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Experimental Investigation of Amine- and Triazine-Based Biocide Effectiveness in Polymer-Based Fluids

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ABSTRACT

Polymer-based fluids are widely used in geothermal drilling operations due to high efficiency and decreased costs associated with them. Most of these polymers have various specimens of bacteria used during their fermentation process. This bacterial activity needs active monitoring. Increased bacterial activity, if not controlled, not only decreases fluid's performance during drilling but also increases the risk for rig personnel by H_2S formation. Moreover, uncontrolled bacteria existence results in plugged pores and reduced permeability of the formation. These, in the end, result in higher chemical treatment costs and increased drilling and well-completion times. In this paper, we investigated two essential types of biocides (amine- and triazine-based) and their effectiveness in tackling different classes of bacteria in polymer-based fluids.

Extensive laboratory experiments were conducted for the analyses. Sterile polyethylene bottles with thioglycolate broth were used for waste drilling fluid sampling to keep all aerobic and nonaerobic bacteria alive during transportation from the well-site to the laboratory. Dilution methods determined the concentration of bacteria in the fluid samples. Spread plate technique was applied with the mineral salt medium. The inoculated plates were inverted onto their lids. A 9.0 cm Whatman filter paper was placed in each plate and saturated with sterile waste drilling-fluid as the carbon source. All of these plates were incubated at 35°C (±2°C) for 7 days. The microorganisms growing on the agar plates were purified and isolated. Semi-automated Analytical Profile Index (API) test and fully-automated Phoenix systems were used in defining the bacteria classification. Amine- and triazine-based biocides were tested to investigate the effectiveness of them against all different classes of bacteria by the quantitative dilution method.

Sixteen different types of bacteria were observed in the polymer-based drilling fluid samples. Eleven of these were identified as gram-negative while the other five were identified as gram-positive bacteria. In the oxygen demand, it was observed that the aerobic bacteria existed in the samples taken from shallow zones. They were replaced with facultative anaerobe and anaerobe bacteria at deeper zones. It was also observed that amine-based biocide, for more than 50% of the strains, was much more effective in tackling the bacteria formation on gram-negative strains than Triazine-based biocide. Amine- and triazine-based biocides were both equally effective on gram-positive strains.

This paper summarizes the experimental results of the most common microorganisms in polymer-based drilling fluids and the effectiveness of amine- and triazine-based biocides to control the bacteria activity. Making a better selection of chemicals increases the effectiveness of biocide, therefore decreasing overall drilling fluid maintenance and well completion costs.

1. INTRODUCTION

Microbiological phenomena and their practical application in exploration and production activities saw an increased interest in recent years (Turkiewicz, Brzeszcz, et al. 2015) (Gul and Aslanoglu 2018). Some of this biogenic process is beneficial e.g. in environment protection, degradation of wastes and polymer effectiveness. However excessive and uncontrollable development of microorganisms can be detrimental (Turkiewicz 2011).

An excessive amount of bacterial activity can cause contamination, which can be harmful in three main ways. First, contamination decreases the performance of drilling fluids by deteriorating the mud (biodegradation) (Johnson, et al. 2018). If not controlled by appropriate agents (biocides), this results in over-treatment of drilling fluids which increases the chemical additive costs. Second, contamination can increase the H_2S and organic acid content of the fluid, which is hugely detrimental to the field personnel. Third, anaerobic bacteria can plug the reservoir pore spaces, which lowers the permeability and decreases production rates (Hong, Manchao, & Jichu 2010).

To completely control microorganism development, it is beneficial to understand them first. An early study in the subject (Sand, 2003) focused on the relationship between the properties of geothermal waters' and microbiological activity. The first reason for microbiological activity is the mineral types in geothermal waters. The most common minerals that microorganisms require for existence are Nitrogen (N), Sulfur (S), Phosphorus (P), Potassium (K), Calcium (Ca), Magnesium (Mg) and Iron (Fe), which are available in high concentrations in geothermal waters. Second, geothermal waters are contained at extremely high temperatures in wellbores. Previous studies show that microorganisms can stay alive in the range of –30°C to 116°C. At temperatures higher than 130°C, spontaneous decomposition of nucleic acids and proteins starts; therefore bacteria existence is not expected (Sand 2003).

