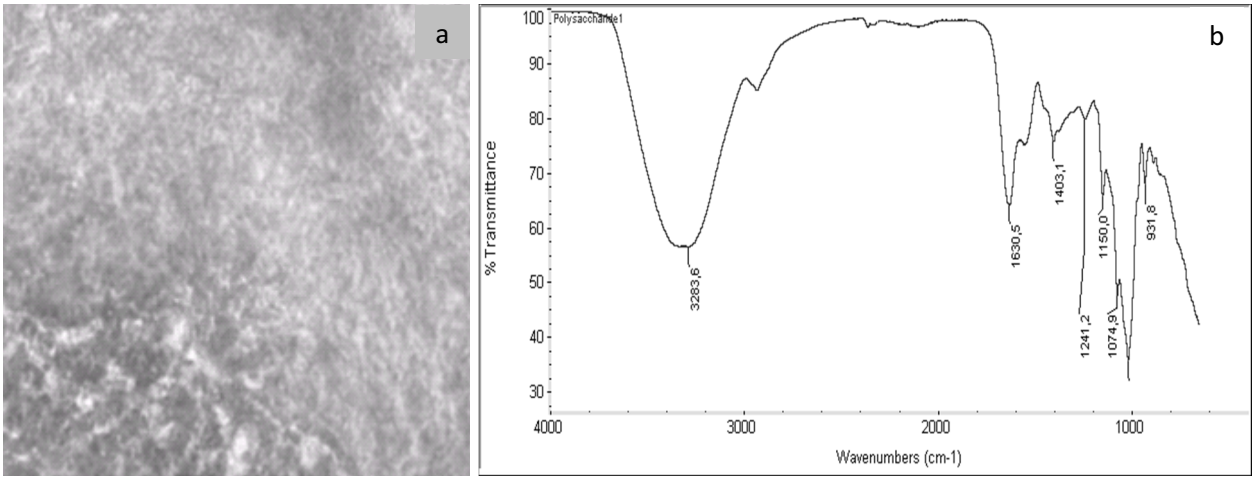


Figure 8: Microscopic image of biofilm as examined under microscope (x40) and the FT-IR spectrum; a. the microscopic image and b. the FT-IR spectrum of the dried biofilm.

Growth in formation waters

The result indicated that gas production began in Gorm formation water (salinity 38 g/L) during the first 24 h while in Harald formation (110 g/L) it was after 48 h suggesting a longer lag period for bacteria growth and metabolism. The cumulative gas produced from Gorm formation water was about 500 mL, while 260 mL was produced from the Harald formation water after 120 h (Figure 9). pH of the Gorm and Harald media were also reduced from 6.81 and 6.08 to 4.81 and 5.82 respectively ; that corresponds to optical

density (OD_{650}) of 1.48 and 1.00 at the end of 120 h. Blank experiment with both Gorm and Harald formation waters and molasses without strain 90F did not produce any measurable amount of gas which shows that the gas was produced by *Clostridium* strain 90F and not by microbes in the formation waters.

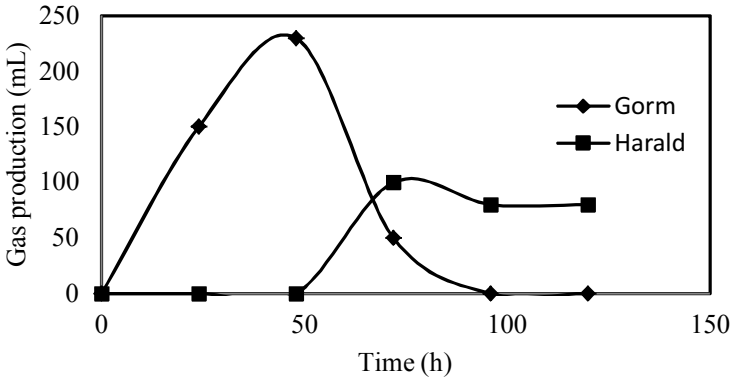


Figure 9: Gas production in Gorm and Harald production waters

These results confirmed bacterial growth and the production of gases in both media. The Danish fields have high salinities as many of them are adjacent to salt domes with the salinity of formation waters increasing from south to north to 300 g/L (Gran et al. 1992). The salinity measurements of the two formation waters showed 38 g/L and 110 g/L respectively which are much lower than the published values probably because the salty formation waters were diluted with sea water injected into the formations. This result gave an indication that this adapted strain can grow and produce gas in some part of the Danish fields of the North Sea.

Oil recovery experiment

The objective of the packed column experiment was to assess the recovery enhancement resulting after inoculation with strain 90F at a relatively high salinity (90 g/L). The summary of result for the oil recovery experiment in sandstone and granulated chalk samples packed columns is shown in Table 1. As can be seen from the table, the average oil recovery after one week incubation at temperature of 37 ^{o}C is about 39 % for sandstone column and 25 % for chalk column designed to stimulate oil reservoirs. The observed additional recovery in these experiments is believed to be a direct result of the in situ growth and metabolism of injected bacteria. The control experiment (without nutrient and bacteria) did not show any significant release of oil after 7 days when water flooded during secondary flooding.

Table 1: Summary of the oil recovery experiment

Parameters	Sandstone	Chalk
Pore volume	1PV=170 mL	1PV= 200 mL
Initial oil saturation	120 mL	145 mL
Residual oil saturation	33 mL	60 mL
Nutrient injected	0.4PV	0.4PV
Inoculum	0.2PV	0.2PV
Incubation	7 days (37 ^{o}C)	7 days (37 oC)
Secondary water flooding	7 PV	4.5 PV
Oil displaced after secondary water flooding	13 mL	15 mL
% oil recovery after microbial treatment	39 %	25%

The difference in the percentage recovery between sand and carbonate columns could be explained by the fact that less amount of water could be used during secondary water flooding for the chalk sample as it became increasingly difficult to pass water through the column for oil displacement. This was probably due to non-uniformity of the chalk packed in the column as compared to the sandstone packed column.The effluent samples collected during secondary flooding to displace oil in the bacteria inoculated sand and chalk packed columns when examined under microscope indicated presence of bacteria, however, it can be anticipated that this represents only a small percentage of the total population of cells.

Temperature adaptation

In this study, an adapted strain of *Clostridium tyrobuytricum* (DSMZ 663) froze over a period of 6 months at -80 ^{o}C and labeled as 90F was evaluated for microbial enhanced oil recovery potential. The first set of experiment was to test the temperature increment on the growth of this salinity adapted strain, since the final aim was to have a strain that can withstand both high salinity and high temperature conditions. The temperature adaptation results for salinity adapted strains 90F is shown in Figures 10. The graph indicates the measured height of the gas at different temperature and the time taken to displace the wax. At 40 ^{o}C and time of 96 h, the displace wax was the highest with an average height about 1.5 cm. This is followed by the height at 43 ^{o}C and time of 96 h with an average of about 0.5 cm. The lowest heights were seen at 45 ^{o}C at a time of 168 h with an average of 0.3cm. The displaced wax corresponds to the amount of gas produced. In this case it can be inferred that no gas was displaced at 48 ^{o}C at a time 240 h. It is therefore suggested that with increase temperature, the amount of gas produced is decreasing, an indication of reduced bacteria metabolism. It was also observed during the experiment that the time taken to displace the wax which corresponds to gas production increased with increasing temperature.

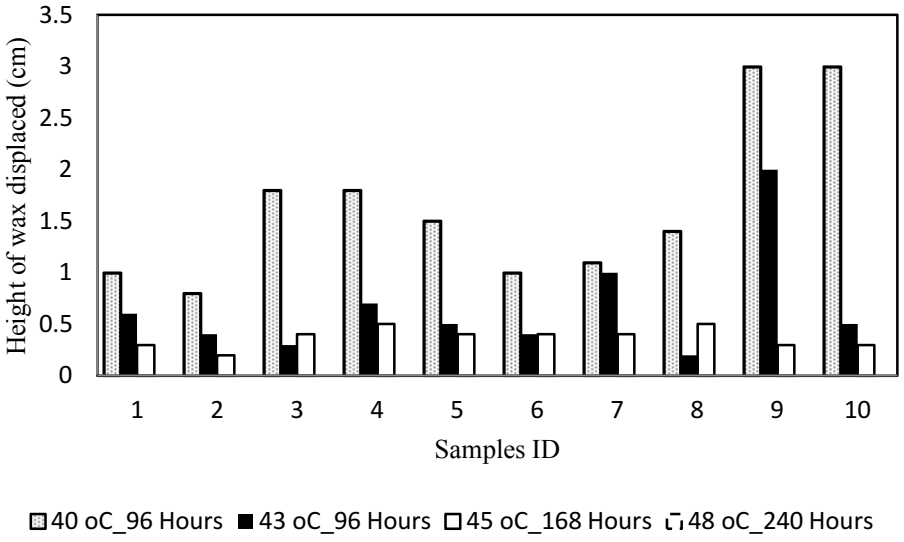


Figure 10: Comparison of gas height at different temperature for adapted strain

Furthermore, the gas production ability of the pure strain (DSMZ 663), the salinity adapted strain (90F) and the temperature adapted strain (90F-45) were compared in different experiment. The result obtained over 120 h is shown in Figure 11. The highest cumulative gas production was recorded with 90F however the temperature adapted strain (90F-45) was able to show even better gas production at relatively high salinity of 90 g/L compared to strain 90F. This was a significant improvement over the initial ability of the pure strain that was only able to grow till salinity of between 40-50 g/L.

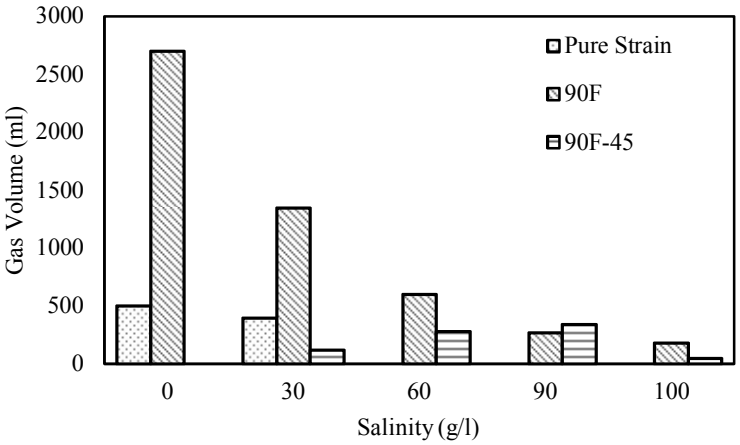


Figure 11: Comparison of gas production potential for pure strain, salinity adapted strain (90F) and temperature adapted strain (90F-45).

Additionally the cell structures of the pure 90F-45 was compared with that of salinity adapted strain 90F and pure strain (DSZM 663) as shown in Figure 12 (Jimoh et al. 2012b). The result indicated that the contents of the cells were different at certain wavelengths. The most significant spectral differences come from the N-H stretching of proteins around 3400-3278 cm^{-1}, the amide bands of protein at 1675 cm^{-1} and

the point which represent the C=C-O-C, C-O, C-O-P, P-O-P vibrations of polysaccharides at 1072 cm^{-1} modes, respectively. This is based on the values of the intensities of the major bands corresponding to these functional groups. In the ranges of 3400-3278 cm^{-1} and 600-900 cm^{-1}, the transmittance is decreasing as follows: pure culture, 90F, and 90F-45. The reverse trend can be observed in the range of 2900-3000 cm^{-1}. The sequence of curves clearly separates adapted strains from pure culture.

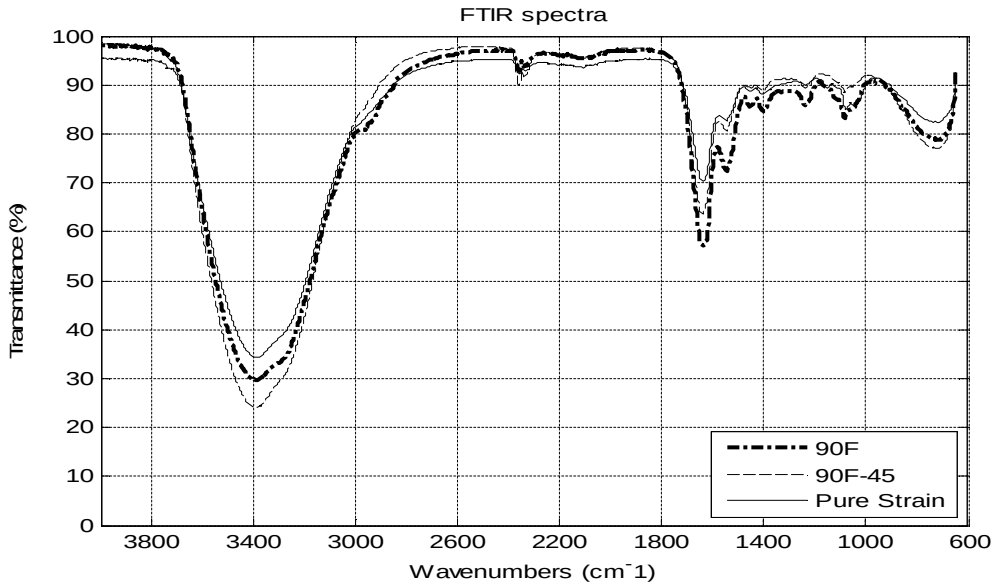


Figure 12: Spectra for dry harvested cells of pure strain, salinity adapted strain and temperature adapted strain of *Clostridium tyrobutyricum.*

Conclusion

It was found out that pure culture of bacteria can become adapted to high levels of salinity and moderate temperature after a careful and repeated sub culturing over time. This adaptation process could be monitored by gas production. The main metabolites produced by salinity adapted strain of *Clostridium tyrobutyricum* labeled as 90F include gas, bio-acids, biomass and biofilm when cultured in molasses based media. Strain 90F was able to produce gas and biofilm to salinity of 100 g/L. When exposed to formation water of salinity of 110 g/L, this strain also showed stable gas production. The quantities of some of the metabolites was found to have direct relationships with salinity of the medium as it was observed that the acid yield generally increases with salinity while the amount of gas produced decreases with salinity. Although presence of chalk facilitated higher gas production in the experiments with microbial solutions, recovery of 39 % was greater from sandstone compared to 25 % from chalk. This was thought to be partly due to a more complex porous structure of chalk.

The temperature adapted strain improved performance of the salinity adapted strain and demonstrated ability to produce more gas at salinity 90 g/L. Microscopic observations make to deduce that bacteria formed aggregations of cells as an adaptation mechanism. Spectroscopic analysis proved to be a tool able to make a distinction of the different strains. The most significant spectral differences between pure and adapted strains came from the N-H stretching of proteins.

References

Aparna A, Srinikethan G, Smitha H. 2012. Isolation, screening and production of biosurfactant by *Bacillus claussi* 5B, Research in Biotechnology 3(2): 49-56.

Almehaideb R, Zekri AY. 2002. Laboratory investigation of parameters affecting optimization of microbial flooding in carbonate reservoirs. Petroleum Science and Technology 20 (3-4):377-392.

Beckman JW. 1926. The action of bacteria on mineral oil, Industrial Engineering Chemical News 4:3.

Behlulgil K, Mehmetoglu MT. 2002. Bacteria for improvement of oil recovery: a laboratory study. Energy Sources 24: 413-421.

Brown FG. 1992. Microbes: the practical and environmental safe solution to production problems, enhanced production and enhanced oil recovery, paper SPE 23955 presented at the 1992 SPE Permian Basin Oil and Gas Recovery Conference, Midland, Texas, March 18-20.

Bryant SL, Lockhart TP. 2002. Reservoir engineering analysis of microbial enhanced oil recovery. Soc. Pet. Eng. Reservoir Eval. Eng. 5: 365-374.

Canganella F, Wiegel J. 2000. Continuous cultivation of *Clostridium thermobutyricum* in a rotary fermentation system. Journal Indust. Microbiol. Biotechnol 24:7-13.

Chen WM, Tseng ZJ, Lee KS,Chang JS, 2005. Fermentative hydrogen production with *Clostridium butyricum* CGS5 isolated from anerobic sludge. International Journal of Hydrogen Energy 30: 1063-1070

Donaldson EC, Chilingarian CV, Yen T. 1989. Microbial enhanced oil recovery. In: Developments in Petroleum Sci. 22:1–12.

Hall EK, Singer GA, Kainz MJ, Lennon JT. 2010. Evidence for a temperature acclimation mechanism in bacteria: an empirical test of a membrane-mediated trade off. Functional Ecology,24:898-908.

Gran K, Bjorlykke K, Aagaard P. 1992. Fluid salinity and dynamics in the North Sea and Haltenbanken basins derived from well logs. Geological Society, London, Special Publications 65: 327-338.

Gray MR, Yeung A, Foght JM. 2008. Potential microbial enhanced oil recovery processes: a critical review. SPE 114676, SPE Annual Technical Conference and Exhibition, 21-24 Denver, Colorado, USA.

Grula EA, Russel HH, Bryant DM, Kenaga M, Hart M. 1983. Isolation and screening of *Clostridium* for possible use in MEOR. In: Donaldson EC, Clark JB, Editors. Proc. Int. Conf. MEOR, Afton Okla., May 16-21, 43-47.

Grula MM, Russell HH, Janloo SM Conway T. 1991. Effects of sodium chloride on growth and metabolism of two strains of Clostridium. In: Donaldson EC Editor. Microbial enhancement oil recovery-recent advances. Elsevier Science Publishers B.V, Netherlands. 183-206.

Hiorth A, Kaster K, Lohne A, Siqveland OK, Berland H, Giske NH, Stavland A, 2007. Microbial enhanced oil recovery-mechanism. Int. Symposium of the Society of Core Analysts, Calgary, Canada, September 10-13.

Hugh R, Leifson E 1953. The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria. J. Bacteriol. 66:24-36.

Janshekar H. 1985. Microbial enhanced oil recovery processes. In: Zajic JE, Donaldson EC Editors. Microbes and Oil Recovery. Bioresources Publications, El Paso, Texas, 54-84.

Jiang L, Wang J, Liang S, Wang X, Cen P, Xu Z. 2009. Butyric acid fermentation in a fibrous bed bioreactor with immobilized *Clostridium tyrobutyricum* from cane molasses. Bioresource Technology 100 (13): 3403-3409.

Jimoh IA, Rudyk SN, Søgaard EG. 2011. Microbial fluid rock interactions in chalk samples and salinity factor in divalent Ca^{2+} ions release for microbial enhanced oil recovery purposes. Chemical Engineering Transactions 24: 889-894.

Jimoh IA, Søgaard EG, Rudyk SN. 2012a. Evaluation of produced volumes of carbon dioxide from the concentration of gas absorbed in the media during microbial fermentation for enhanced oil recovery purposes. Chemical Engineering Transactions, 27: 97-102.

Jimoh IA, Søgaard EG, Rudyk SN. 2012b. Spectroscopy and image analysis characterization of adapted strains of Clostridium tyrobutyricum for microbial enhanced oil recovery purposes. GSTF Journal of Biosciences, 1(2): 36-41.

Lazar I, Petrisor IG, Yen TF. 2007. Microbial enhanced oil recovery. Petroleum Science and Technology 25: 1353-1366.

Lin SC, Goursaud JC, Kramer PJ, Georgiou G, Sharma MM. 1991. Production of biosurfactant by Bacillus lincheniformis strain JF-2. In: Donaldson EC Editor. Microbial enhancement oil recovery-recent advances. Elsevier Science Publishers B.V, Netherlands. 219-226.

Liu L, Xu Q, Li Y, Shi Z, Zhu Y, Du G, Chen J. 2006. Enhancement of pyruvate production by osmotic-tolerant mutant of Torulopsis glabrata. Biotechnology and Bioengineering, 97 (4): 825-832.

Liu X, Zhu Y, Yang S. 2006. Butyric acid and hydrogen production by *Clostridium tyrobutyricum* ATC 25755 and mutants. Enzyme and Microbial Technology, 38 (3-4): 521-528.

Martin JR, Petitdemange H, Ballongue J, Gay R. 1983. Effects of acetic acid and butyric acids on solvents production by Clostridium Acetobutylicum. Biotechnology Letters 5 (2): 89-84.

McInerney MJ, Duncan KE, Youssef N, Fincher T. et al. 2005. Development of microorganisms with improved transport and biosurfactant activity for enhanced oil recovery. Petroleum Microbiol. 11: 215-237.

Michel-Savin D, Marchal R, Vandecasteele JP. 1990a. Control of the selectivity of butyric acid production and improvement of fermentation performance with *Clostridium tyrobutyricum*. Appl. Microbiol. Biotechnology 32:387–392.

Oren A. 2001. The bioenergetics basis for the metabolic diversity at increasing salt concentrations: an implications for the functioning of salt lake ecosystem. Hydrobiologia 466: 61-72.

Rachman MA, Furutani Y, Nakashimada Y, Kakizono T, Nishio N, 1997. Enhanced hydrogen production in altered mixed acid fermentation of glucose by enterobacter aerogenes. J. Ferment. Bioeng., 83 (4) 358-363.

Roberts MF. 2005. Organic compatible solutes of halotolerant and halophilic microorganisms. Saline Syst., 1:5.

Rudyk SN, Søgaard EG. 2011. Microbial enhanced oil recovery. In: Whitby C, Skovhus, TL Editors. Applied microbiology and molecular biology in oilfield systems: microbial EOR, Springer-Dordrecht 179-187.

Russel JB, Dombrowski DB. 1980. Effect of pH on the efficiency of growth by pure cultures of rumen bacteria in continuous culture. Applied and Environmental Microbiology 39 (3): 604-610.

Russell NJ 1984. Mechanisms of thermal adaptation in bacteria: blueprints for survival.Trends in Biochemical Sciences 9 (3): 108-112.

Singh A,Van-Hamme JD, Ward OP. 2007. Surfactants in microbiology and biotechnology: part 2-application aspects. Biotechnology Advances, 25: 99-121

Suthar H, Hingurao K, Desai A, Nerurkar A. 2008. Evaluation of bioemulsifier mediated microbial enhanced oil recovery using sand packed column. Journal of Microbial Methods, 75: 225-230.

van de Vossenberg JLCM, Driessen AJM, da Costa MS, Konings WN. 1999. Homeostasis of the membrane proton permeability in Bacillus subtilis grown at different temperatures. Biochimica et Biophysica Acta 1419: 97-104.

