ASERS

Journal of Environmental Management and Tourism

Quarterly

Volume XI

Issue 5(45)

Fall 2020

ISSN 2068 – 7729

Journal DOI

https://doi.org/10.14505/jemt

ASERS Publishing

Fall 2020
Volume XI
Issue 5(45)

ASERS Publishing
http://www.asers.eu/asers-publishing
ISSN 2068 – 7729
Journal DOI: https://doi.org/10.14505/jemt

Table of Contents:

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DOI: https://doi.org/10.14505/jemt.11.5(45).19

Biological Effectiveness of Constructed Consortia in MEOR

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Suggested Citation:

Kaiyrmanova, G. *et al.* (2020). Biological Effectiveness of Constructed Consortia in MEOR. *Journal of Environmental Management and Tourism*, (Volume XI, Fall), 5(45): 1222 - 1230. DOI:10.14505/jemt.v11.5(45).19

Article's History:

Received 28th of May 2020; Received in revised form 23rd of June 2020; Accepted 24th of July 2020; Published 24th of August 2020.

Abstract:

Kazakhstan is one of the producers of oil and gas in the world. Also, Kazakhstan is a country which uses traditional methods of oil development. These methods are not economically beneficial in the development of oil since two-thirds of the oil remains in the ground. In this regard, 16 indigenous microorganisms were studied from the production water of the "Akingen" field located in Western Kazakhstan. It is known that biological preparations containing several symbiotic strains of microorganisms are important because they are more effective and improved with biotechnology properties than monobacterial preparations. Antagonistic activity of 16 indigenous strains of bacteria was examined by cross streak. In addition, the activities of the created consortia were studied (oil emulsification, production of acids and gas). 5 bacterial consortia were selected based on the study of the antagonistic relationships of strains and their activeness, including: 2 consortia consisting of 2 monostrains - *P. aeruginosa* D6: *Bacillus sp.* SR 1; *P. aeruginosa* D6: *Bacillus sp.* CL1; 2 consortia of 3 monostrains - *P. aeruginosa* D6: *Bacillus sp.* SR1: *Bacillus sp.* CL1; *P. aeruginosa* D6: *Bacillus sp.* CL1: *Bacillus sp.* D1X and one consortium of 4 monostrains *P. aeruginosa* D6: *Bacillus sp.* SR1: *Bacillus sp.* CL1: *Bacillus sp.* D1X.

Keywords: consortium; oil reservoir water; antagonistic activity; emulsifying activity; acid production; microbial enhanced oil recovery.

JEL Classification: L71; O13; Q32.

Introduction

Oil is the most important energy resource and chemical feedstock. Moreover, oil has been the engine for development of contemporary world and intensive global economic development in the last century. Despite the demand for renewable energy sources, oil will continue to be one of the dominant energy sources (Nikolova and Gutierrez 2019, 5). Modern and environmentally friendly technology is required in order to extract oil. One of the latest and most popular methods of oil extraction is tertiary enhanced oil recovery method, more precisely microbial enhanced oil recovery (She *et al.* 2019, 1–16).

Kazakhstan is currently experiencing a period of late-stage development and needs modern methods. Most deposits in Kazakhstan have high viscosity oils, high level of permeability which leads to low efficiency of recovery and also has a high-water salinity, which can affect polymer stability. There are also complex geological structures characterize (Bealessio *et al*., 2020).

In present study, we were focused in the potential of a reservoir's indigenous bacteria to use a microbial enhanced oil recovery (MEOR) process. The site chosen for the study was oil reservoir water of the "Akingen" field, located in Western Kazakhstan. The antagonistic properties were studied of 16 active strains. Then we created consortia based on the results and studied their activities.

1. Literature Review

MEOR are eco-friendly and economically effective (Nikolova and Gutierrez 2019, 5; Veshareh *et al.* 2019, 8; Camara *et al.* 2019, 2333–41). The theme of MEOR process study and involving into the oil recovery technics is presently widely developing among modern scientists (Hosseini *et al.* 2020, 147-158; Nami and Ahmadi 2020, 1-5; Monteagudo and Huang, 2020; Saravanan *et al.* 2020, 120777; Sari *et al.* 2020 716).