Another reason for the existence of microorganisms in geothermal drilling is related to the polymer-based drilling fluids. The most commonly used polymer additives in the fluid design are xanthan gum (viscosifier), starch (fluid-loss control agent) and polyanionic

cellulose (PAC - viscosifier and fluid-loss control agent) (Gul, Kuru and Parlaktuna 2017). These polymers are not only drilling fluid additives but also rich sources of carbon and sulfate for microorganisms (Ozyurek and Bilkay 2017). Some of these polymers also have various species of microorganisms in their formulation (e.g., xanthan gum derives its name from Xanthomonas campestris – the bacteria used in its fermentation process).

Bacterial species can be classified according to different categorizations. The main focuses on geothermal drilling and production are cell wall structure, their oxygen dependence, temperature requirements, and pH levels.
The primary classification is as called gram staining which is a conventional technique used to differentiate two large groups of bacteria based on their different cell wall constituents. The gram stain procedure distinguishes between gram-positive and gram-negative groups by coloring these cells as red or violet. Gram-positive bacteria stain violet due to the presence of a thick layer of peptidoglycan in their cell walls. Alternatively, gram-negative bacteria stain red, which is attributed to a thinner peptidoglycan wall. That does not retain the crystal violet during the decoloring process (Bruckner 2019).
Another classification for microorganisms is their oxygen dependence (Sand 2003). They can be classified into three main groups as below:

- strict aerobes (fully oxygen-dependent),
- facultative aerobes (can use both oxygen or electron acceptors and organic compounds),
- strict anaerobes (cannot use oxygen since it is toxic to their metabolisms).

On the other hand, **Table 1** shows the microorganism classification in various temperature ranges and approximate depth range of the dynamic formation temperature (DFT) (Sand 2003).

Table 1: Microorganism classifications according to temperature ranges and conditions (Sand, 2003).

Description	Temperature Range	Conditions
Pyschro, or cryophiles	0°C up to 20°C	Make-up water
Psychrotrophs	0°C up to 30°C	Mud Pit and Make-up water
Mesophiles	10°C up to 40°C	Mud Pit and depth shallower than 300m
Moderate Thermophiles	35°C up to 55°C	Depth between 200m-600m
Thermophiles	50°C up to 80°C	Depth between 500m-2500m
Extreme Thermophiles	75°C up to 95°C	Depth between 2000m-3500m
Hyperthermophiles	> 90°C	Depth @ deeper than 3000m

In the classification regarding the pH levels, they are called as moderate acidophiles ($2 < pH < 5$), neutrophils ($5 < pH < 9$) or alkaliphiles ($9 < pH < 12$). According to Sand (2003), the major concentration of bacteria in geothermal waters are neutrophils with the minority being alkaliphiles.

The primary way to control the contamination is by using an appropriate additive called “biocide.” In this study, two main types of biocide used in the drilling industry were examined:

1. Amine-based biocide
2. Triazine-based biocide

Amine-based biocide is defined as a water-soluble quaternary ammonium compound-based bactericide. Triazine-based biocide is defined as a highly active bactericide/bacteriostatic and fungistatic containing triazine as the active ingredient (Stable DF 2019). They are both used to provide control of aerobic and sulfate-reducing bacteria in water-based drilling fluids. These biocides help to maintain fluid rheology and fluid loss control by preventing the biodegradation of natural and semi-natural polymers in water-based fluids. They also help reducing corrosion by preventing the production of acid gases due to bacterial growth.

To sum up, there are various kinds of microorganisms which exist in different conditions together with several kinds of biocides to control them. However, the current application of biocide in the field practice is very random. The knowledge of concentration or type of biocide treatment in different situations is limited. No study in the literature studied the effectiveness of different biocides in various bacteria types. In this study, we collected fluid samples from a geothermal well during drilling with polymer-based fluids and analyzed them in the laboratory for the existence of microorganism types. Afterward, two common biocides (amine-based and triazine-based) were tested to understand the performance of each against all the bacteria types observed. In the last section of this paper, a method of choosing the correct biocide type to control the bacterial activity of polymer-based fluids is provided as a function of the gram staining and oxygen demand of identified bacteria. Making a better selection of chemicals will increase the effectiveness of biocide, therefore decreasing overall drilling fluid maintenance and well-completion costs.