Wagner M. 1985. Microbial Enhancement of oil recovery from carbonate reservoirs with complex formation characteristics. In: Donaldson EC Editor. Microbial enhancement oil recovery-recent advances. Elsevier Science Publishers B.V, Netherlands.

Wagner M. 1991. Microbial enhancement of oil recovery from carbonate reservoirs with complex Formation characteristics. Dev. Petroleum Sci. 31: 387-398

Wagner M, Lungerhausen D. 1995. Development and application of a new biotechnology of the molasses in-situ method- detailed evaluation of selected wells in the Romashkino carbonate reservoir. In: Proceeding of Conference, 5th International Conference on Microbial enhanced oil recovery and related biotechnology for solving environmental problems. Tulsa, U.S.A.

Zhang X, Xiang T. 2010. Review of microbial enhanced oil recovery technology and development in China. International Journal of Petroleum Science and Technology, 4 (1): 61-80.

Zobell CE 1947. Bacterial release of oil from oil-bearing materials, Part I and II. World Oil, 126 (13): 36-47 (I); 35-41 (II).

Paper IV

Microbial fluid-rock interactions in chalk samples and salinity factor in divalent Ca^{2+} ions release for microbial enhanced oil recovery purposes

Ismaila A. Jimoh, Svetlana N. Rudyk and Erik G. Søgaard

Section for Chemical Engineering, Esbjerg Institute of Technology, Aalborg University, Niels Bohr Vej 8, 6700 Denmark.
Telephone: +45791272622, E-mail: sr@bio.aau.dk

In this study core laboratory experiments were performed on chalk samples from Danish sector of the North Sea to study microbial fluid-rock interactions with carbonate rock and to evaluate the dissolution of rock matrix ($CaCO_3$). Result showed that the average concentration of Ca^{2+} ions after microbial fluid interactions with chalk samples in media of salinity 40-100g/l increased from initial average concentration of 203 mg/l at the start of the experiment to 1178 mg/l in 28 days. 3-D surface plot (salinity, $Ca^{2+,}$ pH) with time revealed delineation of the measured salinity into two groups with a boundary between 70 - 80 g/l. In the low salinity group (40, 50, 60, 70 g/l) pH increased but decreased in the high salinity group (80, 90, 100 g/l) at the end of the experiment. The highest Ca^{2+} ion concentrations was measured at highest salinities at the end of experiment which means that the dissolution rate of chalk increased with increase in salinity due to the lower values of pH indicative that higher salt concentration facilitates more acid production by bacteria.

1. Introduction

In the last few years oil production in the Danish sector of the North Sea started declining. It was estimated that an increase of only 1% in oil recovery will provide Denmark additional oil for two years (OCD, 2007). The method of microbial enhanced oil recovery (MEOR) is considered for application to the North Sea oil bearing chalk reservoirs; however, microbial fluid interaction in subsurface formations is governed by complicated physical, chemical, and biological phenomenon. Although several attempts have been made to describe microbial processes, no model has yet fully incorporated all of the complex phenomena that are believed to be important thus it requires close coordination between laboratory mechanistic studies and oil displacement experiments under carefully controlled conditions to develop and validate a computer model (Bryant et al., 1992).

Development of such models must incorporate all the physical, chemical and biological processes that occurred during microbial enhanced oil recovery processes such as adsorption, interaction between microorganisms and substrate, biomass formation and ions release. Many works have been carried out to document different aspect of MEOR processes; however the release of divalent Ca^{2+} ions and the role played by the reservoir brine remains one of the least studied parts of microbial fluid rock interaction during microbial enhanced oil recovery. Only few reports refer to ions release during MEOR process in carbonate rocks. During microbial fluid interactions with carbonate rocks, divalent cations are being released as a consequence of dissolution of the rock matrix (Udegbunam et al, 1991). Adkins et al., 1992b have reported similar phenomenon in their work where they used a halophilic bacteria in-situ growth in a packed columns with crushed carbonate rocks. However, they did not mention the concentration of Ca^{2+} ions released. Divalent cations such as Ca^{2+} can influence biofilm formation and can possibly enhance adhesion of bacteria to the rock particles in the medium (Marshall, 1985). 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.

In the present study, we describe the microbial fluid interaction with chalk samples at different salinity and the release of calcium ions from this interaction. Incorporation of such information can increase our understanding of models that attempts to describe fluid-rock interaction during microbial enhanced oil recovery.

2. Experimental procedure

The chalk samples used in this investigation were obtained from Dan-chalk. 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. This strain was already adapted to grow till salinity range of 100 g/l. Detail of the adaptation process is given in (Rudyk and Søgaard, 2011). The bacteria had earlier been cultivated on a reinforced clostridia media (RCM). 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 increased 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).

3. Results and discussion

Fig.1. shows the relationship between Ca^{2+} concentrations with time. A significant content of Ca^{2+} was found for all the media and this correlates with the period of immersion. The average concentration at 7, 14, 21, and 28 days for the salinity measured was 611, 822, 2100 and 3257 mg/l respectively. This amount is significantly different when compared to the average value of 203 mg/l for control experiment (the average starting point of all salinity measured without chalk samples) and average background value of about 50 mg/l for blank experiment (chalk samples and salt solutions at 90 g/l) over the same period of time.

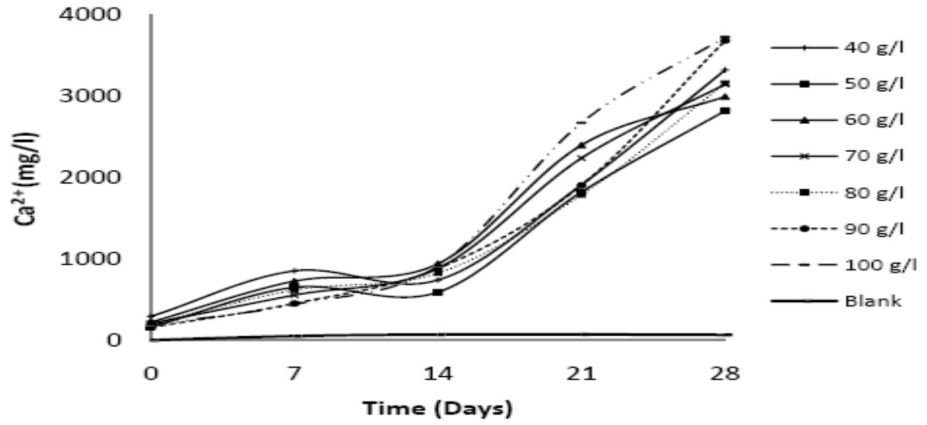


Figure 1. Ca^{2+} ions concentration variation with time at different salinity

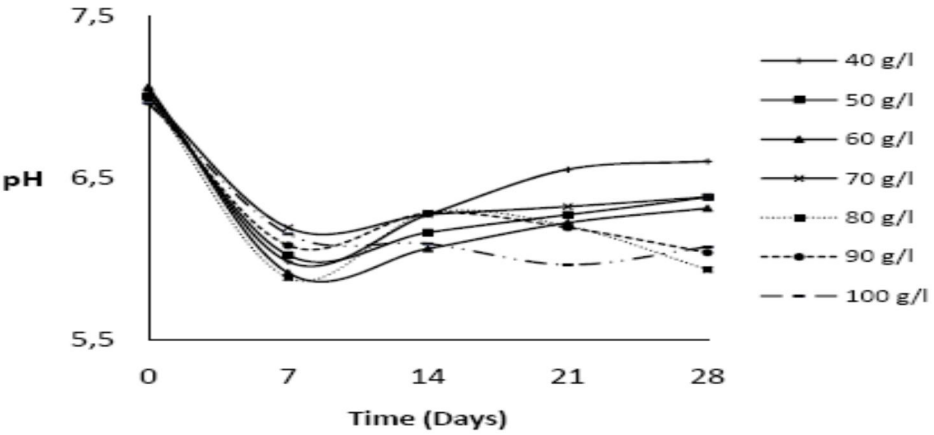


Figure 2. pH variation with time at different salinity

The change in pH of the media with time is shown in Fig. 2. 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. These results appeared to separate the salinity range into two groups i.e. 40, 50, 60, and 70 g/l and 80, 90 and 100 g/l. However correlation between the Ca^{2+} ion content in the fluid and salt concentration cannot be ascertained as it became very irregular.

Increase in the pH of the media with time is indicative of carbonate mineral dissolution as represented by equation (1). This will lead to formation of water soluble bicarbonates.

$$CaCO_3 + CO_2 + H_2O = Ca^{2+} + 2HCO_3^{2-} \quad (1)$$

As the concentration of HCO_3^{2-} increases, the media become less acidic and the pH increases. The opposite effect noticed at salinity (80 -100g/l) from day 14 which is more acidic can be attributed to effect of salinity on metabolism of bacteria that promotes more acid production and reduces the influence of HCO_3^{2-} on pH as it is shown in Fig. 3 for salinities 40 and 100 g/l as example.

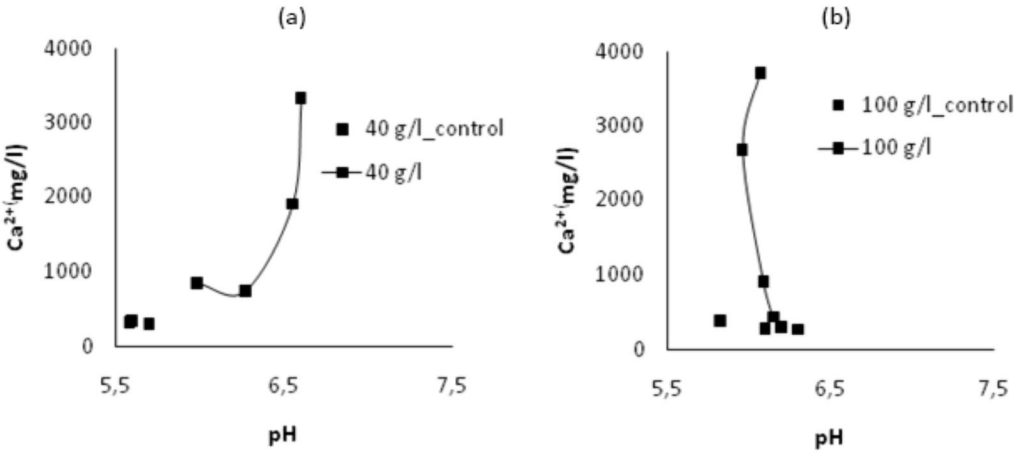


Figure 3.Comparison of media dynamics at (a) 40 g/l and (b) 100 g/l

The results for all salinity measured put together is shown in Fig 4. A strong correlation can be observed between pH and Ca^{2+} concentration with time for the measured salinity range (Fig.4). The equations show a negative correlation for different period of time and an almost equal gradient for dissolution of Ca^{2+} ions which is reflected in parallel lines and almost equal constants in equations. The system is limited with lines between 40 and 100 g/l showing that low salinity corresponds to high pH and high salinity corresponds to low pH. This negative correlation can be an indication of increasing acidic environment with increasing salinity despite relatively higher concentration of Ca^{2+} ions. For more clear presentation of this multivariate system, 3D surface model was made as shown in Fig.5.

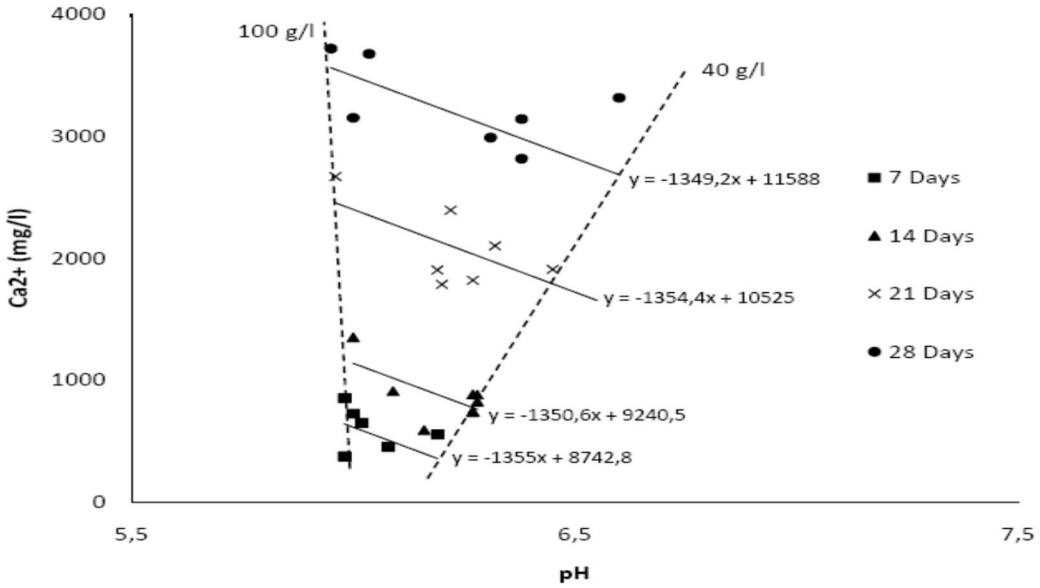


Figure 4.Overall dynamics of the media based on Ca^{2+} ions concentration and pH

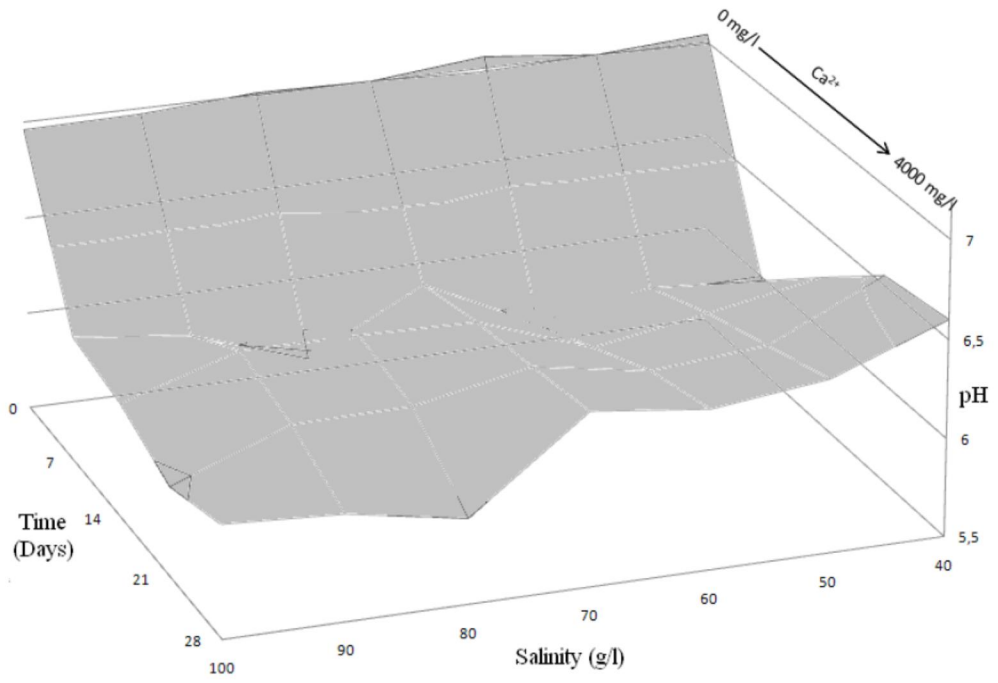


Figure 5. Surface representation of the media parameters

3-D surface plot for all the media (i.e. 40 -100 g/l) as shown in Figure 5 revealed an interesting result. 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 has an elevated plane surface corresponding to higher pH while the high salinity group has a lower plane surface that corresponds to lower pH than the low salinity group. The model thus confirms the deviations corresponding to the low salinity and high salinity groups as in Fig.3. These deviations could be related to the effect of different rates of metabolic process of the bacteria in the different ranges of salinity. At high salinities, the condition in the medium was more acidic. It is also likely that a part of pH change comes from salinity alone 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).

4. Conclusion

The results from this study suggest that incongruent dissolution of chalk samples by microbial solution at different salinity enriched the solution with Ca^{2+} ions about 20 times more than the background value. However the concentration of Ca^{2+} ions in the media did not have clear correlation with salinity. The highest release of Ca^{2+} ions due to higher acidity was observed at harsher environment for bacteria at the highest salinity. The 3-D surface plot was able to distinguish a possible partitioning of pH along two salinity groups. This effect on pH is clearly related to the concentration of Ca^{2+} ions in the media over time

due to bacteria metabolism or those coming from salinity alone. These parameters should be taken into consideration when modeling for microbial fluid rock interactions in carbonate rocks.

References

1. Adkins J.P., Tanner R.S., Udegbunam E.O., McInerney M.J. and Knapp R.M., 1992b, Microbially enhanced oil recovery from unconsolidated limestone cores, Geomicrobiol. J. 10, 77-86.
2. Bryant R.S., Bertus K.M., Stepp A.K., Chang M. and Chase K.L., 1992, Laboratory studies of the parameters involved in modeling microbial oil mobilization, SPE/DOE enhanced oil recovery symposium, 22-24 April, Tusla, Okl., USA.
3. Marshall K.C., 1985, Bacterial adhesion mechanisms and physiological significance: Mechanisms of bacterial adhesion at solid-water interfaces, Eds. Savage D.L. and Fletcher M., Plenum, New York, 133-157.
4. Offshore Centre Denmark (OCD), 2007, Yearbook: Offshore industry in Denmark.
5. Rudyk S. and Søgaard E., 2011, Applied microbiology and molecular biology in oilfield systems: Microbial enhanced oil recovery, Eds. Whitby C. and Skovhus T.L., Springer, Dordrecht, 179-187.
6. Udegbunam E.O., Adkins J.P., Knapp R.M. and McInerney M.J., 1991, Assessing the effects of microbial metabolism and metabolites on reservoir pore structure, SPE22846, Proceedings of SPE annual technical conference and exhibition, Society of Petroleum Engineers, Richardson, Texas.

Paper V

Microbial fluid-rock interactions in 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 , Esbjerg Institute of Technology
Aalborg University Niels Bohrs Vej 8 6700 Denmark, egs@bio.aau.dk

In this study laboratory scale experiment was used to investigate the effect of microbial treatment on chalk samples from Danish sector of the North Sea. Microbial media consisted of *Clostridium tyrobutyricum* in reinforced clostridia media supplemented with molasses as carbon and energy sources. After 28 days of incubation, bacteria treatment was found to increase porosity of all rock samples. The mean porosity of all the samples increases from 40.0% to 44.0%. Additional results showed that the bulk volumes correlates with the biomass retained ($r = 0.70$). Further results from 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 the 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 irrespective of their salinity condition 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. However the correlation observed with calcium ions released and the measured electrical conductivity is weak. 3-D surface plot for the range of salinity measured revealed delineation of measured 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 indicates the dissolution rate of chalk was faster at higher salinity. This 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. Microorganisms alter rates and mechanisms of chemical and physical weathering and clay growth, thus playing fundamental roles in soil and sediment formation. For example microbes contribute to dissolution of insoluble secondary phosphates, possibly via release of organic acids (Banfield et al., 1999).

During microbial growth, the pore environment is altered in many ways which include modification of rock texture, pore size, pore shape and grain surface roughness (Atekwana et al., 2006). 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, J.W., 1967) and 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 pure 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. Understanding of how bacterial treatment and their metabolic products affects reservoir rock properties will allow for good application of microorganisms for enhanced oil recovery.

Several workers have documented the plugging of permeability modification by bacteria to plug the zone of high permeability to increase 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, curtailing the prospect of premature abandonment and Effective modification of reservoir sweep 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 carbonates 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, M., 1996, Marshall, K.C., 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* species and to evaluate the modification with time.

2. Materials and method

The chalk samples used in this investigation were obtained from Dan-chalk. 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. This strain was already adapted to grow till salinity range of 100 g/l. Detail of the adaptation process is given in (Rudyk and Søgaard, 2011). The bacteria had earlier been cultivated on a reinforced clostridia media (RCM). 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 increased 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).

Porosity Determination

For the determination of porosity, rock samples were pretreated 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) is determined. The dry weight of the sample (W_d) is also determined. The pore volume (V_p) is determined 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 bulk 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.

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;

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

For biomass 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 dry complete at a temperature of 100 ^oC. The mathematical expression for biomass is given by;

$$Biomass = W_F - W_I$$

3. Results

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

Table 1: Summary of result for chalk samples before and after microbial treatment

Salinity (g/l)	Sample ID	Initial ϕ	Final ϕ	Change in ϕ	Initial W (g) (W_1)	Final W (g) (W_2)	Biomass Acc.(g) (W_2-W_1)	Initial B.V (cm^3)	Final B.V (cm^3)	Initial Dens.	Final Dens.
40	1	0.40	0.46	0.06	19.36	20.09	0.73	8.80	8.59	2.20	2.33
50	2	0.40	0.44	0.04	16.38	17.07	0.69	8.75	8.59	1.87	1.99
60	3	0.42	0.46	0.04	18.08	18.97	0.89	9.30	9.00	1.94	2.09
70	4	0.41	0.46	0.05	15.74	16.41	0.67	7.90	7.69	1.99	2.14
80	5	0.40	0.45	0.05	14.79	15.39	0.60	6.78	6.43	2.18	2.43
90	6	0.39	0.43	0.04	16.43	17.11	0.68	7.92	7.47	2.07	2.28
100	7	0.40	0.45	0.05	16.74	17.58	0.84	8.93	8.59	1.87	2.05

ϕ =porosity, B.V = bulk volume, Dens. = density, W = weight

From Table 1, it is evident that there is a change in porosity value of each rock sample after bacteria treatment. The increase in average chalk porosity for set of samples increased by the end of experiment. The average porosity increase value was about 0.04 representing an increase of about 11% per sample. Also there is attachment of biomass to rock samples giving the difference in the dry weight before and after microbial treatment. Another property that was modified is the density of the samples. The density increased after the microbial treatment. This is due to the bulk volume of the samples that generally reduced due to dissolution of the rocks. When biomass accumulation is plotted against bulk volume, there is a strong correlation between the two parameters as shown in Figure 1. This suggests that the bigger the bulk volume the bigger the amount of biomass retained on the rock.