The process of MEOR has two methods. The first method ex situ involves obtaining microbial metabolites in the laboratory conditions and then, infusion of the metabolites to oil reservoir. The second method in situ is development of microbiological processes in oil reservoir conditions (Cui *et al.* 2017, 2044–50). In the second case, the method is successful when using indigenous microbes to increase oil recovery. Selective indigenous microorganisms along with nutrients are introduced into the reservoir along with high-permeability thief zones. Microorganisms grow in the reservoir and can produce various metabolites such as biosurfactants, biogas, bioacids, biopolymers and biomass (Sharma *et al.* 2018, 5; Du *et al.* 2019, 1–12). In situ are used different cultures as monocultures or mixed consortium. Microbial community in oil reservoirs can be considered as closed and semiclosed systems and in this ecosystem, oil is the main source of energy for microorganisms. Microbial communities in oil reservoirs are diverse and rich, numerous varieties of microorganisms have been isolated from different oil reservoirs such as Clostridium, Bacillus, Pseudomonas, Micrococcus, and Rhodococcus (Yernazarova *et al.* 2016, 46–58; Shibulal *et el.* 2014, 1–12; Rossiana *et al.* 2018, 4; Geetha *et al.* 2018, 23). Microorganisms often produce biopolymers (i.e. xanthan, welan, polysaccharides), biosurfactants (*i.e.* surfactin, lichenizin), organic acids (*i.e.* acetic, propionic and fatty acids), gases (*i.e*. methane, CO2 etc.) and solvents (*i.e.* ethanol, acetone), which are useful in oil recovery processes (Phetcharat *et al.* 2019, 385; Phetcharat *et al.* 2018; Gao 2016, 549; Purwasena *et al.* 2019). The microbial metabolites can decrease the surface, interfacial tensions and wettability, viscosity reduction, permeability alteration, and emulsification of crude oil. Metabolites have a number of advantages, for example can be derived from renewable resources, biodegradable, active in a wide range of harsh environmental conditions, low toxicity (Nielsen *et al.* 2016, 567; Uzoigwe *et al.* 2015, 4).

2. Methodology

Bacterial strains: isolation and identification. 16 microbial cultures isolated from flooded oil reservoir water of the "Akingen" field located in Western Kazakhstan were used in the work. Identification of these bacterial cultures was conducted by the molecular method by determining the direct nucleotide sequence of a 16S rRNA gene fragment, followed by determination of the nucleotide identity with the sequences deposited in the Gene Bank international database. The microorganisms we used are as follows: (1) Bacillus sp. D1X; (2) P. aeruginosa D5; (3) P. aeruginosa D6; (4) P. aeruginosa D7; (5) P. aeruginosa T2; (6) P. aeruginosa T3; (7) Bacillus sp. M2; (8) Bacillus sp. A5; (9) Bacillus subtilis subsp. spizizenii S1; (10) Bacillus sp. S2; (11) Bacillus sp. D7X; (12) P. aeruginosa D8; (13) Bacillus sp. SR1; (14) Bacillus licheniformis SR2; (15) Bacillus sp. CL1; (16) B. licheniformis CL2.

Nutrient media. The following nutrient media were used to study the biological properties of microorganisms: Nutrient Agar, Nutrient Broth (HiMedia Laboratories, Mumbai, India) and Synthetic medium E8 (g/l: KH_2PO_4 - 0,7; $(NH_4)_2HPO_4$ - 1,5; $MgSO_4$ - 0,8; NaCl - 0,5; pH=6,6-6,7).

Antagonistic activity of strains. Antimicrobial action was studied among 16 strains with high target activities such as oil emulsification, production of acid and gas. The cross-streak method was used to study the antagonistic activity (Lertcanawanichakul and Sawangnop 2008, 161). Bacterial strains were inoculated as a line on the surface of a nutrient media and incubated at 30 °C for 24 h. Then test-culture was inoculated as a perpendicular line to bacterial strains. The plates were incubated for 24 h at 30 °C. The antagonistic activity was detected as a zone of bacterial growth inhibition.

Creating a consortium and their activity for MEOR. Firstly, in different bottles the biomass of each strain was grown. On the day of the experiment, all strains were mixed in a sterile tube in the ratio 1:1, 1:1:1, 1:1:1:1. The obtained microbial suspension was used in experiments to study oil emulsification, production of acid and gas.

pH meter C931P was used to measure the pH of the medium (Guiomar *et al.* 2007, 447). The pH measured during at intervals of 24 h during the fermentation process was to be compared with initial pH condition of each medium. And gas production was studied visually using the Durham tubes (Hayward 1957, 10).