2. MATERIALS AND METHODS

The samples are taken from mud pit which contains drilling fluid waste and formation cuttings. Mud pits are the final destination of the circulation system. Drilling fluid is circulated through mud pump into the well, carries the cutting to the surface, then eliminated by solid control systems to mud pits. The drilling fluid type is polymer-based, and it contains Xanthan Gum, Starch, PAC and Caustic Soda in different concentrations. **Figure 1** shows the samples source.

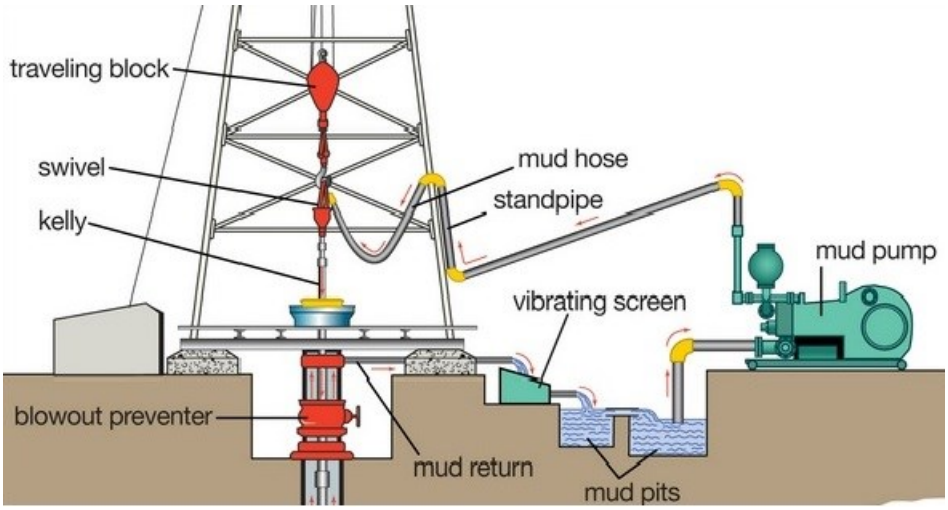

Figure 1: Mud circulation system (image retrieved online from Britannica)

2.1. Bacterial Identification

Extensive laboratory experiments were conducted to analyze the bacteria types in fluid samples and the effectiveness of tested biocides. Sterile, wide-mouthed polyethylene bottles with thioglycolate broth (containing pancreatic digest of casein, dextrose, yeast extract, sodium chloride, sodium thioglycolate, L-Cystine, resazurin, and agar at pH of 7.1) were used for waste drilling fluid sampling to keep all aerobic and nonaerobic bacteria alive while transportation from the well-site to the laboratory. The concentration of bacteria in the fluid samples were determined by the agar dilution method. Spread plate technique was applied with the mineral salt medium (MSM, content: NaCl, $MgSO.7H_2O$, KCl, KH_2PO_4, Na_2HPO_4, $NaNO_3$, agar and distilled water). The medium was adjusted to a pH of 7.4 (Mills, Breuil, & Colwell, 1978). It was also sterilized by autoclaving at a temperature of 121°C under 15 psi pressure for 15 min before dispensing into sterile Petri dishes. The inoculated plates were inverted onto their lids. A 9.0 cm Whatman filter paper was placed in each plate and saturated with sterile waste drilling-fluid as the carbon source. All of the plates were incubated at 35°C (±2°C) for seven days. The microorganisms growing on the agar plates were purified and isolated. Semi-automated API 20E test and fully-automated Phoenix system (**Figure 2**) were used in defining the bacteria.

Figure 2: Fully automated Phoenix system used in experiments for bacteria determination (image retrieved online from ThalesNano).