The bacteria solution 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 salinity. The average concentrations at 7, 14, 21 and 28 days were 611, 822, 2100 and 3527 mg/l respectively.

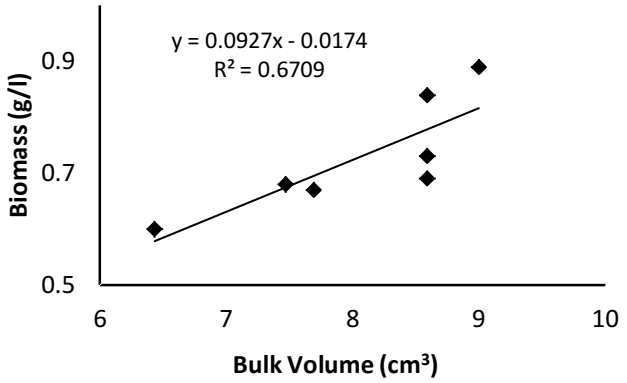


Figure 1: Biomass and bulk volume relationship for the chalk samples

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). The relationship observed did not show a clear correlation between salinity and amount of calcium ions released. 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.

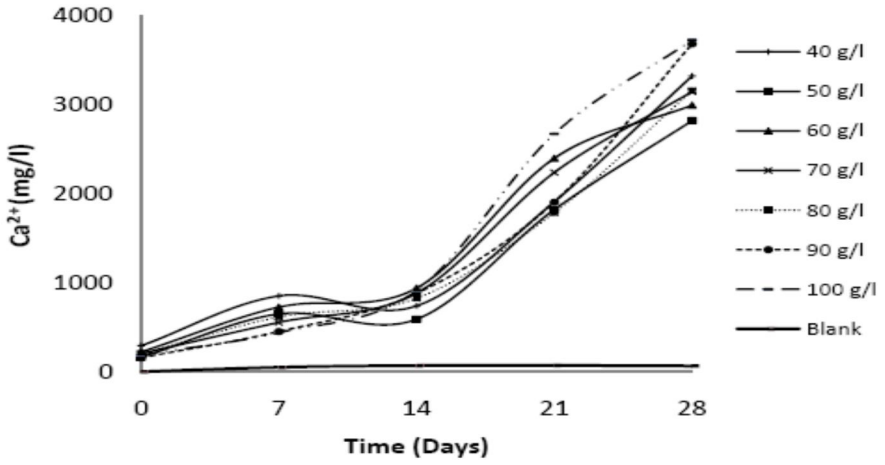


Figure 2:Ca^{2+} ions concentration variation with time at different salinity

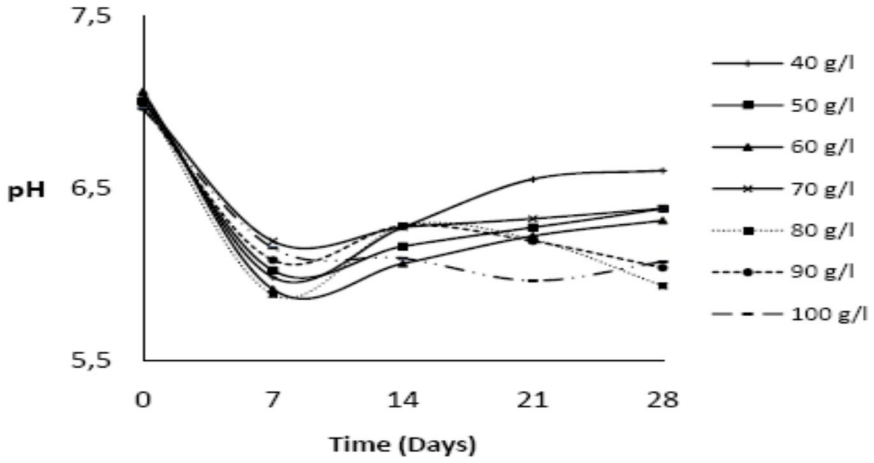


Figure 3: pH variation with time at different salinity

The release of calcium ions also has effect on the electrical conductivity of the media. The measured electrical conductivity increases over time when compared with the electrical conductivity measurement at the start (Figure 4) for the range of salinity measured.

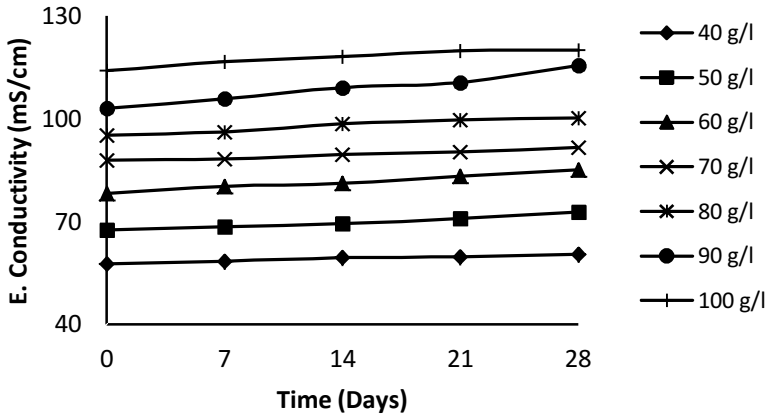


Figure 4: Electrical conductivity variation with time at different salinity

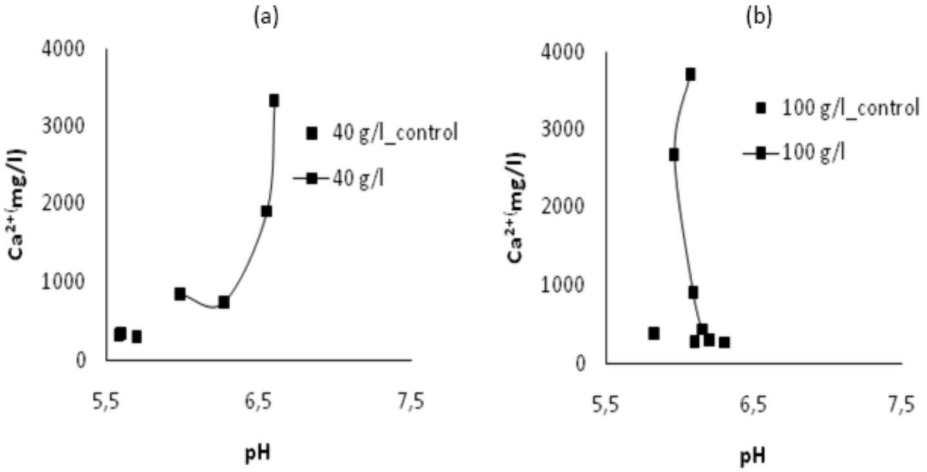


Figure 5: Comparison of media dynamics at (a) 40 g/l and (b) 100 g/l

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.

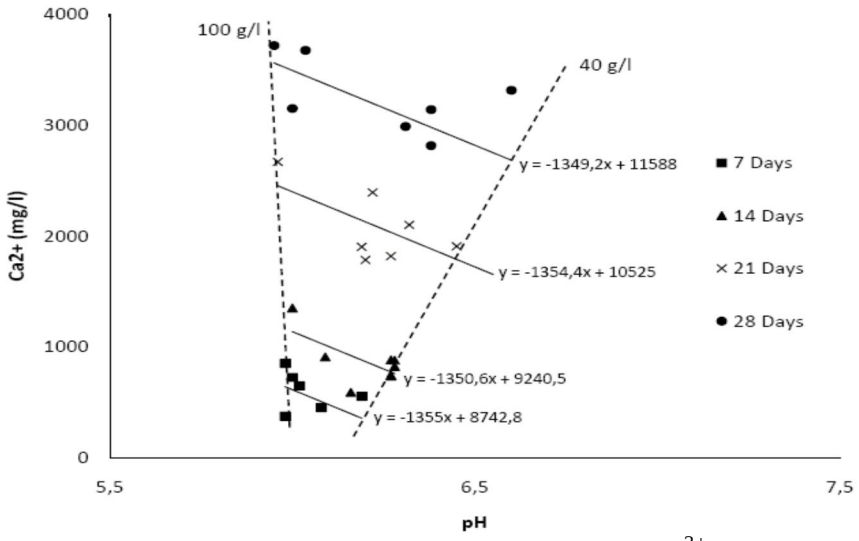


Figure 6: Overall dynamics of the media based on Ca^{2+} ions concentration and pH

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 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). This partition suggests the influence of salinity on microbial metabolism in the different media

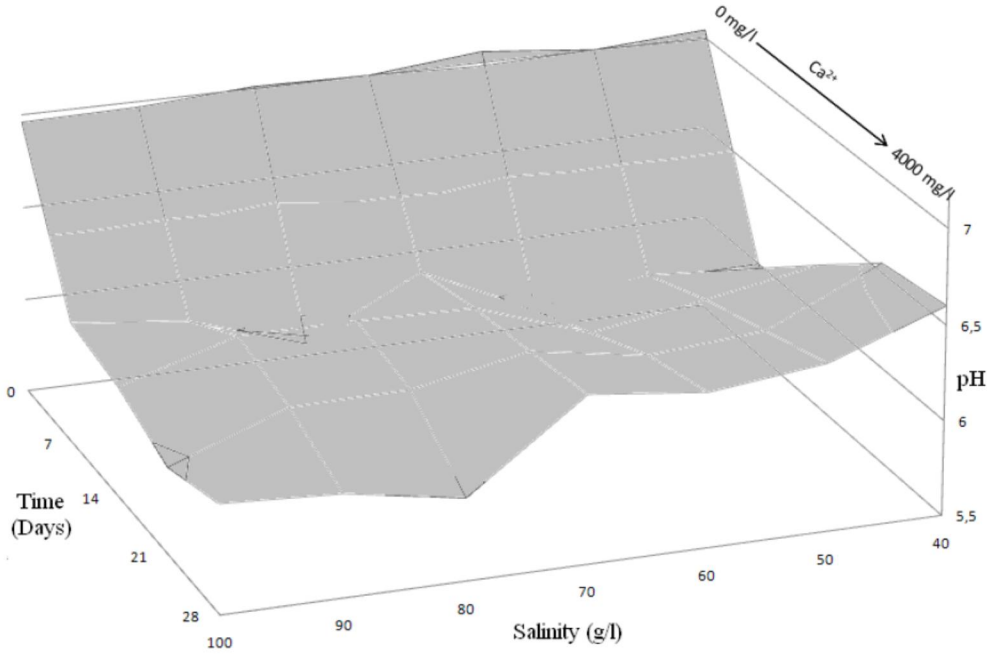


Figure 7: Surface representation of the media parameters

4. Discussion

Microbial modification of carbonate rocks was investigated in this study. The average porosity values increased at the end of incubation period. This is directly related to the microbial activities, suggesting organic acid production and carbon dioxide formation both processes that leads to rapid dissolution of rock mass leading to porosity increase. 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. It has been shown that permeability in chalk depends on porosity and rock surface area (Mortensen et al., 1998, Rogen and Fabricius, 2002). Increase porosity can also results in increase 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 The increase in porosity can also have the advantage of internal pores which can be colonized. Huysman et al., 1983 has found out that the favourable conditions for biofilm formation in a porous material are the size of the pores and the degree of porosity.

The bulk 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 and enabling bacteria adhesion that can encourage the growth of biofilm. Biofilm can grow on the surface of the porous rock which may lead to a change of surface properties and/or a decrease in permeability (Gandler, 2006). Permeability change cannot explain the increased oil production from water wet cores; however the change in surface properties inside the porous rock can thus lead to change in wetting properties causing mobilization of oil (Hiorth, 2007).

Calculation of the density of the rock samples before and after the microbial treatment using the dry mass showed that there is increase in density of the rock samples as a result of amount of biomass retained in pores of the rock samples. 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.

The result showed the release of calcium ions into the bacteria media that increases with period of immersion. This release of ions can possibly enhance adhesion of bacteria to the rock particles in the medium. 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. On the order hand, it not known what the influence of these ions would be during the separation process for produce water after the oil has been separated, since some rock matrix will be produced along oil. Recently there are concerns about the fate of metallic ions such as calcium ions during separation process based on laboratory experience. However adequate separation techniques can overcome this problem.

The dynamics of the rock dissolution and microbial activity can also be inferred from the changes observed in the pH of the medium and the electrical conductivity measurement (Figure 6). These 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 (1) with formation of water soluble bicarbonates.

$$CaCO_3 + CO_2 + H_2O \rightarrow Ca^{2+} + 2HCO_3^{2-} \quad (1)$$

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. 3-D surface plot for all the media (i.e. 40-100 g/l) as shown in Figure 7 revealed that the plane angle of the salinity measured was partition 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 due to effect of salinity on bacteria metabolism resulting in different rate of metabolic process. Another possible explanation is that part of pH change comes from salinity alone 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.

The variation of electrical conductivity of the medium over time shows a slight increase when compared to the start value however it 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). Sierakowski and Leczyeka (1983) have reported that 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 means some of the calcium ions that would have responsible for significant increases in electrical conductivity of the solution are bound by the basic microbes.

5. 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 carbonate rocks with a media inoculated with a strain of *Clostridium tyrobutyricium* indicated porosity increase in the course of the experiment. The porosity increase observed in all the rock samples was mainly due to significant dissolution of carbonate by the organic acids produced during microbial metabolism. The pattern of dissolution lead to reduction in the bulk volume with pore volume slightly reduced or generally remains the same. The density of the carbonate rocks was also increased due to the fractions of biomass retained in the pore spaces. Further results suggest incongruent dissolution of chalk samples by microbial solution at different salinity enriched the solution with Ca^{2+} ions about 5 times on the average more than starting Ca^{2+} concentration in the media. 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. 3-D surface plot was able to distinguish a possible partitioning of pH along two salinity groups low salinity (i.e. 40, 50, 60 and 70 g/l) and high salinity group (80, 90,100 g/l). The effect on pH is clearly related to the concentration of Ca^{2+} ions in the media over time due to bacteria metabolism or those coming from salinity alone, 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 inside the reservoir rock and inducing wettability that can improve oil recovery.

References

1. Adkins J.P., Tanner R.S., Udegbunam E.O., McInerney M.J. and Knapp R.M., 1992b, Microbially enhanced oil recovery from unconsolidated limestone cores, Geomicrobiol. J. 10, 77-86.
2. Atekwana E.A., Werkema D.D. and Atekwana E.A., 2006, Biogeophysics: The effects of microbial processes on geophysical properties of the shallow subsurfaces. Applied Hydrogeophysics, Vol.71, 161-193.
3. Banfield J.F., Barker W.W., Welch S.A. and Taunton A. 1999, Biological impact on mineral dissolution:
4. Application of the lichen model to understanding mineral weathering in the rhizosphere. Proc. Natl. Acad.Sci. USA, vol.96, 3404-3411
5. Fletcher M., 1996, Bacterial Attachment in Aquatic Environments: A Diversity of Surfaces and Adhesion Strategies, Bacterial Adhesion. Molecular and Ecological Diversity, New York: Wiley-Liss, 1996, 1-25
6. Gandler G.L., Gbosi A., Byrant S.L. and Britton L.N., 2006, Mechanistic understanding of microbial plugging for improved sweep efficiency, SPE/DOE Sympossium on Improved Oil Recovery Tusla, Oklahoma USA 22-26 April 2006, Paper No. 100048.
7. Harbaugh J.W., 1967, Carbonate rock reservoirs, Developments in Sedimentology, Vol.9, Part1, 1967, 349-398.
8. Hiorth A., Kaster K., Lohne A., Siqveland O.K.,Berland H.,Giske N.H. and Stavland A., 2007, Microbial enhanced oil recovery- mechanism, Int. Symposium of the Society of Core Analysts, Calgary, Canada, 10-13 Sept., 2007.
9. Hustrulid W.A. and Johnson G.A., 1990, Rock mechanics: Contributions and challenges, Proceedings of the 31st US Symposium, Colorado School of Mines, Golden 18-20 June, 1990.
10. Husyman P., Van Meenen P., Van Assche P. and Verstraete W., 1983, Factors affecting the colonization of non porous and porous packing materials in model upflow methane reactors. Biotechnol. Lett. 5: 643-648.
11. Marshall, K.C., 1985, Mechanisms of Bacterial Adhesion at Solid-Water Interfaces, Bacterial adhesion. Mechanisms and physiological significance, New York: Plenum, 1985, 133-157.
12. Meehan D.N., Menzie D.E. and Crichlow H.B., 1978, A laboratory study of water immobilization for improved oil recovery, J. Pet. Tech. 29: 205–209.
13. Mortensen J., Engstrøm F. and Lind I., 1998, The relation among porosity, permeability and specific surface of chalk from the Gorm Field, Danish North Sea, SPE Reservoir Evaluation and Engineering 1, 245- 251.

14. Parsons L.B and Sturges W.S., 1926, The possibilities of conductivity method as applied to studies of bacterial metabolism. Jour. Bact., 11, 177-188.
15. Rogen B. and Fabricius I.L., 2002, Influence of clay and silica on permeability and entry pore pressure of chalk reservoirs in the North Sea, Petroleum Geosciences, 8, 287-293.
16. Rudyk S.N. and Sogaard E.G., 2011, Applied microbiology and molecular biology in oilfield systems: Microbial enhanced oil recovery, Eds. Whitby C. and Skovhus T.L., Springer, Dordrecht, 179-187.
17. Sierakowski S and Leczycka E., 1983, Aenderungen der electrischen leitfahigkeit un bacterienkulturen. Zentr. Bakt. Parasitk. Und Infektionsk., 127, 486-492
18. Stepp A.K., Bailey S.A., Bryant R.S. and Evans, D.B., 1996, Alternative methods for permeability modifications using biotechnology. SPE Annual Technical Conference and Exhibition, Denver, Colorado, USA
19. Udegbunam E.O., Adkins J.P., Knapp R.M. and Mclnerney, M.J., 1991, Assessing the effects of microbial metabolism and metabolites on reservoir pore structure, SPE22846, Proceeding of SPE annual technical conference and exhibition, Society of Petroleum Engineers, Richardson, Texas.
20. Wagner A., Lungershausen D., Nowak, U. and Ziran B., 1992, Microbially improved oil recovery from carbonate reservoirs.

Paper VI

Evaluation of produced volumes of carbon dioxide from the concentration of the gas absorbed in the media during microbial fermentation for enhanced oil recovery purposes

Ismaila A. Jimoh, Erik G. Søgaard *, Svetlana N.Rudyk

Section of Chemical Engineering, Aalborg University Esbjerg, Denmark. Telephone +4599409821
egs@bio.aau.dk

Carbon dioxide produced by microbes during microbial enhanced oil recovery process (MEOR) promotes oil displacement and productivity through re-pressurization of the oil field and dissolution of the rock matrix. In the laboratory studies conducted using an adapted strain of *Clostridium tyrobutyricum* (DSMZ 663*)* in fermentation media of 500 mL, the volume of the produced gas, the concentration of the dissolved gas determined using titrimetric method and pH of the media as a result of microbial metabolic activities at different salt concentrations (0, 30, 60, 90 and 100 g/L) were measured after 24, 72 and 120 h. The volume of produced gas decreased from about 3000 mL at 0 g/L to 250 mL at 100 g/L. The rate of absorption, volumetric mass transfer coefficient and partial pressure were then related quantitatively as a function of salinity and were compared. The result shows that the rate of absorption decreases exponentially with salinity suggesting a strong correlation with R^2 value of 0.75-0.98 at constant coefficient of 0.0002. The volumetric mass transfer coefficient for carbon dioxide at 0 g/L is approximately 6 times greater than at 100 g/L suggesting the influence of the salinity of the media. The correlation of the gas concentration in the solution with the gas bulk volumes produced at different salinities after 120 h (R^2 = 0.97) suggests an accurate tool for the estimation of the amount of gas produced by microbes.

Introduction

Microbial enhanced oil recovery (MEOR) involves the use of specific bacteria capable of producing useful metabolites in-situ such as gases, acids, surfactants, solvents and polymers in order that their presence will aid further reduction of residual oil left in the reservoir after secondary recovery (Lazar, et al. 2007). The production of biogenic gases as earlier mentioned creates a free gas phase that can account for incremental oil recovery in MEOR processes either by reduction of the oil viscosity by solution of the gas in the oil, or by repressurization of the reservoir causing displacement from trapped capillaries and enhancing mobilization of the oil to the producing wells (Sen, 2008). The most important gas-producing bacteria are *Clostridium, Desulfovibrio, Pseudomonas*, and some methanogens (Behlülgil and Mehmetoğlu, 2002). The composition of the biogenic gas from bacteria metabolism can include carbon dioxide, hydrogen, methane and nitrogen.

However, certain factors can affect gas production by either reducing the volume of gas produced or the amount that can go into solution. One of such factor is salinity of the media. Salinity is known to affect the solubility of gas in liquid because of the salting out effect (Duan and Sun, 2006). Oilfield brines can have a wide range of concentrations of a large variety of cations and anions with concentrations ranging from less than 100 mg/L to more than 200,000 mg/L (Donaldson et al. 1989).

During fermentation process under anaerobic condition, gases are produced by conversion of carbon source such as molasses which is a cheap and universally available nutrient. The effectiveness of the gas produced to enhance oil recovery will depend on how much of it can be transferred into the system. Evaluation of the amount of gas that can dissolve into the solution during microbial process is thus important. This can give an idea of how much of the gas produced goes into solution for displacement of oil at the oil-water-rock interface. However, all these processes happen inside the reservoir which can be very difficult to determine and monitor. Therefore, there is a need for simple system to determine measurement to handle fluid samples from reservoirs. Measurement of concentration of gas produced in situ can give an indication of effectiveness and limit of bacteria metabolism. For example, a fixed amount of bacteria inoculum under laboratory conditions when grown in a medium produces gas, of which the concentration in the solution can be determined by chemical methods and correlated with the measured volume of the produced gas. If the concentration of the gas in the fluid sample from a reservoir is known, then it will be possible to predict the volume of gas produced. Correspondingly, this information would be crucial in simulation of MEOR performance as the salinity level is different for many reservoirs.

For this reason, a simple experimental procedure was used to assess the gas production and evaluate the concentration of carbon dioxide in media of different salinity in order to estimate how much of such can go into solution. Another objective is to find correlation between gas concentration in the media and volume of gas produced.