Emulsification activity was determined by Cooper (Cooper and Goldenberg 1987, 224–29). The culture supernatant was mixed with hydrocarbon substrate (crude oil) in the ratio of 3:2 and mixed high on the laboratory shaker at 250 r/min during 20 min for receiving a stable emulsion. Subsequently, the test-tubes were kept in a vertical position at a room temperature. Emulsifying activity was expressed in a percentage, computing it as a value of attitude of altitude of emulsifying layer to total altitude of liquid in test-tube.

3. Results and Discussion

Screening for antagonistic interactions among microorganisms. Developed oil reservoir water is extreme underground ecosystems, which is characterized by aerobic and anaerobic conditions, enormous pressure, high temperature and salinity. Microorganisms isolated from such extreme ecosystems with unique adaptive and metabolic properties are promising objects for use as bioagents in MEOR.

To obtain consortia of microorganisms, studying antagonistic interactions between strains of microorganisms is a necessary stage in the construction of mixed cultures. Determination of antimicrobial activity was tested using the cross-streak method: the test strain (dominant) was applied in the form of a stroke along the diameter, after incubation for 1-2 days, test objects (associates) were sown perpendicular to the strains. Antimicrobial substances delayed the growth of microorganism's sensitive to them, which was manifested in the formation of zones of no growth of microbes (mm). The antagonistic activity was studied 16 selected microorganisms against each other.

Table 1 shows the results of testing the antagonistic activity of selected microorganisms for consortium.

As can be seen from the studied 16 strains of microorganisms, in particular, 5 strains of *P. aeruginosa* (D5, D6, D7, T2, T3) and representatives of bacilli: *Bacillus sp*. A5, *B. subtilis subsp. spizizenii* S1, *Bacillus sp*. SR1 have different degrees of antagonistic activity in relation to the studied strains, however, these cultures did not show antagonistic activity to all the studied *Pseudomonas* strains. In this instance, we considered the antagonistic activity only in relation to the presented bacillaries.

Growth inhibition zones were taken into account after 24 and 48 hours of cultivation at 30 ° C. *P. aeruginosa* T2 showed high antagonistic activity against 5 strains of *Bacillus*: *Bacillus sp.* D1X, *Bacillus sp.* M2, *Bacillus sp.* A5, *B. subtilis subsp. spizizenii* S1, *Bacillus sp.* S2 – growth inhibition zone was 22-24 mm. 3 strains of *P. aeruginosa* D5, D7 and T3 had a medium degree of antagonistic activity against *Bacilli* (Figure 1). Weak antagonistic activity was shown by all 5 strains of *Pseudomonas*, as well as the following 3 strains of bacilli: *Bacillus sp.* A5, *B. subtilis subsp. spizizenii* S1 and *Bacillus sp.* SR1. The remaining 8 strains of bacilli did not show antagonistic activity to any of the studied 16 microorganisms.