Additionally, eight of the bacterial species were counted after the application of amine- and triazine-based biocides to investigate biocidal efficiency. Bacterial colonies were counted by agar dilution method, and bacterial count in samples was reported as cfu/ml. Contamination is calculated by colony forming unit bacteria volume on 1 mL fluid. Less than 10^5 cfu/ml is classified as low, and over 10^6 cfu/ml is reported as highly contaminated as suggested by Turkiewicz (2011).

2.2. Biocidal Activity Test

After the microbiologic activity was assessed, the effect of two significant biocides' (triazine- and amine-based) antibacterial activity was measured numerically. To obtain the biocidal activity; *Staphylococcus aureus* ATCC (American Type Culture Collection) 6538, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 8739, *Achromobacter sp*. ATCC 21942, *Acinetobacter baumannii* ATCC BAA-1710 standard strains were included in the study. *Aeromonas*, *Proteus* and *Arthrobacter* species that were isolated from waste pit were also examined.

All microorganisms were suspended in phosphate-buffered saline solution (PBS) and adjusted turbidometrically to 0.5 MacFarland standard. Microbial suspensions were inoculated in 4.95 mL biocide solutions. All samples were incubated at room temperature for 5 min. After the incubation period, all test samples were diluted to 1:10 into Dey-Engley Neutralizing Broth before inoculations (content: tryptone, yeast extract, dextrose, sodium thioglycollate, sodium thiosulphate, sodium bisulfite, lecithin, Polysorbate 80, bromocresol purple. Final pH 7.6±0.2). Dey-Engley Neutralizing Broth was used in disinfectant testing since neutralization of the antiseptics and disinfectants was important for determining its bactericidal activity. All the test samples were serially diluted, and all dilutions were plated on MSM. Similar to previous analysis, the dilutions were incubated at 35°C for 7 days. Sterile saline suspensions were used for growth control.

Biocidal activity was interpreted by Reduction Factor (RF), which is the logarithmic difference of the number of microorganisms before disinfectant exposure (N_1) and after treatment with disinfectant (N_2). The high value of RF correlates positively with the disinfectant activity of the tested solution. Colony counts were compared with saline control to determine log reductions (Kilvington, et al. 2011). As also illustrated in **Table 2**, bacterial inhibition is directly proportional to RF. **Eq. 1** explains the calculation method for RF.

RF: log N_1-Log N_2……..……(1)

Table 2: Reduction factor and bacterial inhibition relationship.

Reduction Factor	1	2	3	4	5
Bacterial Inhibition	90%	99%	99.9%	99.99%	99.999%

Serial dilution method was applied to count the bacterial colonies. The goal of the serial dilution process was to obtain plates with concentrations in the range of 30-300 cfu/ml. The process involved several dilutions in multiples of 10 to simplify calculations. **Figure 2** shows a serial dilution example.