Materials and methods

Fermentation set up

Solutions of different salinity were prepared in fermentation bottles of 1 L volume by accurately weighing specific amount of sodium chloride and dissolving in 500 mL of demineralized water to make solution of salinity 0, 30 60, 90 and 100 g/L. The fermentation bottles and media were initially purged of air by passing pure nitrogen for about 5 min. This was to create anaerobic conditions in the bottles. The nutrient consists of 50 mL of molasses and was added into already prepared 500 mL of salt solution of different salinity; the molasses acted as the substrate for the bacteria. Each set up was inoculated with 10 mL inoculum of an adapted strain of *Clostridium tyrobutyricum* (DSMZ 663) cultivated on reinforced clostridia medium after growth of 48 h indicated by change in turbidity. The full process of adaptation for this strain is described in Rudyk and Søgaard, 2011. The set up was completed with water displacement apparatus for gas collection and measurement of volume produced. The whole process was maintained in water bath at 37 ^{o}C. Cumulative gas production was calculated as total sum of gas produce during the period of 120 h. Liquid samples were carefully taken out from the fermentation bottles every 24 h for pH measurement using a pH meter (Model: PH 2000 Radiometer Analytical).

Gas analysis

The gas composition analysis was carried out using gas chromatography equipment (Model: Clarus 500 Perkin Elmer). The sample amount was 1.0 mL with a dilution factor of 1.0. Each sample was run for 3 cycles. The gas samples analyzed were collected after a fermentation period of 48 h.

Titration

The determination of concentration of dissolved CO_2 in the liquid sample was carried out using the method of end point pH with the titration manager (Model: ABU 901 Radiometer Analytical). 10 mL of samples were carefully taken from fermentation bottle at intervals of 48 h. 5 mL of each sample was titrated with a standard sodium hydroxide solution. Free CO_2 reacts with the sodium hydroxide to form sodium bicarbonate. The completion of the reaction is indicated automatically at end point pH of 8.3. The equivalent concentration of CO_2 in each sample is indicated after the completion of the reaction. All the

samples were corrected for any dissolved CO_2 in the molasses by subtracting the background value in molasses from the measured values of dissolved CO_2 at different time.

The concentration of the dissolved CO_2 determined by the titration method as described above can be expressed by Henry's equation (Yagi and Yoshida, 1977) as given in equation 1 below.

$$[CO_2] = K_H \, pCO_2 \qquad (1)$$

where K_H is Henry's law constant (mol/L.atm) and pCO_2 is the partial pressure of CO_2 in the gas phase (atm). The value of Henry's constant used for calculation is 3.4 x 10^{-2} (mol/L.atm) at temperature of 37 ^{o}C.

As shown by (Dixon and Kell, 1989), due to stepwise increase of the pCO_2 in the gas phase, the rate of gas absorption (R_s) can be written as:

$$R_s = dC / dT \qquad (2)$$

C is the concentration of the gas in the bulk liquid, and T is the time. It follows that the rate of absorption R_s of CO_2 can be calculated from the slope of the graph of CO_2 concentration and time and this is the basis of the work to be described.

The volumetric mass transfer coefficient $K_L a$ of CO_2 in the fermentation bottle is calculated from equation 3 below.

$$K_L a = R_s / Hp_g \qquad (3)$$

H is the Henry constant already described above, and p_g is the partial pressure of CO_2 in the gas phase (assumed to be 1 bar). The transfer rate of the gas produced is dependent upon the volumetric transfer coefficient $K_L a$.

Result

During fermentation process of molasses by *Clostridia tyrobutyricum* in solutions of different salinity, gas was produced. Figure 1 illustrates the cumulative gas production at different salinity per 50 mL of molasses in each fermentation bottle. At salt concentration of 0 g/L, the cumulative gas production reached 2900 mL. With increasing salinity, the cumulative gas decreases and is about 250 mL at the highest salt concentration of 100 g/L measured in the experiment within 120 h. The cumulative gas production at 30, 60 and 90 g/L are in between 500-1200 mL. It can be inferred that there is an influence of salinity on the total amount of gas produced as indicated by volume reduction with increase salinity.

The pH variation in the fermentation medium over time at different salinity is shown in Figure 2. The initial pH values fall significantly in the first 24 h at all measured salinity. The decrease of pH values was probably due to acid formation and production of gas. At salinity 0 g/L the pH appeared to increase slightly from 72 h after the process started, while at other salinity ranges it appeared lowered or remain constant after 72 h.

The composition of the gas phase was determined and main constituents are shown in Table 1 as percentage composition. The other gases in Table 1 are assumed to be mixture of hydrogen and methane. With the result of gas composition supporting that main constituent is carbon dioxide, further analysis was done using equations 2 - 3 to evaluate those parameters described.

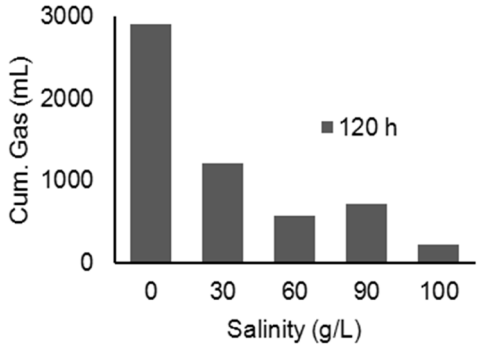


Figure: 1 Cum. gas production at 120 h

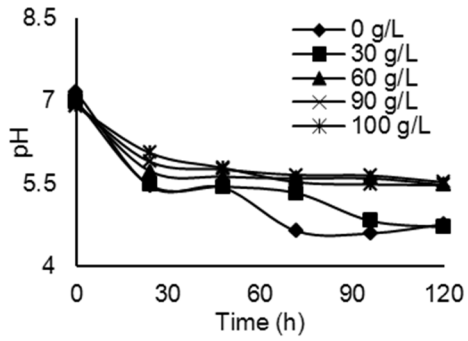


Figure 2: pH variation with time at different salinity

Figure 3 shows the measured amount of dissolved CO_2 in the fermentation medium at different time and salinity. The highest concentrations were found at 120 h. If the salt concentration of the media is considered, irrespective of time, concentration of dissolved CO_2 in the medium is in decreasing order of 0, 30, 60, 90 and 100 g/L respectively. The measured values for 90 g/L and 100 g/L are similar due to the closeness of their salinity range. This result further suggests the effect of salinity on dissolution of CO_2 as well. From the slope of the graph in Figure 3, the rate of absorption was calculated using equation 2 and the result is shown as function of salinity in Figure 4.

Table 1: Average gas composition for gas sample from fermentation of molasses

Component	% Composition
Carbon dioxide	83.66
Others	16.23
Nitrogen	0.11
Total	100.0

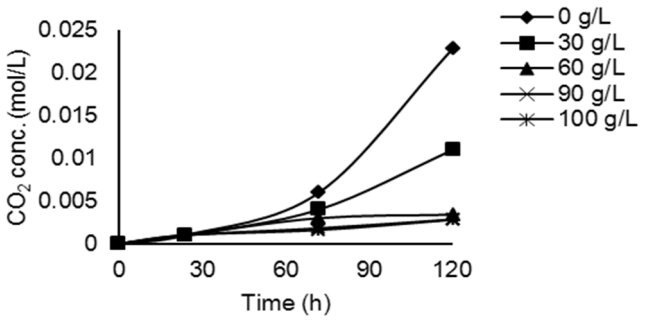


Figure 3: Concentration of dissolved carbon dioxide with time at different salinity

The rate of absorption (R_s) decreases exponentially with salinity with R^2 value of 0.88 - 0.98. This suggests a strong correlation between rate of absorption and medium salinity. However the highest rate occurred at 24 h probably because the largest volume contributing to cumulative gas production was produced in the first 24 h. The concentration of dissolved carbon dioxide is plotted against the cumulative gas production after 120 h and is shown in Figure 5.

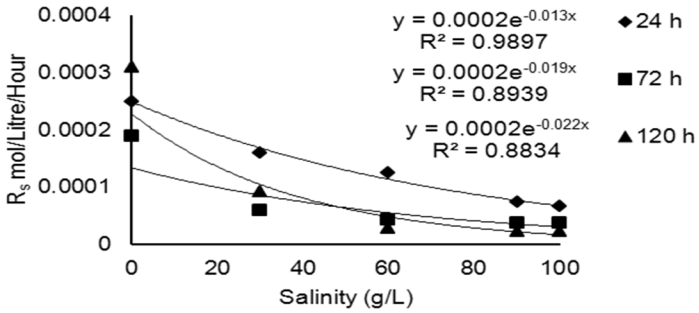


Figure 4: Rate of absorption of carbon dioxide at different salinity and time

The alteration of dissolved CO_2 concentration with salinity correlates with the bulk volumes of the produced gas after 120 h (R^2 = 0.97). The two deviating points are 60 g/L and 90 g/L respectively; however the reason for the deviation is not clear yet. The equation for the relationship is given below in equation (4):

$$[CO_2] = 0.000008 * C_g \quad (4)$$

where C_g is the cumulative gas produced after 120 h.

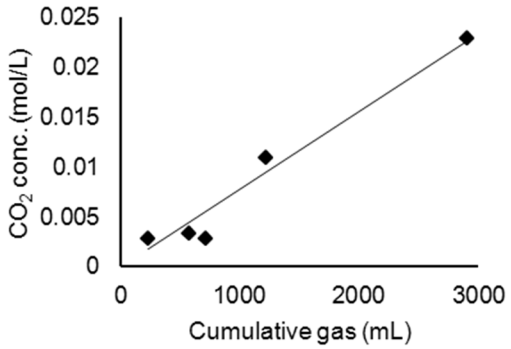


Figure 5: Correlation between concentration of dissolved CO_2 and cumulative gas after 120 h

Table 2 shows the result of transfer coefficient as calculated using equation 3. The result shows that value of volumetric mass transfer coefficient decreases with increase salinity. It was observed that the trend in $K_L a$ followed that of the absorption rate CO_2 quite closely.

Table 2: Result of calculated volumetric mass transfer coefficient at different salinity

Salinity (g/L)	0	30	60	90	100
$K_L a$ (H^{-1})	0.010	0.0042	0.0026	0.0018	0.0017

Discussion

In the production of gas by an adapted strain of *Clostridium tyrobutyricum* (DSMZ 663) grown on molasses, we have shown that the main constituent of the gas phase is carbon dioxide (Table 1). The measured pH values also decreases and it is comparable to our earlier result (Jimoh et al. 2011) where we reported a decrease of pH for this strain of bacteria as indication of gas and acid production. The cumulative volume of gas produced decreases with salinity as a result of the inhibition of the bacterial growth. The volume of cumulative gas at salinity 0 g/L is approximately 13 times greater than at 100 g/L. However, the rates of gas production of approximately 300-700 mL from initial volume of media of 500 mL at salinity range of 90-100 g/L showed that this strain underwent adaptation process successfully and can be productive even at such high salt concentrations.

Furthermore, the calculated values of volumetric mass transfer coefficient of carbon dioxide (Table 2) show a significant influence of salinity of the medium. Thus at high salinity (low carbon dioxide production), a low volumetric mass transfer coefficient of carbon dioxide can be expected. The measured concentration of dissolved CO_2 from titration experiment decreases with salinity of the medium also (Figure 1). The concentration of dissolved CO_2 in the media by the end of the experiment at different salinity probably settles at a value that provides the absorption rate which is directly related to the volume of gas produced.

When the concentration of dissolved CO_2 is plotted against the cumulative gas produced at 120 h there is a strong correlation between the two parameters with R^2 value of 0.97. If concentration of dissolved CO_2 correlates almost at 100 % with the total produced gas, salting out effect could be considered to be minimal taking into account low gas volumes produced. This probably suggests that a reliable estimate of the amount of gas produced can be made from the measurements of the concentration of dissolved CO_2 in the fluid sample and applied to practical purposes of the monitoring of the microbial gas production during MEOR operations.

Conclusion

The adapted strain of *Clostridium tyrobutyricum* (DSMZ 663) is able to produce gas at high salinity. It was shown that about 84 % of the gas produced during fermentation process of molasses is composed of carbon dioxide and the absorption rate decreases with increase salinity. Further evaluation showed that from the concentration of the dissolved gas in microbial media measured from titration method, the estimation of volume of gas produced can be made.

References

1. Behlülgil, K., Mehmetoğlu, M.T., 2002, Bacteria for Improvement of Oil Recovery: A Laboratory Study, Energy Sources, 24,413
2. Dixon, N.M., Kell, D.B., 1989, The Inhibition by CO_2 of the Growth and Metabolism of Microorganisms, Journal of Applied Bacteriology, 67, 109 -136.
3. Donaldson, E.C., Chilingarian, C.V., Yen, T., 1989, Microbial Enhanced Oil Recovery, Developments in Petroleum Science 22, 1-12.
4. Duan, Z., Sun R., 2006, A model to Predict Phase Equilibrium of CH_4 and CO_2 Clathrate Hydrate in Aqueous Electrolyte Solutions, Am. Mineralogist, 91, No. 8-9 1346-1354
5. Jimoh, I.A., Rudyk, S.N., Søgaard, E.G., 2011, Microbial Fluid-Rock Interactions in Chalk Samples and Salinity Factor in Divalent Ca^{2+} ions Release for Microbial Enhanced Oil Recovery Purposes, Chemical Eng. Transactions, Vol., 24, 889-894.
6. Lazar, I., Petrisor I.G., Yen, T.F., 2007, Microbial Enhanced Oil Recovery, Petroleum Science and Technology, 25, 1353-1366.
7. Rudyk, S.N., Søgaard, E.G., 2011, Applied Microbiology and Molecular Biology in Oilfield Systems: Microbial EOR, Eds. Whitby C. and Skovhus T.L., Springer, Dordrecht, 179-187.
8. Sen, R., 2008, Biotechnology in Petroleum Recovery: The Microbial EOR, Prog. Energy. Combust. Sc., 34, 714-724.
9. Yagi, H., Yoshida, F., 1977, Desorption of CO_2 from fermentation broth, Biotech. Bioeng. 19, 801- 819.

Paper VII

Spectroscopy and image analysis characterization of adapted strains of *Clostridium tyrobutyricum* for microbial enhanced oil recovery purposes

Ismaila A. Jimoh, Erik G. Søgaard, Svetlana N. Rudyk and Sergey V. Kucheryavskiy, Section for Chemical Engineering Aalborg University Denmark, Telephone +45 99407662, e-mail: egs@bio.aau.dk

Abstract— In this study, spectroscopy and image analysis have been employed to characterize the difference between pure strains of *Clostridium tyrobutyricum* and the strains adapted to salinity and temperature for microbial enhanced oil recovery purposes. The pure strain and adapted bacteria strains have been metabolically characterized by gas production, pH and morphologically by FT-IR spectroscopy and image analysis. The result of FT-IR analysis was able to distinguish the adapted strains from the pure strain based on cell content. Further results from multivariate data analysis using PCA method showed that the morphological and physiological changes were induced with increasing salt concentration that correlates with gas production and change in pH. It was found that the adaptation probably occurred due to the formation of bacterial clusters at extremely high salinity. High density of bacteria cells occurring as single cells and rod like shape were found in low to medium salinity in contrast to high salinity where the proportion of bacteria cells were found in clusters, round shaped but with decreasing cell density. The shifts in the morphological as well as the physiological composition of the bacterial assemblage in the media allow for a clear subdivision into different salinity groups.

Index Terms—Adaptation, FT-IR spectroscopy, image analysis, salinity

Introduction

Microbial enhanced oil recovery (MEOR) applies injection of bacteria to the depleted oil reservoir to create in-situ production of gas and other metabolic products in order to increase oil extraction [6]. However, there are some challenges that must be overcome for MEOR application to be successful. Challenges in MEOR are mainly the extreme conditions in oil reservoirs. Two of such conditions are high salinity and temperature. However, it has been suggested that careful adaptation of bacteria to such conditions can be carried out under laboratory conditions [1], [10].

To enable them to survive and grow in extreme environments, bacteria have evolved a complex range of adaptations to all of their cellular components, including cell membranes, energy-generating systems, protein synthesis machinery, biodegradative enzymes and the components responsible for nutrient uptake

[11]. Adaptation to salinity by bacteria is achieved in different ways. The first and the most important one involve the accumulation of organic compatible solutes within the cytoplasm without the need for change of intracellular proteins. This method is called 'organic osmolyte strategy' [8], [9]. The second adaptation strategy is intracellular accumulation of high concentration of K^+. This method is referred to as 'salt in cytoplasm strategy', and this requires extensive adaptation of the intracellular enzymatic machinery and is therefore energetically expensive [8]. The objective of this study is not on adaptation mechanism of bacteria. Details of adaptation strategy by bacteria to environmental temperature changes can be found [3], [13].

As pointed out from many reviews on MEOR that reservoir temperature and pressure may not be as restrictive to MEOR as salinity, this however still remains to be solved by carefully conducted laboratory and field experiments [2]. One operational strategy that can be applied to minimize the effect caused by salinity and high temperature is to gradually adapt the microorganisms to high salinity and temperature levels. But questions still remain on how the changes that occurred during adaptation process can be linked to metabolic characteristics.

In this study, *Clostridium tyrobutyricum* which is a Gram-positive rod shaped, spore-forming, obligate anaerobic bacterium have been used in molasses fermentation process that generates energy-rich metabolic products. This genus of bacteria has earlier been used by some researchers [4, 15], because of their ability to produce gases that include carbon dioxide and hydrogen and also butyric and acetic acids which are

useful in microbial enhanced oil recovery process from various carbon sources [14]. The results presented in this paper form a part of our ongoing investigation to characterize adapted strain of bacteria for microbial enhanced oil recovery purposes. Additionally, not much work has been carried out on how to distinguish the strains of pure culture from adapted mutants. The changes that occur in the properties of bacteria cells can be advantageously used in monitoring their metabolic potentials. Gas production has been used as a measure of successful adaptation to salinity and temperature [4]. For evaluation of morphological modifications, a combination of image analysis technique and FT-IR spectroscopy were employed. The two methods are extremely rapid, non-destructive and they can provide quantitative information about structure of investigated objects as well as the interactions between non-visual properties of the objects [12]. Both FT-IR spectroscopy and image analysis have been applied in microbiology in different ways for quantitative and qualitative analysis and can fulfill the requirement of this study.

Materials and methods

Cell harvesting

Salinity adapted strain (90F) and temperature adapted strain (90F-45) were used for comparison with the pure strain (PS) of *Clostridium tyrobutyricum* (DSMZ 663). The cells of pure strain, strains from salinity and temperature adaptation were harvested just after 72 h of their growth. The broth solutions were filtered to separate cells from the suspended solid materials. The liquid containing the cells was measured into a graduated plastic centrifuge cylindrical bottle (50 mL) for separation of the cells by centrifugation at 10,000 x g for 10 mins using centrifuge model (Biofuge Primo, Heraeus Instruments).

The broth was carefully discarded and the cell pellet was rinsed 3 times with deionized water to remove any trapped ions. The cell pellet was dried at room temperature so as to record the FT-IR spectra. For each cell harvesting at specific salinity, the total amount of broth used was 300 mL and the amount of dry cell collected varied from 2.0 to 4.0 g. Fresh cells mass was generated to have enough quantity for experimentation. The dry cells were later used for spectra collection with FT-IR spectrometer.

Spectra recording

FT-IR technique was employed for this study. The infrared spectra of the samples of the pure strain (PS), 90F and 90F-45 were recorded using Avatar 370 FT-IR spectrometer. Samples were placed directly on the crystal which uses DTGS-KBr as detector. 10 spectra each of the different strains were recorded to verify reproducibility. The number of scan for each spectrum is 32 at a resolution of 4 cm^{-1}.

Zeta-potential measurements

The zeta-potential measurements of the different strains investigated were made with Zetaphoremeter IV (CAD Instrumentation) equipped with a microscope after about 1 h conditioning. 1 mL of the bacteria solutions used for measurement was diluted by a factor of 200 using sterilized distilled water to reduce the number of bacteria cells in the solutions for effective measurement. All the measurements were at a constant ionic strength of 0.01 M using KCl. The zeta potential of the bacteria cells and the pH of each solution were measured simultaneously.

Image acquisition and analysis

72 h culture samples were drawn from media of 0-100 g/l during adaption process using the test tubes similar to Hugh-Leifson technique [5]. The bacteria were cultivated on reinforced clostridia media (RCM). The image acquisition was performed in 640 x 476 pixels and 8-bit format with 40 x magnifications. Around 30 images per sample were acquired in a Nikon Eclipse E-200 (Nikon) coupled to a NIS-elements F2.3 (fotoprogram Nikon).

The image processing and analysis program for bacteria characterization during salinity adaptation was developed in Matlab (The Mathworks Inc.). Primarily, the image processing steps involved transformation of the binary images from the cultures grown at different salinity, from which the morphological parameters and cell density were evaluated. The main steps of the program comprise the image pre-treatment, segmentation, and outlier elimination. The image analysis program is oriented to the single cell characterization and content determination. With this procedure every image had a vector of features, describing its complexity which was then used for classification into different salinity groups using PCA analysis.

Statistical analysis

The statistical analysis was performed using the Matlab (The Mathworks Inc.,). The obtained spectra were analyzed by PCA method to reduce the spectra into different possible classes. The spectra were first smoothened before processing to the same integrated intensity for the given spectra region which in this case was 650-4000 cm^{-1}. The first derivative using Savitzky-Golay algorithm to minimize baseline variability and to enhance resolution was employed.

Results and discussion

Comparison of different strains

The spectra of the adapted strains (90F and 90F-45) as compared to the pure strain (PS) are shown in Fig. 1. The measurement was performed after air-drying the sample. The intensity of the bands at wavenumbers and the interaction of radiance with the sample are affected differently. The most significant spectral differences come from the N-H stretching of proteins around 3400-3278 cm^{-1} in point A, the amide bands of protein at 1675 cm^{-1} at point B and at point C which represent the C=C-O-C, C-O, C-O-P, P-O-P vibrations of polysaccharides at 1072 cm^{-1} modes, respectively. This is based on the values of the intensities of the major bands corresponding to these functional groups.