Figure 1. Study of antagonistic activity of P. aeruginosa D5

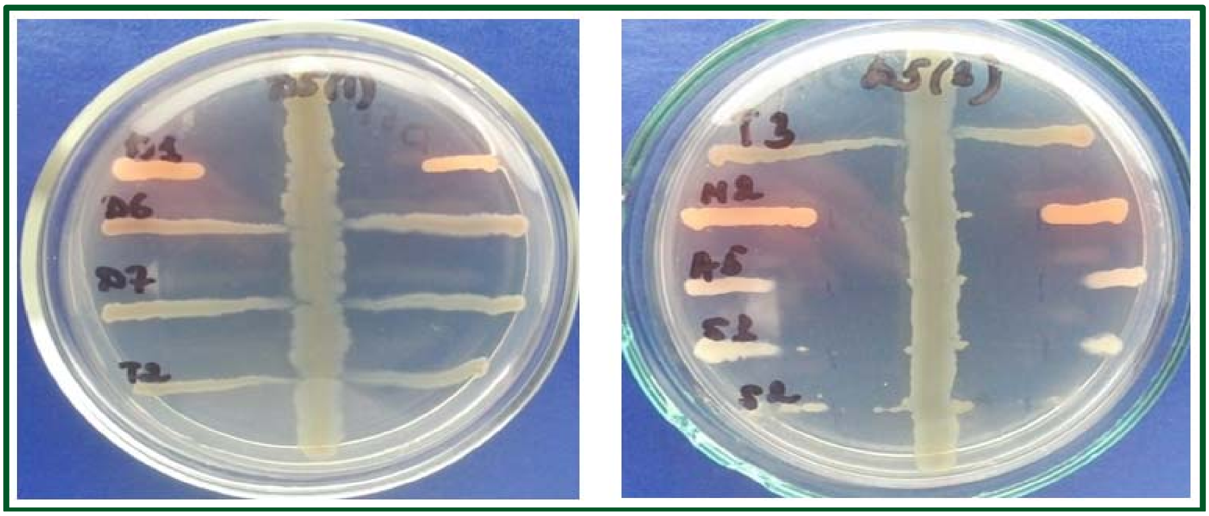


Table 1. Antagonistic activity of microorganisms

Strains	*Zone of growth inhibition, mm*															
Dominant / *Associate*	*Bacillus sp. D1X*	*P. aeruginosa D5*	*P. aeruginosa D6*	*P. aeruginosa D7*	*P. aeruginosa T2*	*P. aeruginosa T3*	*Bacillus sp. M2*	*Bacillus sp. A5*	*B. subtilis ssp.spizizenii S1*	*Bacillus sp. S2*	*Bacillus sp. D7X*	*P. aeruginosa D8*	*Bacillus sp. SR1*	*B. licheniformis SR2*	*Bacillus sp. CL1*	*B. licheniformis CL2*
Bacillus sp. D1X	-	12±0,5	-	15±0,6	23±1,0	10±0,5	-	5±0,2	2±0,1	-	-	-	-		-	-
P. aeruginosa D5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa D6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa D7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa T2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
P. aeruginosa T3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Bacillus sp. M2	-	13±0,6	-	9±0,4	20±1,0	10±0,5	-	5±0,2	-	-	-	-	-	-	-	-
Bacillus sp. A5	-	14±0,7	3±0,2	10±0,5	22±1,0	15±0,8	-	-	-	-	-	-	5±0,2	-	-	-
B. subtilis subsp. spizizenii S1	-	14±0,7	3±0,2	11±0,5	24±1,0	13±0,6	-	7±0,4	-	-	-	-	-	-	-	-
Bacillus sp. S2	-	13±0,6	-	10±0,5	24±1,0	10±0,5	-	-	2±0,1		-	-	-	-	-	-
Bacillus sp. D7X	-	-	-	5±0,2	5±0,2	5±0,2	-	-	-	-	-	-	-	-	-	-
P. aeruginosa D8	-	8±0,4	-	10±0,5	5±0,2	5±0,2	-	-	3±0,2	-	-	-	-	-	-	-
Bacillus sp. SR1	-	8±0,4	-	10±0,5	8±0,4	12±0,5	-	-	6±0,3	-	-	-	-	-	-	-
B. licheniformis SR2	-	10±0,5	-	12±0,5	8±0,4	5±0,2	-	-	5±0,2	-	-	-	-	-	-	-
Bacillus sp. CL1	-	10±0,5	-	12±0,5	6±0,3	10±0,5	-	-	5±0,2	-	-	-	-	-	-	-
B. licheniformis CL2	-	10±0,5	-	10±0,5	5±0,2	8±0,4	-	-	-	-	-	-	-	-	-	-

Note:
vertically-dominant, checked culture
horizontally-the assumed associate
“-”- no growth suppression
“x” mm - zone of antagonistic effect on strains

It is known that the antagonistic activity of bacteria is manifested by their vital activity, that is, by the use of metabolites and cell walls. One example is *Bacilli* and *Pseudomonas*, which have such characteristics. According to information *P. aeruginosa* produces a large number of exoproducts, such as hemolysin, rhamnolipids and pyocyanin (Lau *et al.* 2004, 599). Some strains of *Bacillus* produce bacteriocins, which are effective against bacteria of the same species, others produce antibiotics against Gram-negative bacteria (Moore *et al*. 2013; Meena and Kanwar 2015). Sara Tayeb and their co-workers (Tayeb *et al.* 2015, 362) found that that *P.*

aeruginosa manifested a considerable antagonistic activity against *B. subtilis*, *B. cereus* (30, 22 mm). According to the authors Foysal and Lisa (2018, 387) *Bacillus sp.* moderately inhibited the growth of *Pseudomonas sp.* (9-10 mm).