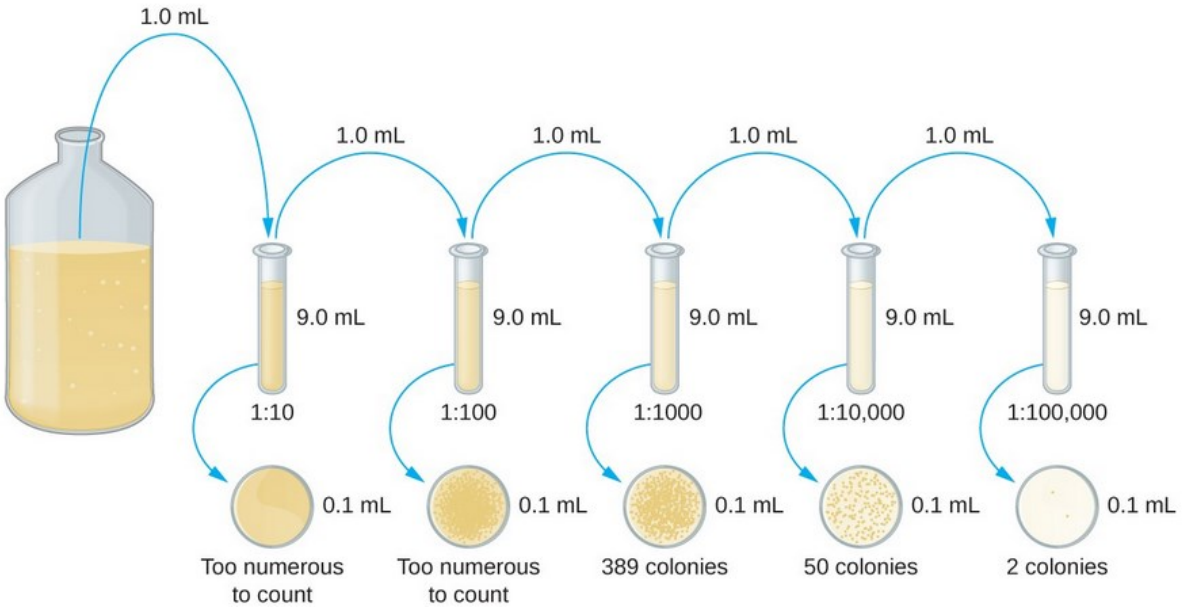

Figure 3: Serial dilution method (image retrieved online from Lumen Microbiology).

Dilution continues until colony number is between 30-300 cfu/ml. Then the second step of dilution verifies the result. After the bacterial suspension, each sample was treated with two types of biocides separately. Then bacterial colony number was counted with serial dilution. One sample was taken non-treated while the other one was treated with the applied biocide. **Figure 3** illustrates the dilution experiment procedure applied in this study.

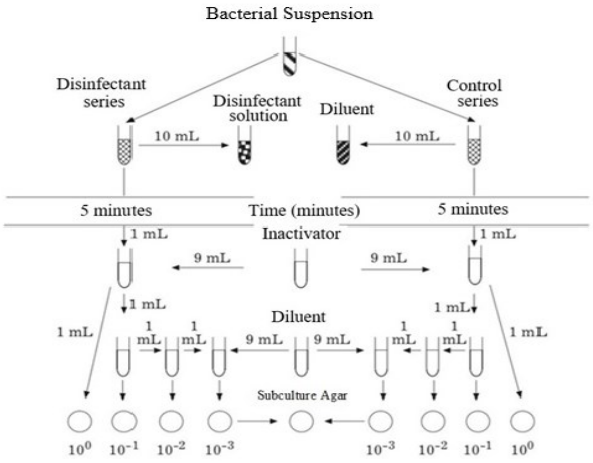

Figure 4: Experiment procedure for biocidal treatment.

3. RESULTS

The main focus of the study was bacterial identification and biocidal treatment of the waste pit, which contains polymer-based drilling fluids and cuttings as well as core samples obtained from the well. Gram staining properties and oxygen demand were the primary classifications for the identified bacteria in both waste pit and the core samples. **Table 3** summarizes identified bacteria names with their gram staining and oxygen demand properties.

Achromobacter sp., *Aeromonas* sp., *Arthrobacter* sp., *Bacillus* sp. were isolated from both core samples and waste pit. The identified bacteria were aerobe, and two of them were gram-negative, while the other two were gram-positive. They are shown in bold letters in **Table 3**. Moreover, *Acinetobacter* sp. *Bacteroidetes* sp., *Brevibacterium* sp., *Chromobacterium* sp., *Clostridium* sp., *Escherichia* sp., *Micrococcus* sp., *Proteus* sp., *Pseudomonas* sp., *Staphylococcus* sp., *Stenotrophomonas* sp. and *Veillonella* sp. were the other bacterial species isolated in the waste pit. 69% of them were gram-negative, and 31% were gram-positive. While the majority (56%) of the bacteria species were aerobe, 25% were facultative anaerobe, and 19% were anaerobe.

Table 3: Isolated bacteria with their gram staining and oxygen demand properties.