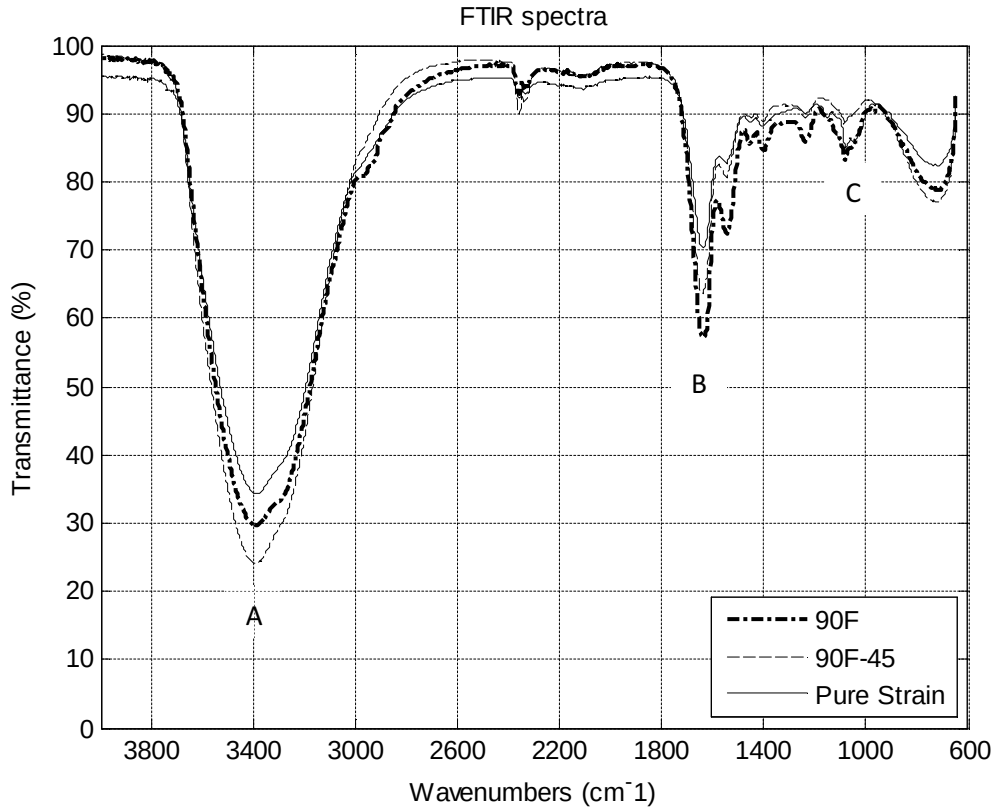


Fig. 1. Spectra for dry harvested cells of pure strain, salinity adapted strain and temperature adapted strain of *Clostridium tyrobutyricum*

Following the initial discrimination between the *Clostridium tyrobutricum* strains based on the FT-IR spectroscopy, data were collected and PCA decomposition was computed. The major PCA results for the spectra are shown in Figs. 2 and 3. Fig. 2a is the loading plot for PC1 while Fig. 2b is the loading plot for PC2. The loading of PC1 (Fig.2a) had a strong negative correlation between 1072-1675 cm^{-1}. The strong positive loading of PC2 is at 1072 cm^{-1} while the strong bands of negative loading were at 600 cm^{-1}, 1250-1275 cm^{-1} and 1675 cm^{-1} respectively (Fig.2b). From PC1 and PC2 loading plots, the significant bands can

be inferred to be 1072 cm^{-1} and 1675 cm^{-1} that corresponds to the vibrations of polysaccharides and amide bands of proteins in the samples. The score plots are shown in Fig. 3. The scatter plot of PC1 against PC2 (Fig. 3a) shows clear differences between the three strains (at 95 % confidence level). The PS samples clustered in the positive direction of PC1 while both 90F and 90F-45 are in the negative direction of PC1; however, 90F had a positive PC2 value and 90F-45 a negative PC2. In the plot of PC2 against PC3 (Fig. 3b), the 90F cells appeared in the quadrant where both PC2 and PC3 were positive and both PS and 90F-45 samples in negative direction of PC2. In summary about 91.3 % of the difference between the samples can be explained by PC1.

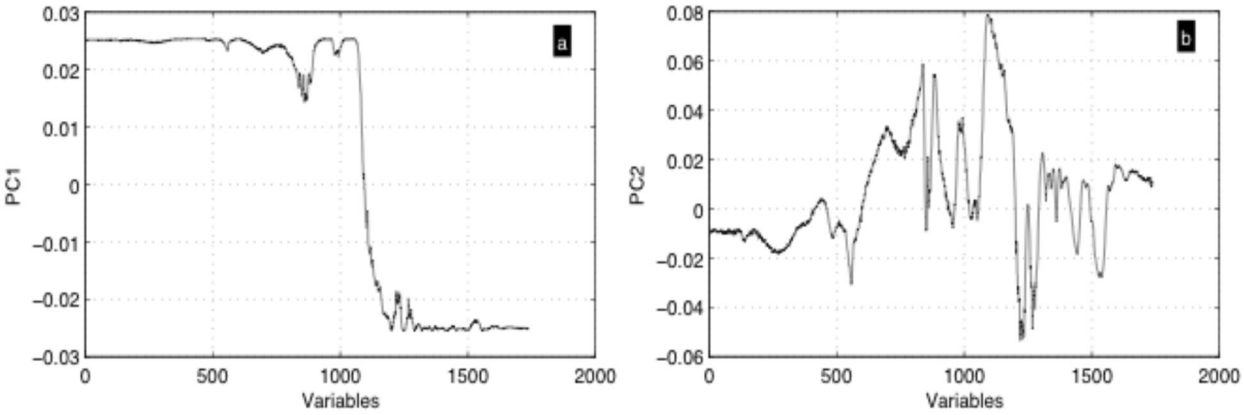


Fig. 2. The score loadings of PC1 and PC2; a — PC1, b — PC2.

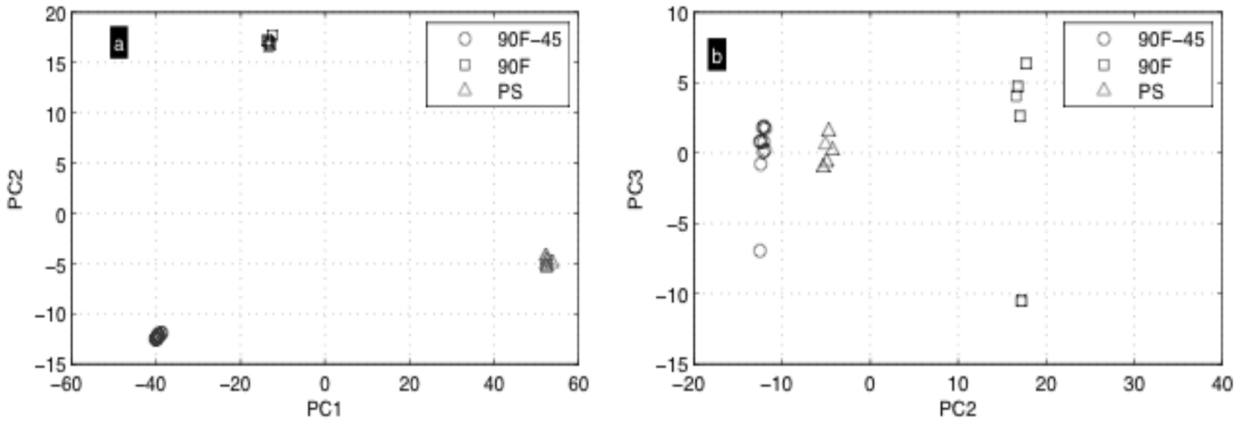


Fig. 3. PCA of the first three PC components showing good classification between different strains of *Clostridium tyrobutyricum* cells; a. — PC1 against PC2, b. — PC2 against PC3.

Attempt was also made to evaluate the difference between the different strains using the zeta potential measurement. The surface of a bacterial cell is charged due to the presence of functional groups such as carboxyl (-COOH), amino ($-NH_2$) and hydroxyl (-OH), originating from the cell wall components of the lipopolysaccharides, lipoproteins and bacterial surface proteins [1]. The zeta potential measurement of the pure strain (PS), 90F and 90F-45 as function of pH is shown in Fig. 4. The result showed that the cells are

negatively charged and the zeta potential values differ substantially and it matches the score plot of PC1 and PC2 (Fig. 3a).

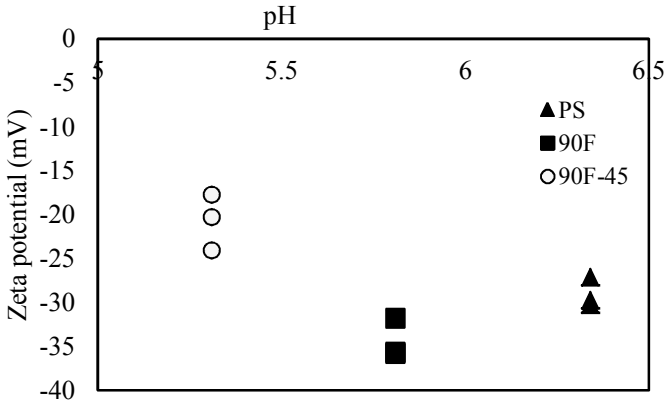


Fig. 4. Zeta potential plot for the different strains of *Clostridium tyrobutyricum*

Changes during salinity adaptation

The representative images for the groups identified from the image analysis are shown in Fig. 5. The salinity group and aggregated morphological structures were subdivided into 3 groups representing the low, medium and high salinity groups. It was difficult to obtain a model that could correspond to each salinity level. Figure 5a shows the representative images of low salinity group (0-30 g/L), Fig. 5b shows the medium salinity group (40-60 g/L) and Fig. 5c belongs to the high salinity group (70-100 g/L). The low salinity group was characterized by isolated bacteria cells, rod like shaped with a dense population of cells. The medium salinity group was characterized by lower cell density, low agglomeration of cells and rod and spherical shaped bacteria cells. The third group that belongs to high salinity showed that the bacteria cells are mostly present in clusters, with very low cell density and spherically shaped.

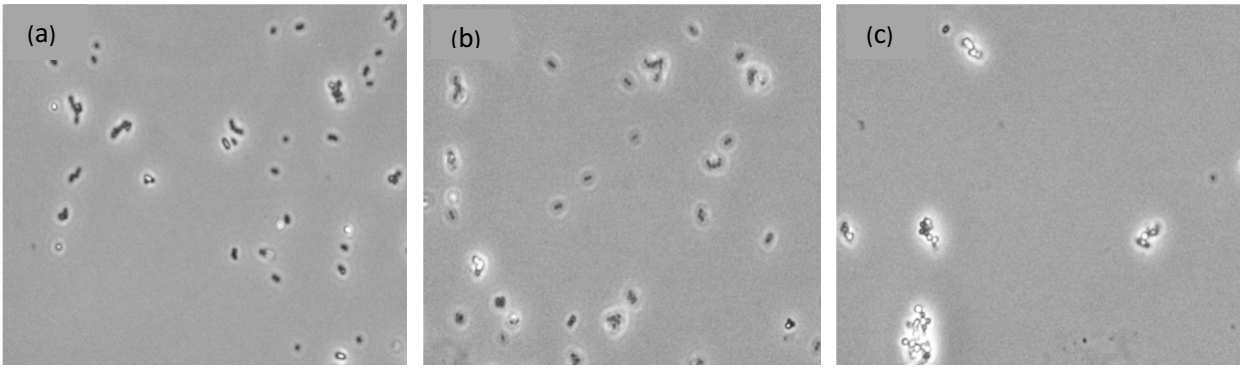


Fig. 5. Representative images of the three sub groups of salinity (magnification:40x); a. — low salinity group, b. —medium salinity group and c. — high salinity group.

The image analysis result that showed morphological difference and cell density was matched with the score plots of the gas production and change in pH of the media for different salinity measured. The result is shown in Fig. 6. It can be seen that for all the figures (Figs. 6a-6d) low salinity group (0-30 g/L) plots in the lower left hand corner, the medium salinity group (40- 60 g/L) plots more or less in the upper right hand corner of the score plot and the high salinity group (70 -100 g/L) plots in the center of the score plot. PC1 was able to explain 83.51% of the variation in the differences between the groups.

Evidence of morphological changes, cells density and aggregations of the bacteria cells as indicated by image analysis probably suggests that with increased salt concentration the bacteria cells shifts to an adaptation strategy with a pronounced change in ability to produce gas. These conform to the observations made earlier as in that increasing salt concentrations can incite physical modifications in bacteria cells [7].

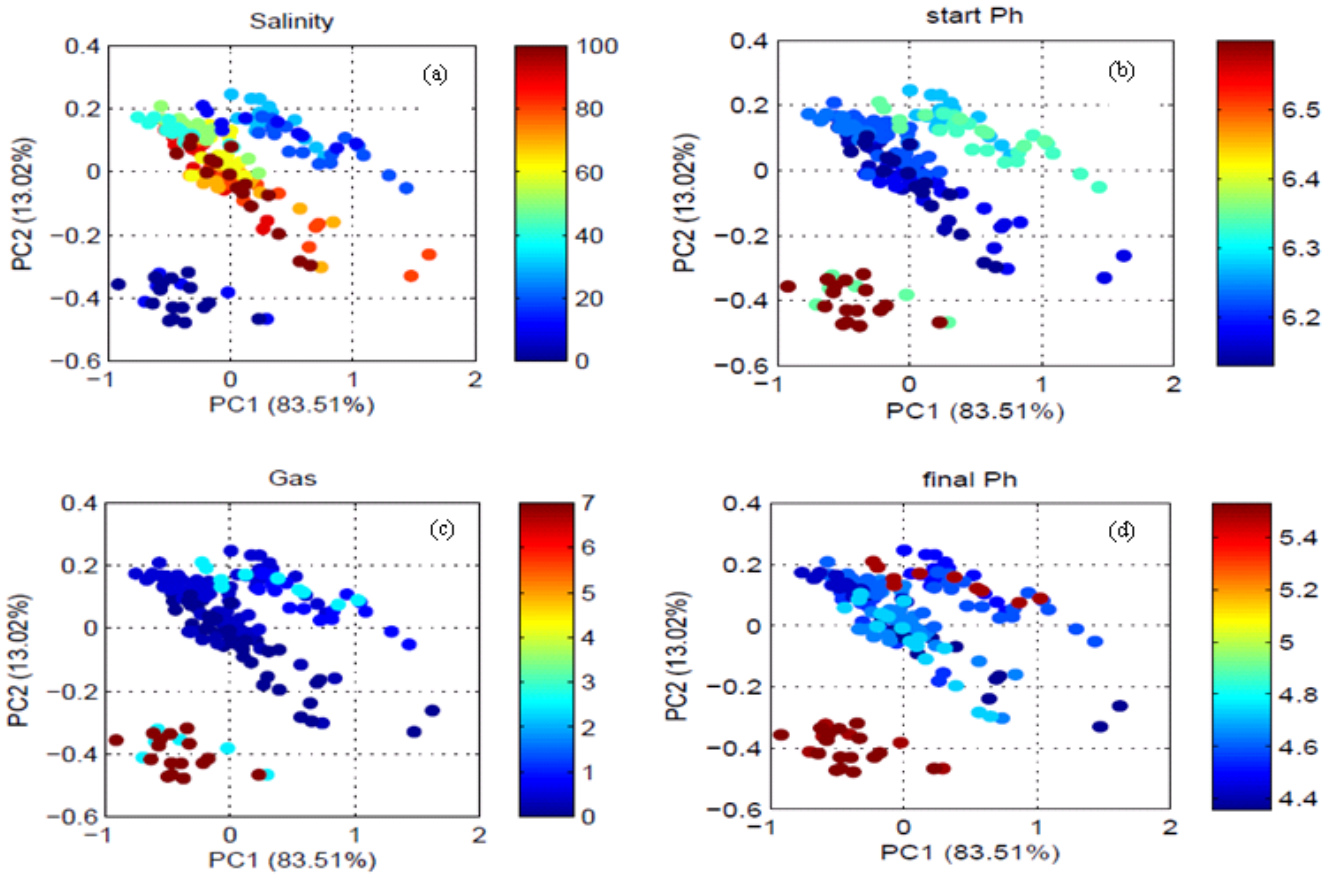


Fig. 6. Scores plots for image analysis, gas production and the pH variation. (a) is the model for salinity of the media, (b) is the model for initial pH measurements, (c) is the model for final pH measurements and (d) is the model based on gas production.

As can be seen from Fig. 5 there are some structural changes on images with different salinity values, these changes can be notified by eye (subjective) and also by mathematical models (objective), Fig. 6. If we match the image analysis with metabolic gas production and pH measurements (Figs. 5 and 6), we can probable discriminate within separate samples with very low and very high salinity (as well as for low and high initial and final pH values, and low and high gas production) which is a good result for such images. Even though three groups can be depicted from the model, there is quite a bit of mixing of some of the samples. This can be due to a few quality problems with some of the images even though many of these quality problems were corrected using various preprocessing algorithms. The lower left cluster of the samples represents the low salinity group (0-30 g/L). However, a small group of samples are intermixed between the medium salinity group and high salinity group. It is expected that with further refinement of this technique, an improved model can be obtained.

Conclusion

The results of this work reinforced the statement that adaptation of bacteria to extreme reservoir conditions of high salinity and temperature is possible to a certain extent. However, for adaptation to be successful, it has to be through a gradual acclimation. The pure strains adapted in this study were able to reach higher level of salinity and temperature but the process of adaptation caused shifts in morphological structure and metabolic potential. The implementation of image analysis and PCA techniques has shown to provide important information for a better understanding of the morphological and cells characteristics at different salt concentration. Moreover, the obtained results explained the strong relationships between the bacteria cells contents, aggregates morphology and gas production. Initial gas production can be affected significantly however bacteria can quickly overcome this shortcoming after proper adaptation. Finally it is shown that a simple and rapid method such as PCA can be used to determine significant changes in the composition of the bacteria cells during adaptation process for microbial enhanced oil recovery.

References

[1] H. J. Busscher, B. van de Belt-Gitter, H.C. van der Mei, "Implications of microbial adhesion to hydrocarbons for evaluating cell surface hydrophobicity: 1. Zetapotential of hydrocarbon droplets", Colloid Surf. B: Biointerfaces 5, 1995, pp. 111-116.

[2] E.C. Donaldson, C.V. Chilingarian, T. Yen, " Microbial enhanced oil recovery", in Developments in Petroleum Sci. 22, 1989, pp. 1–12.

[3] A. Edwards, A.M. Anesio, S.M. Rassner, B. Sattler, B. Hubbard, W.T. Perkins, M. Young, G.W. Griffith, "Possible interactions between bacterial diversity, microbial activity and supraglacial hydrology of cryoconite holes in Svalbard", ISME J. 2010 DOI: 10.1038/ismej.2010.1100

[4] E.A. Grula, H.H. Russel, D. Bryant, M. Kenaga, M. Hart, "Isolation and screening of *Clostridium* for possible use in MEOR " in E.C. Donaldson, J.B. Clark, Eds. Proc. Int. Conf. MEOR, Afton Okla., May 16-21, 1983, pp. 43-47.

[5] R. Hugh, E. Leifson, "The taxonomic significance of fermentative versus oxidative metabolism of carbohydrates by various gram negative bacteria", J. Bacteriol., 66, 1953, pp. 24-36.

[6] I. Lazar, I.G. Petrisor, T.F. Yen, "Microbial enhanced oil recovery", Petroleum Science and Technology 25, 2007, pp.1353-1366.

[7] B. H. Moon, G.T. Seo, T.S. Lee, S.S. Kim, C.H. Yoon, " Effects of salt concentration on floc characteristics and pollutants removal efficiencies in treatment of seafood wastewater by SBR", Water Sci. Technol., 47, 2002, pp.65-70.

[8] A. Oren, "The bioenergetics basis for the metabolic diversity at increasing salt concentrations: an implications for the functioning of salt lake ecosystem," Hydrobiologia 466, 2001, pp. 61-72

[9] M.F. Roberts, "Organic compatible solutes of halotolerant and halophilic microorganisms", Saline Syst., 1, 2005, pp. 5.

[10] S. N. Rudyk, E.G. Søgaard, "Microbial enhanced oil recovery," in Applied Microbiology and Molecular Biology in Oilfield Systems: Microbial EOR. Eds. C.Whitby, T.L. Skovhus, Springer-Dordrecht 2011, pp. 179-187.

[11] N. J, Russel "Molecular adaptations in psychrophilic bacteria, potential for biotechnological applications: Biotechnology of extremophiles," in Biochemical Eng./Biotechnology 61, 1998 pp. 1-21.

[12] J. Schmitt, H. Flemming, "FTIR- spectroscopy in microbial and material analysis," International Biodeterioration and Biodegradation, 4, 1996, pp. 1-11.

[13] J.L.C.M. van de Vossenberg, A.J.M. Driessen, M.S. da Costa, W.N. Konings, "Homeostasis of the membrane proton permeability in Bacillus subtilis grown at different temperatures," Biochimica et Biophysica Acta, 1419, 1999, pp.97-104.

[14] M. Wagner, “Microbial enhancement of oil recovery from carbonate reservoirs with complex formation characteristics,” Development Petroleum Sci., 31, 1991, pp. 387-398.

[15] M. Wagner, D. Lungerhansen , U. Nowak, B. Ziran, “Microbially improved oil recovery from carbonate, ” in A.E. Torma, M.L. Appel, C.I. Brierley Ed., Biohydrometalurgical Technologies,II, 1993, pp. 695-710.