Due to the fact that water flooded oil reservoir is a multicomponent mixture of various petroleum hydrocarbons, using of the metabolic potential of mixed cultures of microorganisms in consortia is more effective than monostrains. However, the use of a large number of microorganism components can make biotechnology more expensive, requiring additional equipment and energy to prepare for the development of microbial biomass. Therefore, based on the study of target properties (oil emulsification, production of acids and gas) and antagonistic relations of microorganisms selected 5 strains, 2 strains of *Pseudomonas* - *P. aeruginosa* D5, *P. aeruginosa* D6 – aerobic and 3 strains of bacteria – *Bacillus sp.* D1X, *Bacillus sp.* SR1, *Bacillus sp.* CL1 – facultative anaerobes, as candidates for the compilation of consortium for the development of MEOR methods.

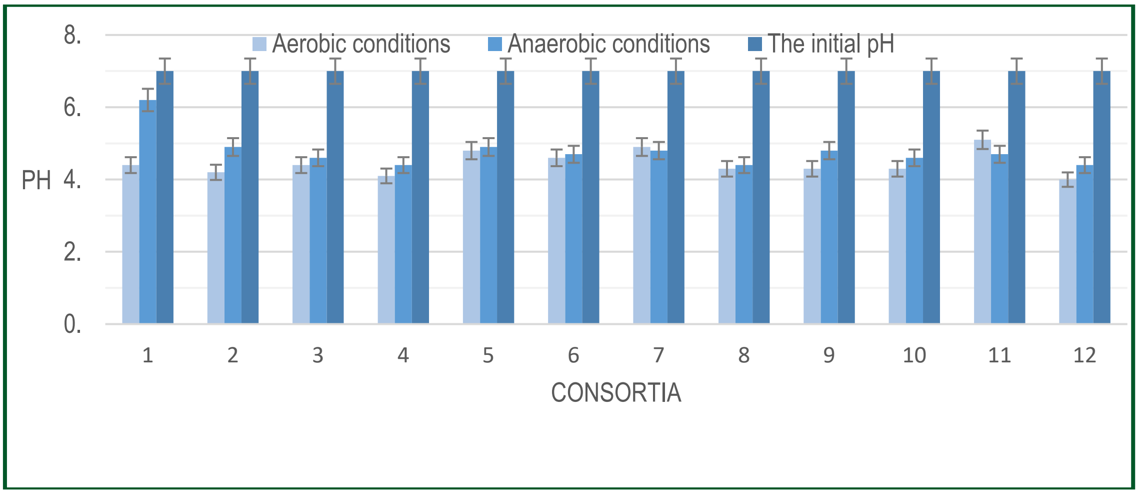
Creating a consortium and their activity for MEOR. Currently, combined biopreparations are in demand in the oil industry, due to the fact that oil is a multicomponent mixture of hydrocarbons of different molecular weight and other chemical compounds. The bacterial strains of the biopreparations are combined into a consortium regarding to the ability of strains to produce various biologically active substances, and they complement or strengthen each other in biological activity to produce oil-displacing and oil-liquiding metabolites in the conditions of the developed oil reservoir (high concentrations of mineral salts, pressure, oxygen deficiency) (Rathi *et al.* 2015, 88115; Rahman *et al.* 2002, 1277).

25 possible consortia were created, excluding weak and medium levels of antagonism between microorganisms. For further study, 12 consortia of microorganisms were selected, including: 7 consortia made up of 2 monostrains; 4 consortia - from 3 monostrains and one consortium – from 4 monostrains. When creating consortia of bacteria were activated in the NB within 48 hours. Then they were mixed in a ratio of 1:1 when using two strains; 1:1:1 and 1:1:1 - when using three and four strains. The resulting consortia of microorganisms (inoculate) was added to the nutrient medium in an amount of 10 % of the volume of the medium.