Paper VIII

Laboratory Investigation of *Thermoanaerobacter brockii* subsp. *lactiethylicus* strain 9801^T for Possible Utilization in Microbial Enhanced Oil Recovery

Julaine T. Enas, Ismaila A. Jimoh, Erik G. Søgaard, Svetlana N. Rudyk

Section for Chemical Engineering, Aalborg University Esbjerg, Niels Bohrs Vej, DK 6700 Denmark Email: egs@bio.aau.dk, Telephone: +45 99407622

Abstract

Thermoanaerobacter brockii subsp. *lactiethylicus* strain 9801^T originally isolated from a deep reservoir environment was evaluated for potential use in microbial enhanced oil recovery (MEOR). Investigation was conducted for finding the optimum environmental parameters for growth (temperature, salinity and pH) and production of metabolites desired for microbial enhanced oil recovery. There was growth in the media with pH from 6-9.5 and salinity range of 0.5-3.5% (w/v) and temperature of 50-60 ^oC. The optimum growth occurred at about pH of 7 and temperature of 55 ^oC. The fermentable substrates included molasses, reinforced clostridia medium, thioglycolate and crude oil with major fermentation end products being lactic acid, volatile fatty acids and some amount of gas that included carbon dioxide. The concentration of lactic acid reached 11.7 g/l with a corrosive effect on core chalk samples that led to dissolution and release of calcium ions approximately 38 times greater than the background value. Additionally, this strain also produced biomass with a yield of between 0.0034-0.052 g/h per 100 ml of broth. Result of biosurfactant production assay indicated an oil displacement area of 3.5 cm^2 and fermentation of crude oil indicated a significant reduction in concentration of long chain alkanes in both light and heavy oils. Finally this strain compared favorably well in terms of metabolites production with other known bacteria strains employed in MEOR thus confirming the potential of *Thermoanerobacter brockii* subsp. *lactiethylicus* 9801^T for utilization in microbial enhance oil recovery.

Key words: *Thermoanaerobacter,* fermentation, degradation, oil recovery, metabolites

1 Introduction

Microbial enhanced oil recovery (MEOR) is an enhanced oil recovery technique that uses bacteria in oil reservoirs through production of specific metabolic processes that can lead to enhance oil recovery. The concept of using microorganisms to enhance oil recovery was first proposed by Beckman [4]. However, it was not until the after the work of Zobell [30] that the research on MEOR gained wider attention. Since that time multiples of microbiological enhanced oil recovery projects have been carried out in different parts of the world. MEOR is a tertiary oil recovery process, where bacteria are supported via injection of nutrients. Some processes involved injecting fermentable carbohydrates into the reservoir. Other reservoirs require inorganic nutrients and oxygen to be introduced into the oil bearing strata in quantities that allow the microbes to release the residual oil into the water phase and substrates for cellular activities.

The mechanisms by which MEOR processes work can be highly complex. In general, the mechanisms of MEOR's action are most probably due to multiple effects of the microorganisms on the environment and oil. These mechanisms include gas formation, and pressure increases, acid production and degradation of carbonate matrices, reduction in oil viscosity and interfacial tension by biosurfactant, solvent production, plugging by biomass accumulation and degradation of large molecules in oil resulting in enhanced oil recovery [9, 14, 19].

The actual mode of MEOR in a particular reservoir will be very dependent on the characteristics of the reservoir and the types of indigenous microbes present in a particular reservoir. MEOR differs from chemical enhanced oil recovery through the method by which enhancing products are introduced into the reservoirs. In MEOR, microbes produce all the necessary chemicals in-situ but generally, the application, conditions and cultures can be targeted to meet a particular oil recovery situation [12].

Although MEOR has many advantages compared to other enhanced oil recovery technologies its successful application requires the selection of microorganisms producing effective quantities of desired metabolites, processes which permits injection and dispersion of these microorganisms, and their supporting nutrients, predictable proliferation, and metabolic activity and an ability for

the new ecosystem to persist for periods consistent with economic profitability [26]. The most active applications of the MEOR process are single well simulation treatments for removal of near wellbore formation damage or oil mobilization in the region around wellbore [5], use of microbial of microbial systems for permeability modifications to improve water flooding sweep efficiency [15], use of microorganisms to produce gas, surfactants, acids and alcohols useful for enhanced oil recovery [6]. Although the use of microorganisms has been studied and tested for many years, the success of its application can be limited by the extreme reservoir conditions.

Thus, finding the right bacteria candidate for MEOR to fulfill the environmental conditions normally encountered in the petroleum reservoirs can be very challenging. This has made the search for the bacteria that will perform the work of releasing residual oil in the reservoir environments a continuous process. One of such group of bacteria targeted for application in microbial enhanced oil recovery are members of the order Thermoanerobacteriales within the Firmicutes which are commonly encountered in oilfields and include isolates belonging to the genera *Thermoanarobacter* [7, 11]. This is because they are conducive for growth in deep seated environment such as oil bearing reservoirs [7]. *Thermoanaerobacter brockii* is one of the thermophilic bacteria under current investigation for evaluation of the potential of these organisms for production of metabolites that can enhance oil recovery. Such metabolites include gas and acid production, surfactants, biomass etc. Their high growth temperature offers process advantage in high temperature oil reservoirs and their broad substrate spectrum may allow utilization of inexpensive carbon sources available such as molasses.

The main goal of this study is to choose thermophilic anaerobic bacteria, *Thermoanaerobacter brockii* subsp. *lactiethylicus* strain 9801^{T} for laboratory investigation of its potential for production of metabolites needed in microbial enhanced oil recovery processes. This strain of bacteria have been studied previously in terms of its characterization and genetic properties [7, 11] but little is known about its potential for enhance oil recovery purposes. It is believe that initial outcome from this study can be specific in determining the mechanisms by which this strain could enhance or improve oil recovery before planning physical simulation experiments and also to determine if adaptation to reservoir conditions such as high salinity will be required.

2 Materials and methods

Microorganisms

The microorganism, *Thermoanaerobacter brockii* subsp. *lactiethylicus* strain 9801T was purchased from the German Culture Collection (DSM) and it was the same strain that was originally isolated from a deep subsurface French oil well at a depth of 2100 m where the temperature was 92 ^{o}C and now deposited at DSM. The details of the oil strata and reservoir conditions have been described [7]. The cells are gram-positive, straight motile rods (0.5 by 2 to 3 microns) with flagella uniformly distributed over the entire surface of the cell. During cultivation, optimum growth occurred between 55 and 60 ^{o}C, the fermentable substrates include glucose, fructose, galactose, mannose, cellobiose, maltose sucrose etc. The products of fermentation of glucose were lactate, acetate, ethanol, hydrogen and carbon dioxide and hydrogen while the DNA base composition is 35 mol % G + C .

Determination of most suitable nutrient broth for growth of Thermoanaerobacter brockii 9801-T

All nutrients broths were prepared by weighing the accurate amount specified on the products bottles and mixed in a volumetric flask to 1000 ml. After all salts were completely dissolved they were divided into 100 ml portions in serum bottles flushed with nitrogen and autoclaved at 121^{o}C for 20 min. The nutrient broths were later inoculated with 0.5 ml and incubated for 42 h at 50 ^{o}C.

Optimization of pH in the substrate

Clostridia nutrient medium and thioglycolate broth with resazurine was prepared according to the given instructions on the bottles and divided into portion where the pH was adjusted with the help of a pH Meter and a calibrated pH electrode and NaOH and HCl. For clostridium medium, the pH was adjusted to 5.00, 6.00, 6.60 (which is the original pH of the base) 7.00, 8.00, 9.00, 10.00, 11.00 and12.00 while for thioglycolate broth, the pH was adjusted to 5.00, 6.00, 7.00 (which is the original pH of the base), 8.00, 9.00, 10.00, and 11.00. 100 ml of each nutrient broth was dispensed into serum bottles, purged with nitrogen for 2 min and autoclaved at 121^{o}C. All the serums bottles were inoculated with 0.5 ml culture with a sterile needle and syringe and incubated in an anaerobic jar at 50 ^{o}C for approximately 42 h, and OD was measured. There were 4 replicates of each pH solution. Graphs were drawn over the average results (standard deviation = 0.08).

Effect of temperature on growth

Clostridia media and thioglycolate broth with resazurine were used in this experiment. The media were dispensed into 100 ml serum bottles, purged with nitrogen and autoclaved at 121^{o}C for 2 min. The pH was adjusted to 6.60 and 7.10 for both media. All the serums bottles were inoculated with 0.5 ml culture with a sterile needle and syringe and incubated in an anaerobic jar at 50, 55, 60, 65, 70, and 75 ^{o}C for 42 h .OD was measured and graphs were drawn over the average results of 4 replicates (standard deviation = 0.1) The temperature of the incubation oven was monitored with a calibrated digital thermometer.

Effect of salinity on growth

Clostridia media was prepared according to specifications and the broth were adjusted to salt concentration of 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6 7 and 10 % (w/v) respectively. 100 ml was dispensed into serum bottles, autoclaved and inoculated with 0.5 ml culture with a sterile needle and syringe and incubated in an anaerobic jar at 50 ^{o}C for 42 h. The optical density (OD) was measured and graphs drawn over the average of 4 results (standard deviation = 0.12). The temperature of the incubation oven was monitored with a calibrated digital thermometer.

Screening for metabolites production

2½ litres of Clostridial nutrient medium (Fluka 27546) was prepared and autoclaved at 121^{o}C according to the given instructions. The pH electrode and temperature sensor were calibrated prior to start of the experiment. The fermentation cultivation vessel-; tubing, temperature sensor, pH electrode and all other utensils were sterilized following standard procedure. After all components were sterilized the reactor system was assembled and the medium was aseptically poured into the vessel and was purged with nitrogen so it was anaerobic and inoculated with 10 ml of a 42 h old culture. The temperature was set at 55 ^{o}C and start pH at 7.28. 4 gas wash bottles were connected to the outlet; the first bottle was empty used as a safety bottle number 2 was with the saturated $Ba(OH)_2$, bottle number 3 was with the 0.1 M $Cu(NO_3)_2$ the last bottles was with water from which a tube ran over to a graduated cylinder (it is very important that the system is 100% anaerobic).These wash bottles were used for qualitative detection of gases. Carbon dioxide when passed through $Ba(OH)_2$ gives a white precipitate of $BaCO_3$, similarly when hydrogen sulphide is passed through $Cu(NO_3)_2$ it gives a reaction which result is a precipitate of CuS. The gas wash bottle with water was used for the purpose of the occurrence of other types of gas which could be detected by the displacement of water. The process was started

and samples were taken for OD analysis and VFA at convenient intervals. The fermentation was continued for 52 h and at the end of the fermentation ethanol and lactic acid was determined.

Cell biomass determination

Triplicate 5 ml samples (fermentation broth) were put in 100 mm centrifuge tubes. The samples were centrifuged for 15 min at 5000 rpm. The supernatant was decanted. The precipitate was washed twice with 10 ml distilled water. The final residue was poured into a tarred aluminum weighing dish which had been previously dried in the oven for 24 h. The dishes were placed in an oven at 105 ^{o}C and dried to constant weight. The aluminum dishes were cooled in a dessicator before weighing. The biomass was determined by calculating the difference in the weight of the full and empty dishes. The final cell biomass concentrations were reported as g of dry biomass per 100 ml of fermentation broth.

Determination of biosurfactant activity and surface tension

A qualitative method to determine the displacement of oil by biosurfactant was employed [28]. The produced biosurfactant was separated from the culture media by centrifugation to get culture supernatant. 50 ml of distilled water was pipetted on a petri dish (18 cm diameter) followed by addition of 20 µl of crude oil to the water surface, and finally 10 µl of culture broth supernatant were gently put on the centre of the oil. The displacement pattern was observed. A clear circle was visible under light. The area of the circle was measured and calculated for oil displacement area (ODA) by the equation (1).

$$ODA = 22/7\ (radius)^{2}\ cm^{2} \quad (1)$$

Fermentation of light and heavy oil

Fermentation processes was carried using light and heavy oil with and without molasses. 1500 ml of seawater, 5% thin oil, and 0.5% molasses was put in a 2000 ml blue cap media storage bottle and a second solution consisting of 1500 ml of seawater, 5% thin oil without molasses was put into another 2000 ml bottle. The two set ups were completed with two outlets on the cap from which samples were taken and the other outlet for gas exhaustion. Both oil solutions were sterilized at 120 ^{o}C for 20 min. Wash bottles were attached to the exhaust outlets with the help of silicone tubing. Like in the screening experiment, the first wash bottle was a safety bottle, the second was with saturated $Ba(OH)_2$ that could precipitate any carbon dioxide produced, the third

wash bottle contain 0.1M $Cu(NO_3)_2$ to absorb any H_2S and the last bottle was with water to detect any other gas produced through displacement of water (Figure 1). The oil mixtures were then inoculated with 10 ml of 48 h culture and both bottles were incubated in a water bath at 55 ^oC. The pH and temperature were monitored with the help of a data logger. Samples were taken at intervals for analysis and also to monitor the culture under microscope. A similar experiment as was carried out with heavy oil for comparison.

Degradation of different alkanes

9 ml aliquot of seawater was dispensed into 8 serum bottles purged with nitrogen and sterilized. Prior to inoculation, 100 µl of vitamins, and 200 µl minerals were injected from sterile stock solutions and 100 µg of different alkanes were put in the serum bottles respectively and identified by a clear label. Repeated experiments were carried out by replacing seawater with reinforced clostridia media to evaluate the effect of additional nutrient on the degradation process.

Bacteria solution in chalk sample

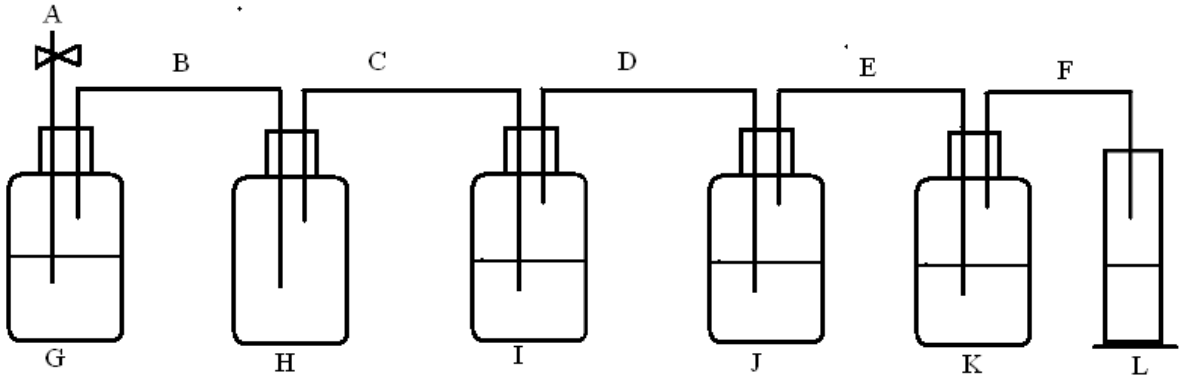
A chalk core obtained from a Danish oilfield with a diameter of 2.1 cm was cut into a piece of 5 cm long. It was sterilized and placed in a sterile core holder and attached to a sterile blue cap bottles with an assembly spout and 200 ml clostridia nutrient media at each end. Before all experimental runs, the flowing tubing and fittings were disinfected with 70% IPA spirits and then completely flushed with sterile 0.9% brine before each start-up. It is very important to be very sure that there is no leaking of media from one chamber to another by ensuring that the core fits tightly. The system was flushed with nitrogen to ensure anaerobic condition. The only way the bacteria and broth can reach the other chamber is to migrate through the pores of the chalk core. One of the bottles was inoculated with 10 ml 48 h culture and incubated at 55 ^oC. The apparatus was monitored for leaks and the system proved to be leak proof. The experiment was carried out twice. The system fulfilled all the requirements for rapidly and effectively identifying a migration of a growing culture which showed potential of being candidates for further testing when time permits [13].

The second experiment with chalk samples was to determine the effect of acid produced by the strain on carbonate rock. Four identical chalk samples with diameter of 2.0 cm and length of 2.5 cm were used for the experiment. Duplicate samples were immersed in flasks containing 100 ml

bacteria solution for 72 h. Duplicate samples were also immersed in flasks containing 100 ml demineralized water for the same period to serve as control. Final result was average of two measurements (standard deviation = 0.023). Background value of calcium ions measured in the reinforced clostridia media was deducted from the measured concentration after 72 h. The initial and final pH of each the media were also measured.

Analytical methods

The optical density measurements were acquired a spectrophotometer (Model: Cecil 7200). The concentration of volatile fatty acid (VFA) and the ethanol produced during fermentation was analyzed using GC Varian 3800 model with column; Varian 25 m, 0.32 mm ID. The carrier gas was helium/10 psi. The lactic acid was determined using bio-analysis kit for lactic acid (Cat. No 10 139 084 035) from Food Diagnostics. The biogas yield was measured by using a lure lock syringe and a needle. Varian GC-MS (Model Varian 3800) was used for chromatographic test of alkanes and crude oil. Viscosity of the oil sample before and after bacteria inoculation was determined using a Brookfield viscosimeter. The surface tension measurements were taken using a modified Wilhelmy surface tensiometer and measurement taken at static mode. The fluid samples were analyzed for calcium ions concentration by atomic absorption spectra (AAS) using Perkin Elmer Optima (DV 3000). All the analyses were performed according to standard methods.



A = sample outlet with valve, B, C, D,E, F = gas outlet, G = fermentation bottle, H = safety bottle, I = barium hydroxide bottle, J = copper nitrate bottle, K = water for gas displacement, L = measuring clinder

Figure 1: Schematic of substrate fermentation process

3 Results

3.1. Growth in nutrients

The ability to utilize various substrates was tested and given as a function of optical density (OD) (Table 1). The OD of the growth showed that the highest growth occurred in the clostridial nutrient media with and without vitamins. The use of thioglycolate broth with vitamins resulted in a better growth than thioglycolate broth without vitamins. The glucose based medium with or without vitamins showed little or no growth. All the results were obtained after 42 h of incubation, the glucose medium when incubated for a period of 4 days longer showed only a minimal extra growth. These results suggest that both clostridial and thioglycolate can be a better substrate than glucose based medium for cultivation of *Thermoanerobacter brockii* subsp. *lactiethylicus* 9801^T as they provide enough nourishment for the bacteria to survive with a reasonable growth rate. Thus, the two media were selected for evaluation of the optimum pH for growth. Figure 2 showed the phase contrast micrograph of *Thermoanerobacter brockii* subsp. *lactiethylicus* 9801^T.

Table 1: The results of the different media used for growth based on optical density measurement

Media	Optical Density (650 nm)
Glucose based medium with 0.2 ml trace elements and 0.2 ml vitamin solution	0.328
Glucose based medium with 0.2 ml trace elements without vitamins	0.156
Glucose based medium without trace elements and vitamin solution	0.051
Glucose based medium without trace elements and with 0.2 ml vitamin solution	0.013
Clostridial nutrient medium without vitamins	1.344
Clostridial nutrient medium with 0.2 ml vitamins	1.270
Thioglycolate broth with resazurine without vitamins	0.904
Thioglycolate broth with resazurine with 0.2 ml vitamins	1.256

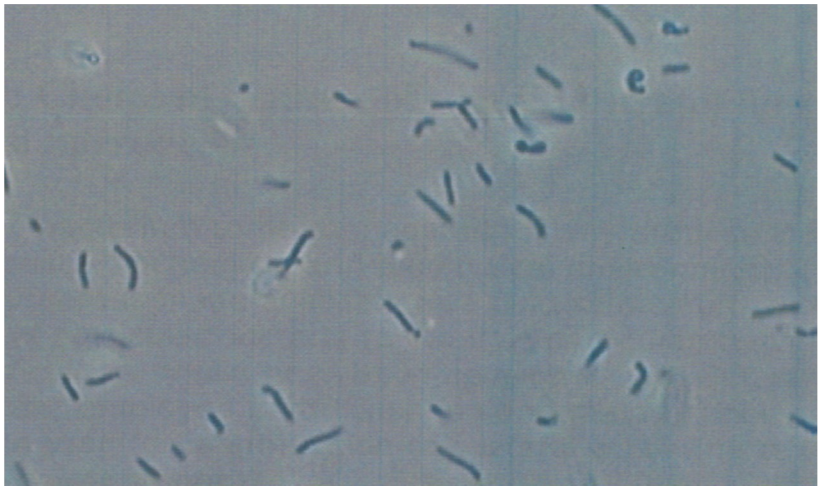


Figure 2: Phase-contrast micrograph of *Thermoanerobacter brockii* subsp. *lactiethylicus* 9801^{T} (x 40)

3.2. Optimization of pH in the substrate

An overlay of the results of the pH in different media is shown in Figure 3. The result shows that there is optimal growth at a pH interval of 6.5-8.5 for clostridia medium with and without vitamins. This suggests there is no need to add vitamins as the growth seem to be the same. When compared to the thioglycolate broth with and without vitamins, it shows that the thioglycolate with vitamins has growth at pH of 7.5-8.7 and thioglycolate without vitamins has an optimal growth at an approximate pH of 6.5-7.5. Combining all the results, it can be deduced that this strain can grow at a pH of 6-9.5.

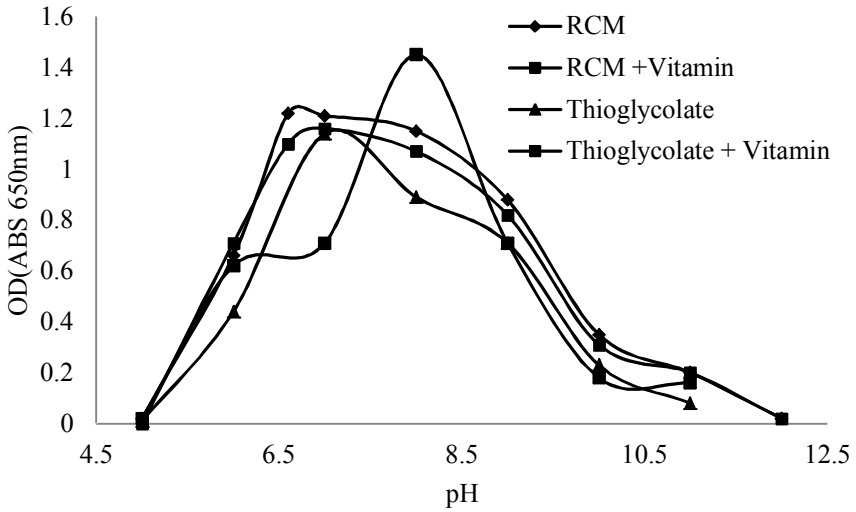


Figure 3: Optimum pH for growth in different media

3.3. Effect of temperature on growth

As shown in Figure 4, growth was observed at a temperature range of 45-70 ^{o}C for the two substrates. The OD was highest at 55 ^{o}C for reinforced clostridia media; however for the thioglycolate the OD was slightly lower at 55 ^{o}C. When the two lines for the broths were statistically compared by running an F-test, both averages was found to be the same. It can be concluded that given a pH of 6.6 affords the best optimal temperature for this strain to be deduced in a substrate. Since the clostridial nutrient medium gives the best result for optimum temperature, further experiments conducted uses this substrate as growth media. It is time saving and affords the opportunity to concentrate entirely on a single substrate.