The main task in biotechnologies for enhanced oil recovery of developed and flooded oil reservoirs is decreasing the viscosity of oil and increasing the pressure in the reservoir using microorganisms and their metabolites (Shibulal *et al.* 2018, 10). Due to the fact that oil is a multicomponent mixture of various petroleum hydrocarbons, it is preferable to use consortia of microorganisms in enhanced oil recovery. Mixed cultures form a number of different metabolites – biosurfactants, gases, acids, the appearance of which in the reservoir system is associated with aerobic-anaerobic degradation of oil (Shibulal *et al.* 2014, 1–12). Aerobic-anaerobic debasement of oil in the oil reservoir is caused by artificial flooding of the well from the surface using secondary methods of oil production. Furthermore, the research of the target activity of the constructed consortia of microorganisms was carried out both under aerobic and anaerobic cultivation conditions.

Figure 2 presents the results of determining two-day emulsifying activity of the consortia of microorganisms during cultivation under aerobic and anaerobic conditions.

Figure 2. The change in pH of consortia of microorganisms



1 – *P.aeruginosa* D5: *P.aeruginosa* D6; 2 – *Bacillus sp.* D1X: *P.aeruginosa* D6; 3 – *P.aeruginosa* D6: *Bacillus sp.* SR 1; 4 – *P.aeruginosa* D6: *Bacillus sp.* CL1; 5 – *Bacillus sp.* SR1: *Bacillus sp.* CL1; 6 – *Bacillus sp.* SR1: *Bacillus sp.* D1X; 7 – *Bacillus sp.* CL1: *Bacillus sp.* D1X; 8 – *P.aeruginosa* D6: *Bacillus sp.* SR1: *Bacillus sp.* CL1; 9 – *P.aeruginosa* D6: *Bacillus sp.* SR1: *Bacillus sp.* D1X; 10 – *P.aeruginosa* D6: *Bacillus sp.* CL1: *Bacillus sp.* D1X; 11 – *Bacillus sp.* SR1: *Bacillus sp.* CL1: *Bacillus sp.* D1X; 12 – *P.aeruginosa* D6: *Bacillus sp.* SR1: *Bacillus sp.* CL1: *Bacillus sp.* D1X

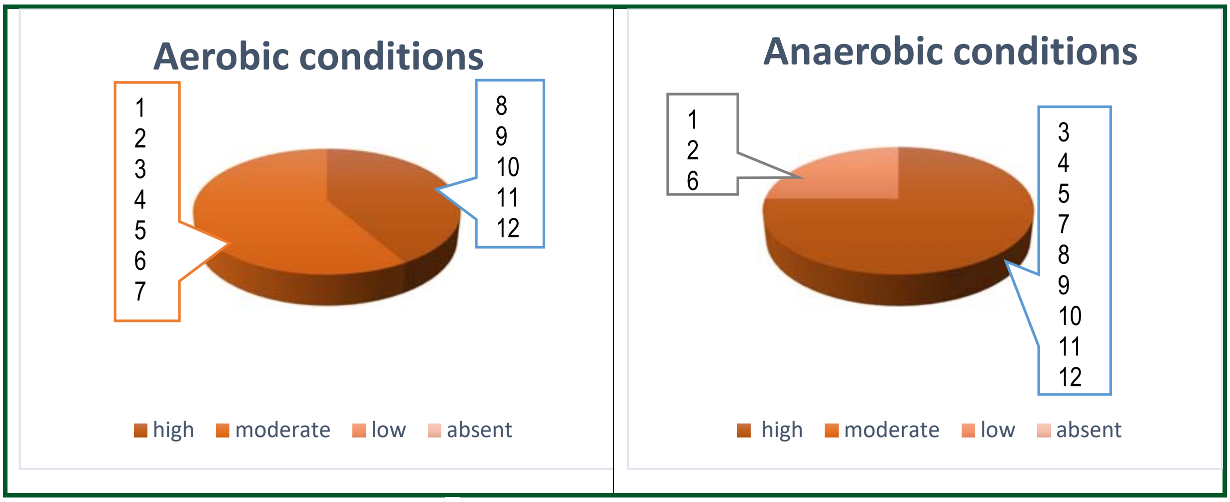
As can be seen from the figure 3, microorganisms under aerobic conditions, active acid formation occurs, so that the maximum decrease in pH of the medium by the end of the experiment is from 7 units. up to 4.0-4.4 units observed for 8 consortia:

- D6: D1 (4.2 units);
- D6: CL1 (4.1 units);
- D6: SR1 (4.4 units);
- D5: D6 (4.4 units);
- D6: SR1: CL1 (4.3 units);
- D6: SR1: D1 (4.3 units);
- D6: CL1: D1 (4.3 units);
- D6: SR1: Cl1: D1 (4.0 units).