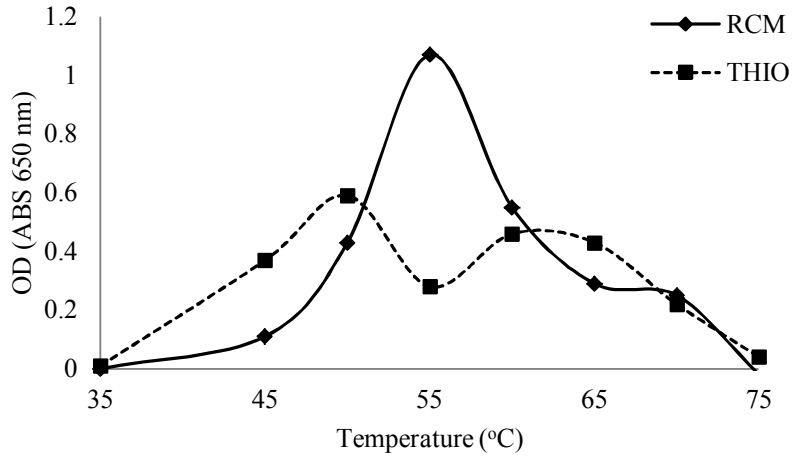


Figure 4: Temperature optimum after 24 h incubation

3.4. Effect of salinity on growth

The effect of salt concentration on *Thermoanerobacter brockii* subsp. *lactiethylicus* 9801^T is shown in Figure 5. The bacteria appear to grow reasonably well in salt concentrations from 0.5% to 3.5% (w/v) which is the concentration of seawater. The optimum growth was found at 1.5% (w/v) concentration. When the concentration becomes greater, the growth slows down, and subsequently results in death of the bacterium culture. Further experiments showed that if this strain is grown repeatedly at 4% (w/v) concentration, they become adapted and subsequent increased by 1% (w/v) of salt concentration at time causes growth till 5% (w/v) of salinity.

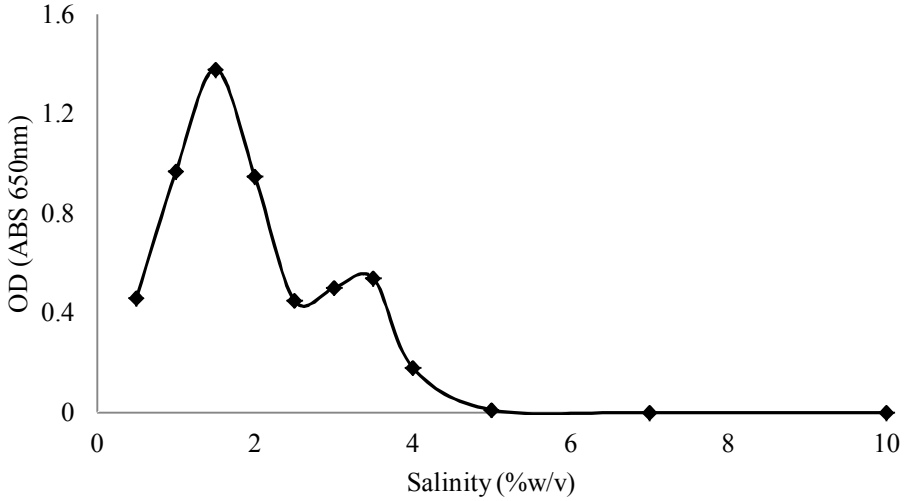


Figure 5: Growth of *Thermoanaerobacter brockii* 9801^T at different salt concentration

3.5. Cell Biomass determination

The result of cell biomass determination is shown in Figure 6. The final cell biomass concentrations are reported as g of dry biomass per 100 ml of fermentation broth. If we consider the cell biomass production rate over time, the cell biomass rate decrease from 0.052 $g.h^{-1}$ in 17 hours to about 0.0034 $g.h^{-1}$ in 46 h. The decrease in the cell biomass rate can be related to decrease in growth rate over time as less and less nutrient is available and suggest that less cell biomass can be harvested as time increase.

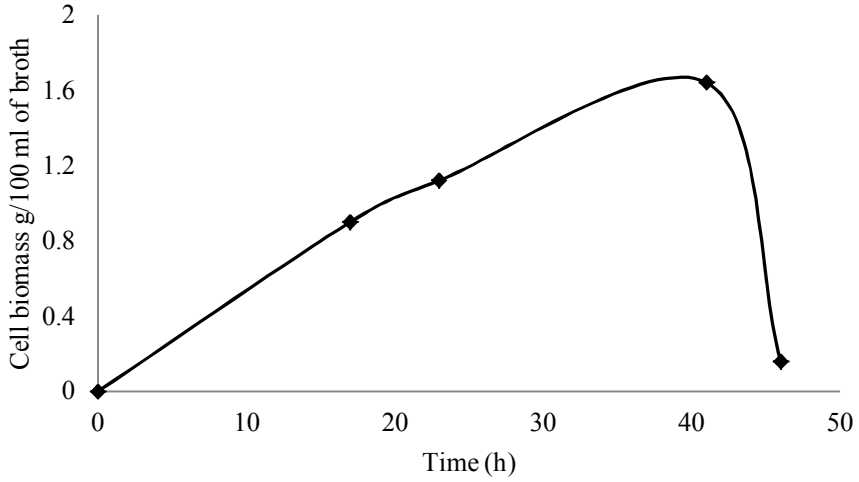


Figure 6: Biomass per 100 ml substrate over 46 h of fermentation

3.6. Fermentation of medium

Table 2 shows the summary of metabolite products from the fermentation process using RCM as substrates. It was observed that a good amount of CO_2 was produced as indicated by precipitate of barium carbonate. The volume produced was not measured. No hydrogen sulphide was detected during the fermentation process, but there was about 100 ml of water displaced which suggests that there was a production of another type of gas, probably methane.

Table 2: Metabolites produced during fermentation process with reinforced clostridia medium

Products	Lactic acid	Ethanol	Acetic acid	Iso-valeric acid	CO_2	H_2S	Other Gas

	(g/l)	(g/l)	(g/l)	(g/l)			(ml)
Amount	11.7		2.5	0.08	P	N.P	100

P=produced, N.P=not produced

The pH fell from 6.8 to 4.48 in the fermentation flask suggesting the productions of acids. Analysis of the samples showed the presence of acetic acid, iso-valeric acid, lactic acid and ethanol. The average concentration of lactic acid produced during the fermentation process was 11.7 g/l. The volatile fatty acids (VFA) that turned out to be acetic acid and of iso-valeric acid had average concentrations of 2.5 g/l and 0.08 g/l respectively. The concentration of the ethanol produced during fermentation was in average about 2.4 g/l.

Furthermore, the effect of ionic strength of the media on gas production per 100 ml of substrate after 48 h was investigated. The results (Figure 7) showed that the specific salt concentration that was present at the optimal growth also were involved in the highest gas production, i.e. 1.5 % (w/v) with the exception of 0.5% (w/v) which gives almost the same amount. The volume of gas produced was between 10-40 ml and it decreases with increasing ionic strength.

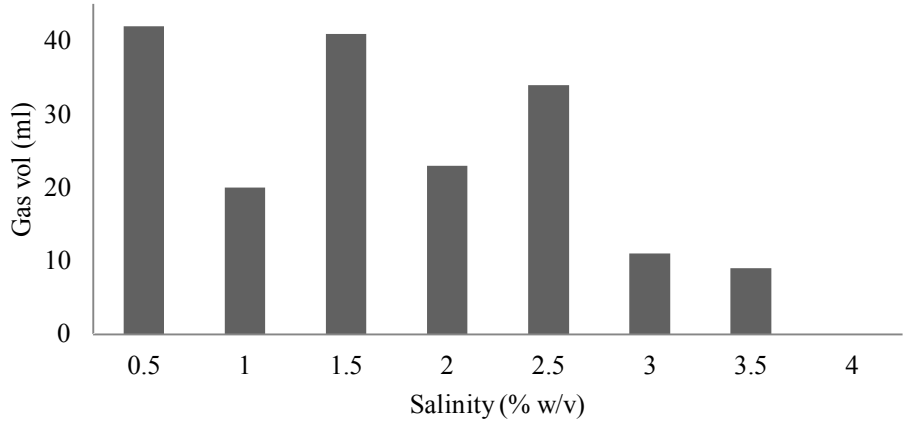


Figure 7: Gas production per 100 ml of substrate

3.7. Biosurfactant production and surface tension measurement

The result of surface tension measurement for the culture grown on different salt concentration is shown in Table 3. The measurement of surface tension gives a rough idea about the

concentration of the biosurfactant in the broth. The surface tension measurement showed almost constant surface tension independent of salinity or culture growth and very similar to the value of pure water at room temperature. Therefore, this method was not able to show increase in concentration of biosurfactant at the relatively high salinities.

Table 3: The result of surface tension measurement for culture grown on different salt concentration

NaCl (% w/v)	0.5	1.0	1.5	2.0	2.5	3.0	3.5
Initial surface tension (N/m)	0.057	0.059	0.057	0.059	0.057	0.057	0.056
Final surface tension (N/m)	0.054	0.055	0.053	0.053	0.056	0.058	0.057

Contrary to this, the bio surfactant activity monitored by oil displacement showed a very distinctive effect visible under light (Figure 8). It can be seen that there was a very distinctive displacement of oil when the culture broth supernatant was put on top of it; an indication of that biosurfactant possibly reduces the surface activity resulting in the formation of a clear zone on the drop of oil. The area of the circle as calculated by equation (1) was 3.5 cm^2 representing about 10% of area of the oil spread. No such effect was observed when distilled water was drop on the surface of the oil. The blank experiment showed that there is no clearing and the drop of oil stayed intact. This test indicated that biosurfactant was produced by *Thermoanerobacter brockii* subsp. *lactiethylicus* 9801^T.

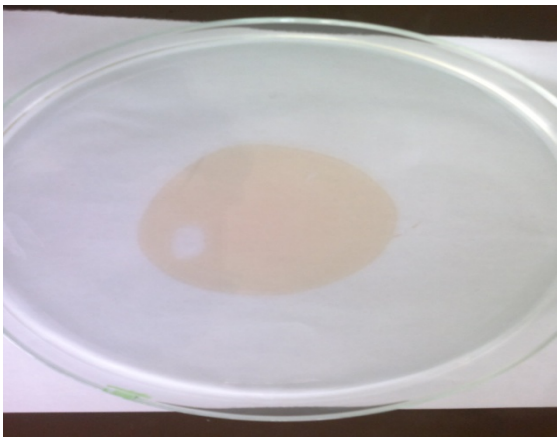


Figure 8: Oil displacement by biosurfactant. The area displaced can be seen as whitish halo within the oil spread.

3.8. Fermentation of hydrocarbon

The results from fermentation of heavy and thin oil showed different characteristics. The viscosity of thin oil did not change during the period of fermentation. The initial viscosity measured was 23 cP at the start of experiment; the final viscosity measured at the end after approximately 130 days is 24 cP indicating almost no change. However, for the thick oil, the viscosity was reduced from initially value of 70 cP to 8 cP, representing an about 9 times reduction in viscosity. Acetic acid, butyric acid and ethanol were also produced as shown in Table 4 during fermentation of crude oil in seawater.

Table 4: VFA and ethanol production in heavy and thin oil after fermentation

Components	Acetic acid	Butyric acid (mg/l)	Ethanol
Thin oil			
Initial conc.	0.0	0.0	0.0
Final conc.	4869.0	795.3	0.30
Heavy Oil			
Initial conc.	0.0	0.0	0.0
Final conc.	700.97	0.0	0.17

The result of the alkane degradation is shown in Figure 9. The results from the GC/MS runs were calculated by taking the top height of the chromatogram from the alkanes and dividing it with the top height of the internal standard. When the results are analyzed as shown in Figure 9, it can be concluded that the ratio of alkanes falls during the period of incubation. C_{15}, C_{16}, C_{18}, C_{20} and C_{28} all show a drastic fall in concentration, while for carbon chains C_{10}, C_{12} and C_{13}, the fall in concentration was minimal.

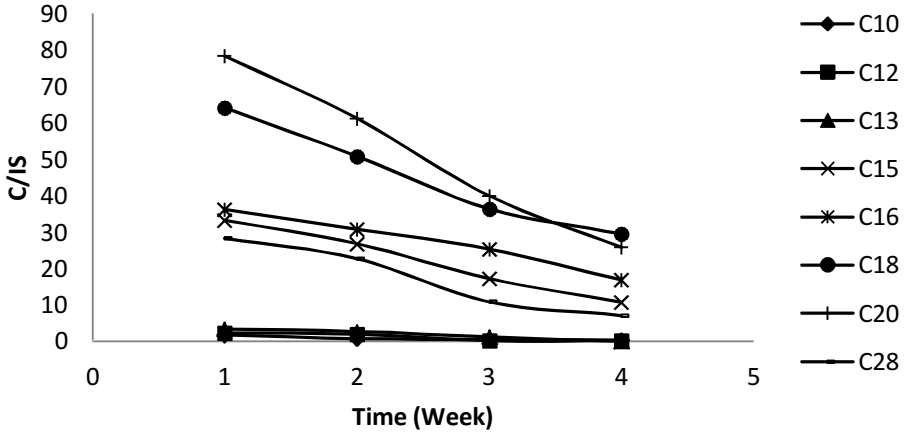


Figure 9: The result of the degradation of alkanes

When the degradation of heavy oil in seawater and in the reinforced clostridium media (RCM) were compared (Figure 10 and 11), the oil in seawater showed a slower degradation than oil in the reinforced media. This suggests there was better metabolism for this strain in the reinforced media and oil as carbon source than with oil and seawater.

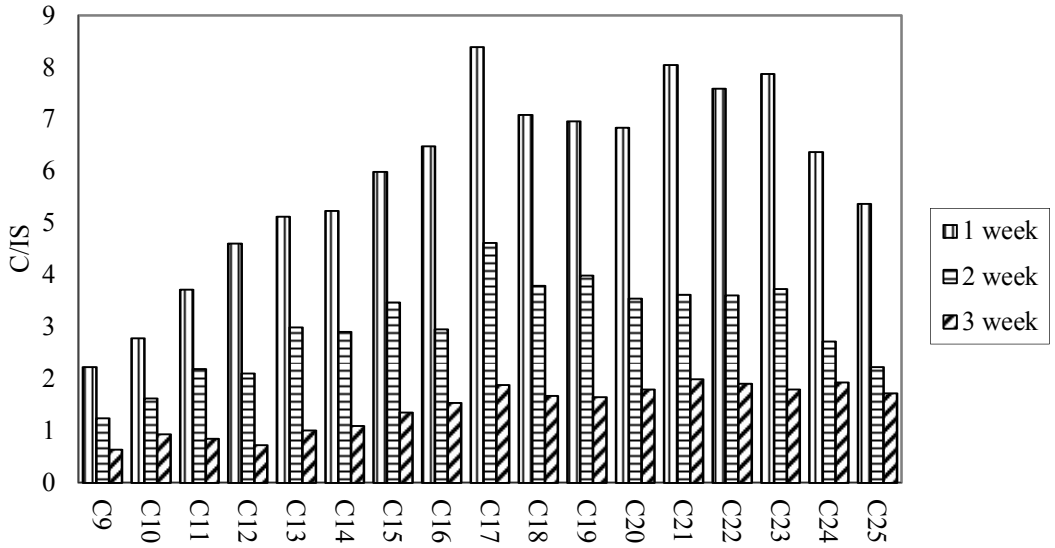


Figure 10: Growth in heavy oil with seawater as substrate

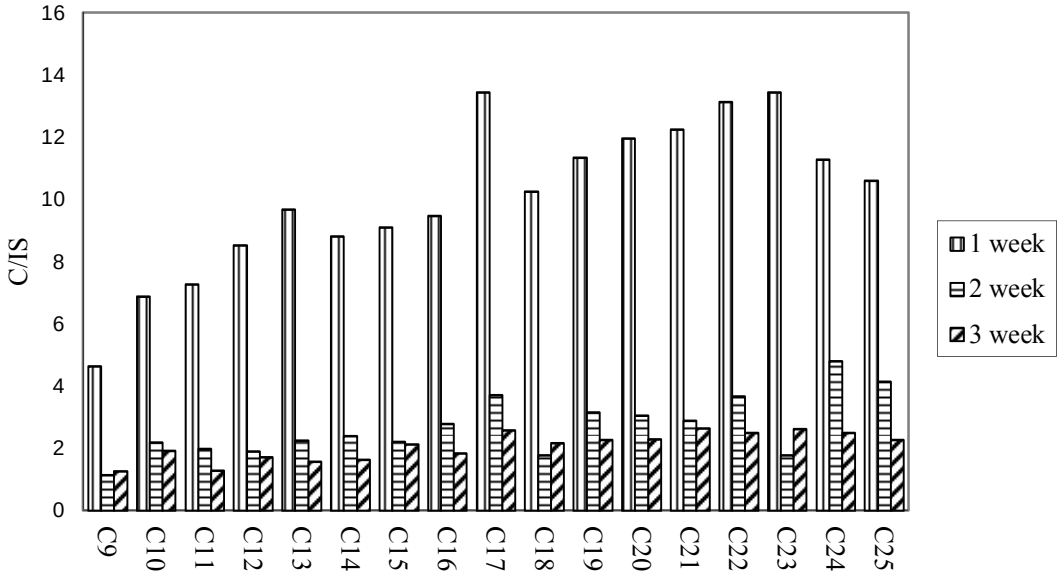


Figure 11: Growth in heavy oil with reinforced clostridia media as substrate

3.9. Bacteria solution in chalk sample

The result of bacteria solution in chalk core showed that bacteria migration through chalk core occurred after 3 days of incubation. There was growth in the media which was not inoculated with culture suggesting a migration of bacteria through chalk core from the inoculated part had taken place which was indicated by change of turbidity of the not inoculated fluid. However,

after further incubation for another week, it was observed when examined under the microscope that there was an aggregation of the cells on the surface ends of the chalk (Figure 12).

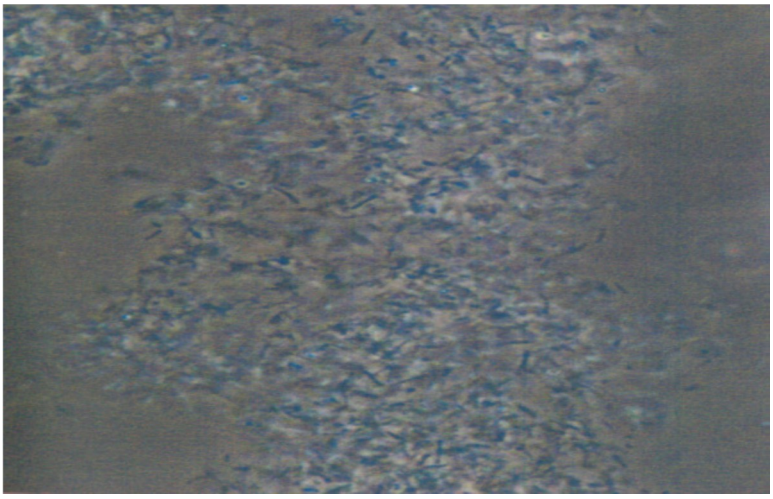


Figure 12: Aggregated cells of at one end of chalk core

The result of the corrosive effect of the acid produced on chalk samples is shown in Table 5. The measured concentration of calcium ions in the fermentation medium of *Thermoanerobacter brockii* subsp. *lactiethylicus* 9801^T was 354.8 g/l compared to 3.1 g/l when chalk samples were immersed only in de-mineralized water. The background value of calcium ions in the bacteria media without chalk samples was only 9.4 g/l an indication that the acid effect increased the calcium ions concentration by a factor of 38.

Table 5: Comparison of chalk dissolution in bacteria medium and demineralized water

Experiment	Medium	Initial pH	Final pH	$[Ca^{2+}]$ (mg/l)
1	Chalk + bacteria solution	6.80	6.15	354.8
2	Chalk + demineralized water	6.34	6.30	3.12
3	Bacteria solution without chalk sample	6.80	5.12	9.43

4 Discussions

In this study, *Thermoanaerobacter brockii* subsp. *lactiethylicus* strain 9801^T an anaerobic, rod shaped, gram positive and thermophilic was found to ferments a variety of carbohydrates and produce lactate ethanol, acetic, CO_2 and other gases. This matches earlier descriptions [7]. Also

the strain utilizes thiosulphate as an electron acceptor to produce sulphide but does not utilize sulphate. Sulphide production makes strain 9801^T a potential bio-corrosive agent in oil wells because of the presence of thiosulphate as it has been mentioned that the *Thermoanaerobacter* species have the ability to reduce thiosulphate during growth on carbohydrates [8, 11].

On the basis of its optimum temperature for growth and sodium chloride requirement, *Thermoanaerobacter brockii* subsp. *lactiethylicus* 9801^T did not grow at temperature above 70 ^oC although the in-situ temperature of the oil well from which it was isolated was 92 ^oC indicating that this microbe may have colonized in the cooler parts of the reservoir. The optimum growth occurred at temperatures of 55 ^oC. This falls within the optimum temperature range of between 55-60 ^oC reported by [7, 11]. Even though many oil reservoirs have initial temperature of greater than 75 ^oC but due to continuous injection of water for oil recovery over time, the temperature in the reservoirs is much less than the initial reservoir temperature as the field matures. MEOR application is usually targeted for matured waterflooded reservoir, therefore there is a potential for this strain to be utilized in MEOR if other properties such as acids, gases and biosurfactant production for improve oil recovery are fulfilled. Compared to mesophilic bacteria such as *Clostridium tyrobutyricum* and *Bacillus* that normally grows at optimal temperature of 37 ^oC, this strain has a potential for application in high temperature reservoirs.