The consortia changed the medium characteristics, which led to a change in the pH medium. It means that molasses and some nutrients inside the medium were used by consortia that favour bacterial growth, increasing their activities. In this case, organic acids can be produced in fermentation process. These acids easily dissolve carbonates and can significantly improve permeability in limestone reservoirs. Similar changes have been shown in the work of Halim *et al.* (2009, 1–8), which presents laboratory studies of the ability of bacteria to live and alter the physical characteristics of crude oil in high-pressure conditions. Medium pH changes during incubation was about 4.5-5.

Figure 3 demonstrates the analysis of gas formation constructed consortia of microorganisms. As you can seen from the data, active gas formation under aerobic conditions is observed for 5 consortia of microorganisms, of which 3 consortia consisting of 2 monostrains: D6: SR 1; SR1: CL1; CL1: D1X and one consortium, consisting of 3 and 4 monostrains: D6: SR1: D1X; D6: SR1: Cl1: D1X. In anaerobic conditions, active gas formation is observed for 9 consortia. Moderate gas formation was observed for the consortium D1: D6: SR1 and there was no gas formation in two consortia: D5: D6 and D6: D1. Bacteria can ferment carbohydrates to form gases such as carbon dioxide, hydrogen, and methane. These gases can contribute to increased pressure in depleted reservoirs (Nikolova and Gutierrez 2019, 5). Some of the reported gas-producing genera are *Clostridium, Desulfovibrio, Pseudomonas* and certain methanogens (Behlülgil and Mehmetoğlu 2002, 413–21; Al-Wahaibi *et al.* 2014, 324–33).

Figure 3 - Analysis of gas formation of constructed consortia of microorganisms



1 – *P.aeruginosa* D5: *P.aeruginosa* D6; 2 – *Bacillus sp.* D1X: *P.aeruginosa* D6; 3 – *P.aeruginosa* D6: *Bacillus sp.* CL1, 4 – *Bacillus sp.* SR1: *Bacillus sp.* D1X; 5 – *P.aeruginosa* D6: *Bacillus sp.* SR1: *Bacillus sp.* CL1; 6 – *P.aeruginosa* D6: *Bacillus sp.* CL1: *Bacillus sp.* D1X; 7 – *Bacillus sp.* SR1: *Bacillus sp.* CL1: *Bacillus sp.* D1X, 8 – *P.aeruginosa* D6: *Bacillus sp.* SR 1; 9 – *Bacillus sp.* SR1: *Bacillus sp.* CL1; 10 – *Bacillus sp.* CL1: *Bacillus sp.* D1X; 11 – *P.aeruginosa* D6: *Bacillus sp.* SR1: *Bacillus sp.* D1X; 12 – *P.aeruginosa* D6: *Bacillus sp.* SR1: *Bacillus sp.* CL1:*Bacillus sp.* D1X

Consequently, from the studied 12 constructed consortia of microorganisms, at least 4 indicators coinciding with 6 target properties (oil emulsification, production of acid and gas under aerobic and anaerobic conditions) the following 5 consortia of microorganisms were selected, of which: 2 consortia from 2 monostrains - D6: SR 1; D6: CL1; 2 consortia of 3 monostrains - D6: SR1: CL1; D6: CL1: D1X and one consortium of 4 monostrains D6: SR1: Cl1: D1X. Thus, as a result of studying the oil-displacing and oil-fluidizing properties of 12 constructed consortia of microorganisms, 5 consortia of microorganisms with high target characteristics under aerobic and anaerobic cultivation conditions were selected.

Conclusion

In summary, the study of the antagonistic relationship of 16 strains selected the following 5 strains: *P. aeruginosa* D5, *P. aeruginosa* D6, *Bacillus sp.* D1X, *Bacillus sp.* SR1 and *Bacillus sp.* CL1. Thus, as a result of studying the oil-displacing and oil-fluidizing properties of 12 constructed consortia of microorganisms, 5 consortia of microorganisms with high target characteristics under aerobic and anaerobic cultivation conditions were selected, including: 2 consortia consisting of 2 monostrains - *P. aeruginosa* D6: *Bacillus sp.* SR 1; *P. aeruginosa* D6: *Bacillus sp.* CL1; 2 consortia of 3 monostrains - *P. aeruginosa* D6: *Bacillus sp.* SR1: *Bacillus sp.* CL1; *P. aeruginosa* D6: *Bacillus sp.* CL1: *Bacillus sp.* D1X and one consortium of 4 monostrains *P. aeruginosa* D6: *Bacillus sp.* SR1: *Bacillus sp.* CL1: *Bacillus sp.* D1X. The results are recommended for further research as promising objects in developing microbiological methods for enhanced oil recovery.

Acknowledgement

This work was sponsored by the project AP 05134797 “Creating a technological scheme for MEOR”.

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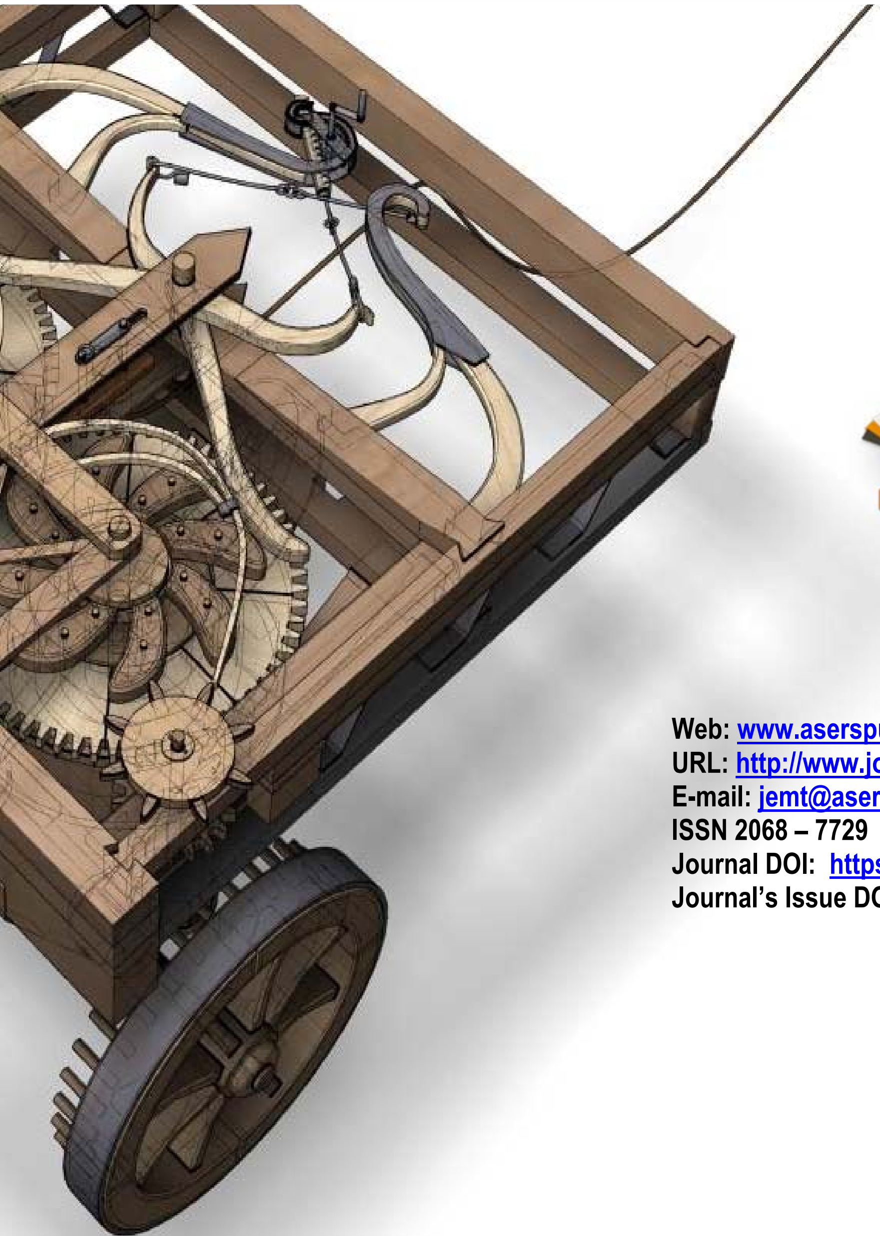

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