There was growth in the media with pH from 6-10 and salinity range of 0.5-3.5% (w/v). The optimum growth was found at a salinity of 1.5% (w/v). However, further experiments showed that if this strain was grown repeatedly at 4% (w/v) concentration of NaCl, they become adapted, but no growth occurred when the concentration was further increased to 7% and 10% (w/v) salt concentration, respectively. It is evident that the current salinity range at which growth occurred for this strain is low compared to salinity condition for many oil wells. For example *Bacillus licheniformis* strain, JF-2 from an oilfiled water injection was found to grow and produced biosurfactant at salinity up to 8% and temperatures up to 45 ^oC [29]. Experience have shown that with careful planning and repeated sub-culturing, it is possible that this strain can grow in higher salt concentrations normally encountered in the reservoirs, given that salinity gradient can vary from low to high, however we did not investigate this further in the current study.

The fermentable substrates include molasses, thioglycolate, RCM and crude oil. However the strain showed little or no growth in glucose medium with or without vitamins which is opposite to earlier observation reported by different workers [7, 11]. The reason for this cannot be fully understood, and raises the question whether the lower pH is actually causing the reduction in growth rate observed in the presence of glucose. It was also found that *Thermoanaerobacter brockii* subsp. *lactiethylicus* strain 9801^T can grow in media containing oil supplemented with either reinforced clostridia medium, molasses or seawater. At a temperature of 55 ^oC the strain was capable of producing biogas and bioacids.

Analysis of the samples for VFA's showed the presence of acetic acid, iso-valeric acid, lactic acid and ethanol. The average concentration of lactic acid produced during the fermentation process was 11.7 g/l. The volatile fatty acids (VFA) that turned out to be acetic acid and of iso-valeric acid had average concentrations of 2.5 g/l and 0.08 g/l respectively while concentration of the ethanol is about 2.4 g/l. Analysis at different intervals indicated an irregular pattern of rise and fall for the net VFA's produced during fermentation suggesting that bacteria can be utilizing some of the acid for metabolism. These results support the earlier findings regarding the metabolic products of *Thermoanerobacter brockii* subsp. *lactiethylicus* 9801^T during fermentation and was comparable to the measured value of organic acids of 9.7 - 42 g/l reported for *Clostridium tyrobutyricum* ATCC 25755 [21] and 11.4 g/l of organic acids by another strain of *Clostridium tyrobutyricum* [27].

The full composition of the biogas formed was not determined, but it was clear that one of the major constituent was carbon dioxide as indicated by the precipitate of barium carbonate. Composition of biogas production from other bacteria strains employed in microbial enhanced oil recovery showed that *Clostridium tyrobutyricum* produces about 80% hydrogen and 15-20% of hydrogen and small amount of methane [18, 27]. However the volume of biogas produced is very low. In comparison, reported range of biogas include between 250-3000 ml by adapted strain of *Clostridium tyrobutyricum* 663 [18], and 9700 ml for *Clostridium* sp. [27]. Explanation for the low volume of gas could be as a result of low volume of fermentation broth and nutrient which was only 100 ml compared to the 500 ml for the results cited. Biogas formation during a

microbial oil recovery process may decrease the oil viscosity and increase reservoir pressure that can force out oil from the rock pores.

This strain was also capable of producing cell biomass. The cell biomass yield per 100 ml of broth decreased from 0.052 $g.h^{-1}$ in 17 h to about 0.0034 $g.h^{-1}$ in 46 h. The decrease in the cell biomass rate can be related to decrease in growth rate over time as less and less nutrient is available and suggest that less cell biomass can be harvested as time increase. The cell biomass yield can be said to be low for this strain as similar experiments showed a biomass concentration of 0.11-0.15 g/g for *Clostridium tyrobutyricum* ATCC 25755 [21] and a value of 1.73 g/l for *Bacillus claussi* [3]. A likely explanation for the observed low cell biomass yields is that most of the energy source consumed is diverted for the production of metabolic products. This argument is supported by the presence of high concentration of lactic acid and VFAs in the fermentation broth.

The presence of biosurfactant produced by bacteria has been shown to reduce the surface tension and interfacial tension (IFT) of the medium [23]. Result of biosurfactant production by surface tension measurement method could not clearly indicate change in surface tension measurement at relatively higher salinities (>2.5% (w/v)) for the RCM cultures. This was consistent with earlier observation that little changes can occur in the surface active properties of biosurfactant with addition of NaCl up to 2.0 mol^{-1} [1]. However, the production of biosurfactant was confirmed by oil displacement test. There was a very distinct displacement of oil when the culture is put on top it, an indication that the biosurfactant reduces the surface activity thereby making a clear zone on the drop of oil. The oil displacement area was about 3.5 cm^2 or a diameter of 1.5 cm in less than 1 h. This result is comparable to that of experiment with a biosurfactant producing *Staphylococcus* with potential application on hydrocarbon bioremediation that measured diameter of 2.5 cm for oil displacement test [10]. We did not measure the concentration of the biosurfactant produced, however, the quality and quantity of biosurfactant production are affected and influenced by the nature of the carbon substrate [22, 24]. Biosurfactant yield from a strain of Bacillus measured 0.015 mol biosurfactant/ mol glucose [29].

The ability to produce biosurfactant can increase the oil degradation potential at a higher rate with possibilities of lowering the viscosity as shown by the results of oil degradation. The viscosity in heavy oil was approximately 10 times thinner than the original oil but no change was observed in the viscosity of thin oil under the same condition.

Moreover, the degradation of alkanes was also very significant probably from the effect of biosurfactant production. All the alkanes both thin and heavy oil form a pattern to show that *Thermoanerobacter brockii* subsp. *lactiethylicus* 9801^{T} can degrade alkanes. Comparison of heavy oil degradation using two different media of seawater and reinforced clostridia medium showed that the seawater had a slower degradation than the oil in reinforced clostridia media which suggests there was better metabolism for this strain in the reinforced media and oil as carbon source than with oil and seawater. It can also be concluded from the results (Figures 9 and 10) that not all the alkanes were equally reduced as a difference was recorded in each graph but it suggested interesting properties for its application in microbial enhanced oil recovery as biosurfactant is one of the most sought after bioproducts in oil and gas industry.

Evidence of bacteria transportation through the chalk core was manifested by growth in the media which was not inoculated with culture suggesting a migration of bacteria through chalk from the inoculated media. This a good property as it demonstrated that after injection, further transport of bacteria during incubation can occur by growth and mobility through the stagnant nutrient medium which fills the porous rock. However, after further incubation for another week, it was observed when examined under the microscope that there was an aggregation of the cells on the surface ends of the chalk. This can be an indication of potential plugging of the inlet surface of the core and can also suggest that this strain may be used to stimulate the in-situ growth of microorganisms in high permeable zone during selective microbial plugging process.

It was earlier shown that the average concentration of lactic acid produced during fermentation process was 11.7g/L the effect of which was confirmed by the corrosion of the chalk samples as indicated by increased concentration of calcium ions in the media after 72 h of immersion in the fermentation fluid (Table 5). The concentration of the calcium ions was increased by a factor of 38. The increased concentration of calcium ions can only be explained by the dissolution of the

chalk samples, which in MEOR application can improve chalk formation porosity and permeability. In comparison, acid and gas production by *Clostridium tyrobutyricum* increased dissolved calcium ions in carbonate chalk samples by a factor of 20 [17], *B. lichenformi*s increased dissolved calcium by 13 % [2], fourfold increase of the calcium ions of the stratum water compared to the initial value was also reported [27].

Success of microbial enhanced oil recovery depends not only on the ability of selected microbes to produce desired metabolites but also on the good understanding of the reservoir conditions and whether the selected strain can proliferate and produced desired metabolites under such conditions. It can be inferred from the experimental results that this strain exhibited some desirable properties in production of metabolites such as acid production, production of biosurfactant, biomass production and ability to degrade alkanes in light and heavy oils and ability to penetrate core pores. The amounts of the metabolites produced are comparable to values cited in the literatures for other strains of bacteria commonly employed in microbial enhanced oil recovery purposes. However, what makes this strain better lies in the ability to grow at high temperature of 55 ^{o}C in comparison to many bacteria isolated and identified for microbial enhanced oil recovery purposes. Many of the bacteria strains used in MEOR usually experienced low efficiency when maintained at higher temperatures but this strain was able to exhibit good potential in production of metabolites at such high temperature. The significant production of lactic acids as well as acetic, iso-valeric acid, ethanol and biomass can be an additional factor in rock modification and oil mobilization. Although, composition of gas was not specifically determined, formation of gaseous products under anaerobic condition is a positive result. Four conditions indicated to be met to achieve successful in situ bacteria growth and core plugging are: the cells must be transported throughout the rock stratum, nutrients must be transported for growth, the microorganisms must be able to grow and reduce the permeability of the rock by biomass and extracellular polymer production, the bacteria growth must not be so rapid that it results in the formation of bacterial plugs farther away from the wellbore [16, 20]. The results of this study have demonstrated that *Thermoanerobacter brockii* subsp. *lactiethylicus* 9801^{T} meets all of these criteria and in addition was able to produce a broad spectrum of metabolites including acids, biomass, biosurfactants, gases whose production can be important in the oil recovery process. The only concern is its low growth at relatively high salinity (>5%) but

it is believed that careful adaptation process can help to overcome this limitation as it has been shown that successful adaptation of bacteria to high salinity condition is possible [25].

5 Conclusions

Thermoanaerobacter brockii subsp. *lactiethylicus* 9801^T has been investigated as a suitable candidate for microbial enhanced oil recovery. From this investigation, it can be concluded that *Thermoanaerobacter brockii* subsp. *lactiethylicus* 9801^T have a potential for possible utilization in microbial enhanced oil recovery. This is based on the ability of this strain to grow at an optimal temperature of 55 ^oC and production of metabolites that can be utilized for MEOR purposes. The strain was capable of producing organic acids that can modify rock properties evident in dissolution of chalk samples. Cells were also able to migrate through pore spaces of carbonate rock sample suggesting possibility of high mobility when injected for microbial enhanced oil recovery purpose. Degradation of the alkanes was also significant. It is evident that this strain offer useful metabolic products such as biosurfactants, biogas, biomass, in addition to bio-acids for enhancing oil recovery. These bio-products can contribute to different microbial systems which can tackle specific problems of oil recovery. However, the finding indicated that the range of salinity at which this strain could grow is low compared to saline content of many oil wells but it could provide an opportunity for further research in terms of its applications in microbial enhanced oil recovery.

References

1. Abouseoud M, Maachi R, Amrane A, Boudergua S, Nabi I, (2008) Evaluation of different carbon and nitrogen sources in production of biosurfactant by Pseudomonas fluorescens. Desalination, 233: 143-151.

2. Adkins JP, Tanner RS, Udegbunam EO, McInerney MJ, Knapp RM, (1992) Microbially Enhanced Oil Recovery from Unconsolidated Limestone Cores, Geomicrobiology J.,10: 77-86.

3. Aparna A, Srinikethan G, Smitha H, (2012) Isolation, Screening and Production of Biosurfactant by *Bacillus claussi* 5B, Research in Biotechnology 3(2): 49-56

4. Beckman JW, (1926) The Action of Bacteria on Mineral oil. Industrial Engineering Chemical News 4: 3

5. Bryant RS, Burchfield TE, (1989) Review of Microbial Technology for Improving Oil Recovery. SPE Reservoir Eng., May 151.

6. Bryant RS, Douglas J, (1988) Evaluation of Microbial Systems in Porous Media for EOR, SPE Reservoir Eng., May, 489-495.

7. Cayol L, Ollivier B, Patel BKC, Ravot G, Magot M et al. (1995) Description of *Thermoanaerobacter brockii* subsp. *lactiethylicus* subsp. nov., Isolated from a Deep Subsurface French Oil Well, a Proposal To Reclassify *Thermoanaerobacter finnii* as *Thermoanaerobacter brockii* subsp. *finnii* comb. nov., and an Emended Description of *Thermoanaerobacter brockii*. Int. Journal of Systematic and Evolutionary Microbiology 45: 783-789.

8. Crolet, JL, Daumas S, Magot M (1993) pH Regulation by Sulfate Reducing Bacteria. Corrosion 93: 1-18

9. Donaldson EC, Chilingarian GV, Yen TF (1989) Microbial Enhanced Oil Recovery, Developments in Petroleum Science 22: 1-12.

10. Eddouaouda K, Mnif S, Badis A, Younes SB, Cherif S et al., (2011) Characterization of a Novel Biosurfactant Produced by Staphylococcus sp. Strain 1E with Potential Application on Hydrocarbon Bioremediation, Journal of Basic Microbiology 51: 1-11.

11. Fardeau ML, Ollivier B, Patel BKC, Magot M, Thomas P et al. (1997) Thermotoga hypogea sp. nov., a Xylanolytic, Thermophilic Bacterium from an Oil- Producing Well, International Journal of Systematic Bacteriology 47, 4: 1013-1019.

12. Hitzman DO (1991) Microbial Enhanced Oil Recovery-The Time is Now. Developments in Petroleum Science 31: 11-20.

13. Hitzman DO, Stepp AK, Dennis DM, Graumann LR (2003) Innovative MIOR Process Utilizing Indigenous Reservoir Constituents, Final Technical Reports.

14. Jack TR (1988) Microbially Enhanced Oil Recovery, Biorecovery 1: 59-73

15. Jack TR, Stehmeier LG, (1998) Selective Plugging of Water Injection Wells In: Bucherfiled TE, Bryant RS, (Eds.), Proc.US DOE Conference 870858, Natl. Technical Information Service Symposium on Application of Microorganisms to Petroleum Technology Springfield, VA.

16. Jenneman GE, Knapp RM, McInerney MJ, Menzie DE, Revus DE, (1984) Experimental Studies of In Situ Microbial Enhance Oil Recovery, Soc. Pet. Eng. J. Feb. 33-37.

17. Jimoh IA, Søgaard EG, Rudyk SN, (2011) Microbial Fluid Rock Interactions in Chalk Samples and Salinity Factor in Divalent Ca2+ ions Release for Microbial Enhanced Oil Recovery Purposes, Chemical Eng. Transactions, 24:889-894.

18. Jimoh IA, Søgaard EG, Rudyk SN, (2012) Evaluation of Produced Volumes of Carbon Dioxide from the Concentration of the Gas Absorbed in the Media during Microbial Fermentation for Enhanced Oil Recovery Purposes, Chemical Eng. Transactions 27: 97-102.

19. Khire JM, Khan MI, (1994a) Microbially Enhanced Oil Recovery (MEOR), Part I, Importance and Mechanism of MEOR. Enzyme Microbiol. Tech, 16: 170-2.

20. Lappin-Scott HM, Cusack F, Costerton JW, (1988) Nutrient Resuscitation and Growth of Starved Cells in Sandstone Cores a Novel Approach to Enhanced Oil Recovery Appl. Environ. Microbiol. 54: 1373-1382.

21. Liu X, Zhu Y, Yang ST, (2006) Butyric Acid and Hydrogen Production by Clostridium tyrobutyricum ATCC 25755 and Mutants, Enzyme and Microbial Technology 38: 521-528.

22. Rahman KSM, Gakpe E, (2008) Production, Characterization and Applications of Biosurfactants-Review, Biotechnology 7: 360-370.

23. Ramsey BA, Cooper DG, Margartis A, Zajic JE (1983) Rhodochorous Bacteria. Biosurfactant Production and Demulsifying Ability. Microbial Enhanced Oil Recovery. 61-65.

24. Raza ZA, Khan MS, Khalid ZM, (2007) Evaluation of Distant Carbon Sources in Biosurfactant Production by a Gamma Ray Induced Pseudomonas putida mutant, Process Biochem 42: 686-692.

25. Rudyk SN, Søgaard EG, (2011) Microbial Enhanced Oil Recovery In: Applied Microbiology and Molecular Biology in Oilfield Systems, Eds. Whitby C. and Skovhus T.L., Springer, Dordrecht, 179-187.

26. Sheehy AJ (1991) Microbial Physiology and Enhanced Oil recovery. Developments in Petroleum Science 31: 37-44.

27. Wagner M, Lungerhausen D, (1995) Development and Application of a New Biotechnology of the Molasses In-Situ Method; Detailed Evaluation for Selected Wells in the Romashkino Carbonate Reservoir, In: Proceeding of Conference ‘’The Fifth International Conference on Microbial Enhanced Oil Recovery and Related Biotechnology for Solving Environmental Problems’’ Tusla USA, 153-173.

28. Youssef NH, Duncan KE, Nagle DP, Savage KN, Knapp RM et al. (2004) Comparison of Methods to Detect Biosurfactant Production by Diverse Microorganisms. J. Microbiol. Methods 56: 339-347.

29. Youssef N, Simpson DR, Duncan KE, Mclnerney MJ, Folmsbee M et al., (2007) In Situ Biosurfactant Production by Bacillus Strains Injected into a Limestone Petroleum Reservoir, Applied Environ Microbiol., 73(4): 1239-47.

30. ZoBell CE (1947) Bacteria Release of Oil from Oil Bearing Materials, Part I and II. World Oil 126, 13: 36-47 (I), 35-41 (II).

4 Conclusion and major findings

4.1 Conclusion

In the present study, strain of *Clostridium tyrobutyricum* (DSZM 663) was employed for investigation of microbial enhanced oil recovery with molasses based fermentation process. The main aspects covered during the investigation were adaptation to reservoir conditions of salinity, temperature and pressure, metabolites production, microbial fluid rock interactions, oil recovery mechanisms and modeling of some of the parameters related to microbial enhanced oil recovery processes. All these have described in details in separate papers that formed the bone of this thesis.

This work established the practicality that pure culture of bacteria can become adapted to high levels of salinity and temperature after a careful and repeated sub culturing over time and that adaptation to high salinity and temperature could be monitored by gas production. The important point to be made here is that efficiency of gas production was found to reflect rapid metabolism and successful adaptation. Inhibition of cell growth can be observed during initial introduction of bacteria cells to high salt concentration however cells were able to recover after a period of adaptation to high salinity. This suggested that continuous sub-culturing can prolong the limit of adaptation for salinity.

Through a detailed microscopic study it was detected that in response to high salt concentration, bacteria cells became round or spherical shaped and formed aggregates of cells compared to rod shape and existence of single cells at minimal salt concentration. The final stage of the adaptation process produced two main strains, strain 90F which is salinity adapted strain and strain 90F-45 which is temperature improved version of strain 90F. The preliminary investigation at a pressure of 200 bar indicated growth but with some morphological changes. It could not however be concluded if metabolite production was affected. A view of overall results revealed that strain 90F that was salinity adapted strain could grow and proliferate at up to minimum salinity of 100 g/L. Temperature adaptation of this strain (90F) was achievable to 45 ^{o}C and confirmed better improve gas ability at a relatively high salinity of 90 g/L. These were significant improvement over the pure culture that can grow and produce metabolites till salinity

of only 40 g/L. FT-IR spectroscopy and image analysis was able to confirm the difference between the pure culture and adapted strains.

The salinity adapted strain produced gas when introduced to different formation waters with salinity of 30 g/L and 110 g/L respectively from the North Sea. This confirmed its readiness for potential application in some of the North Sea oil reservoirs.

Significant reduction in pH of the medium was also observed after experiments with bacteria inoculums. The decrease in pH was due to the production of acids and gases during fermentation of molasses medium. The lowest pH measured was ranged from 4-5 and the onset of lowering of pH was around 15-20 h after incubation after which the pattern of pH can vary depending on the salinity and metabolites production. Experiments indicated that growth and activity of bacteria are dependent on the amount of nutrient available. The composition of the gas indicated a composition > 80% carbon dioxide while the main acids identified were butyric and acetic acids. It was observed that at increasing salinity the acetic acid concentration increases and vice versa for butyric acid.

The enrichment of microbial fluids by calcium ions during microbial fluid rock interactions was due to rock dissolving properties of the organic acids. This later caused neutralization of the acidity and can therefore improve the production of active metabolites in the medium.

Treatment for microbial improved oil recovery from sand and carbonate packed columns by application of molasses and inoculums of adapted strain at a salt concentration of 90 g/L showed 38% improved recovery in sandstone and 25 % in carbonate rock after a shut in period. It was believed that mechanisms of oil recovery were combination of effects from metabolic process since it was not possible to separate individual contributions of each mechanism.

The information reported in this thesis supported the view that a careful laboratory investigation can improve the understanding of bacteria environmental conditions, growth, metabolisms, interactions with reservoir rock and oil release mechanisms. Through this, it will be possible to

effectively control, monitor and utilize microbial activities in the recovery and utilization of oil resources.

4.2 Major findings

The following summary lists the most significant findings in this thesis.

- Adaptation of bacteria to environmental conditions of salinity, temperature and pressure is possible with careful planning.
- Volume of inoculum can have an effect on the lag phase during microbial growth in medium of high salinity.
- Gas, acid and biofilm production showed direct relationships with salinity of the medium.
- pH partitioning was observed between low and high salinity groups with a common boundary at 70 g/L that probably indicate start of salting out effect.
- Fast and robust spectroscopic and image analysis can be performed to determine the changes that occurred during adaptation to salinity and allowed morphological grouping. It can also relate metabolite production (such as gas) to observed morphological and physiological changes.
- Simple method of titrimetric was proposed to quantify the amount of gas (carbon dioxide) produced in-situ during fermentation process. This can be used to determine effectiveness and limit of bacteria metabolism.
- Organic acid can cause release of significant amount of calcium ions during microbial fluid interactions with carbonate chalk samples and these are most likely to neutralize the acidity and promote effective metabolite production.
- Oil recovery resulted from combination of different mechanisms.

5 Future perspectives

The field of microbial enhanced oil recovery is growing every day as the cost of other enhanced recovery methods become larger. Microbial enhanced oil recovery can be applied in different ways to remediate problems during oil and gas production but to increase its chances for successful application more knowledge is needed. Further research within the following areas will help improve the understanding of microbial enhanced oil recovery. On the basis of the conclusions drawn, some future work considerations are presented in the following.

- A complete evaluation and assessment of MEOR from an engineering standpoint based on economics, applicability and performance is required to further improve the process efficiency for writing more success stories.
- Development of adequate thermodynamic models that can quantify bacteria metabolism and mechanisms are need.
- MEOR has been investigated in most details and hence more studies in area of optimization of the processes and reservoir geomicrobiology can be invaluable.
- Mobilization and transport of bacteria in-situ are still often questioned when MEOR is applied; therefore more studies investigating this phenomenon are needed. Most research suggests that bacteria can be transported in pore spaces of the rocks but well documented field tests could elucidate this issue.
- In general well documented and published field scale applications are needed to support and validate the observations from small scale laboratory tests.