No	Bacteria	Gram Staining	Oxygen Demand
1	***Achromobacter sp.***	Gram-Negative	Aerobe
2	*Acinetobacter sp.*	Gram-Negative	Aerobe
3	***Aeromonas sp.***	Gram-Negative	Aerobe
4	*Pseudomonas sp.*	Gram-Negative	Aerobe
5	*Escherichia sp.*	Gram-Negative	Facultative anaerobe
6	*Proteus sp.*	Gram-Negative	Facultative anaerobe
7	***Arthrobacter sp.***	Gram-Positive	Aerobe
8	*Staphylococcus sp.*	Gram-Positive	Facultative anaerobe
9	*Micrococcus sp.*	Gram-Positive	Aerobe
10	*Stenotrophomonas sp.*	Gram-Negative	Aerobe
11	*Veillonella sp.*	Gram-Negative	Anaerobe
12	***Bacillus sp.***	Gram-Positive	Aerobe
13	*Bacteroidetes sp.*	Gram-Negative	Anaerobe
14	*Brevibacterium sp.*	Gram-Positive	Aerobe
15	*Chromobacterium sp.*	Gram-Negative	Facultative anaerobe
16	*Clostridium sp.*	Gram-Negative	Anaerobe

In the second part of the experiments, the first 8 of identified bacteria in **Table 3** were treated with amine- and triazine-based biocides. **Table 4** shows the results as RF for the counted bacteria species from the start time (time = 0) until the biocidal treatment separately for amine- and triazine-based biocides. Also, the gram staining and oxygen demand of the bacteria was reported for more natural interpretation for each case. Treated species caused high contamination up to 10^{10} cfu/ml. Moreover, Arthrobacter sp. and Staphylococcus sp. were inhibited correctly. On the other hand, RF of triazine-based biocide was less than amine-based biocide for other bacteria species.

Table 4: Results of biocidal treatment on various types of bacteria.

			Bacteria Amount (cfu/ml)			Reduction Factor	
Bacteria	Gram Staining	Oxygen Demand	Time = 0	After treated with amine-based	After treated with triazine-based	After treated with amine-based	After treated with triazine-based
Achromobacter sp.	gram-negative	aerobe	10^{8}	10^{3}	10^{4}	5	4
Acinetobacter sp.	gram-negative	aerobe	10^{7}	10^{1}	10^{2}	6	6
Aeromonas sp.	gram-negative	aerobe	10^{9}	10^{1}	10^{6}	8	3
Pseudomonas sp.	gram-negative	aerobe	10^{9}	10^{2}	10^{6}	7	3
Escherichia sp.	gram-negative	facultative anaerobe	10^{10}	10^{2}	10^{7}	8	3
Proteus sp.	gram-negative	facultative anaerobe	10^{7}	10^{0}	10^{6}	7	1
Arthrobacter sp.	gram-positive	aerobe	10^{8}	10^{0}	10^{0}	8	8
Staphylococcus sp.	gram-positive	facultative anaerobe	10^{7}	10^{0}	10^{0}	7	7

Figures 4 and 5 show the effect of amine- and triazine-based biocides on *Escherichia sp* respectively. This sample was chosen for illustration since the contamination was the highest amongst all other samples with a concentration of 10^{10} cfu/ml. While triazine-based biocide provided a decent treatment (RF:3 – **Figure 3**), amine-based biocide treated with an extremely high reduction factor (**RF:8 - Figure 4**).

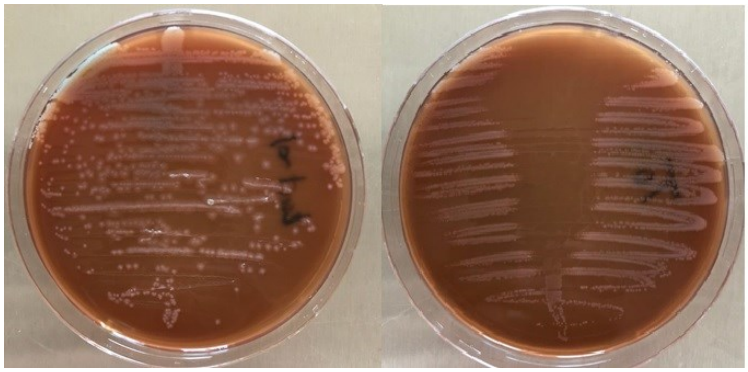

Figure 5: Before (left) and after (right) the treatment against *Escherichia sp by triazine-based biocide (RF: 3).*

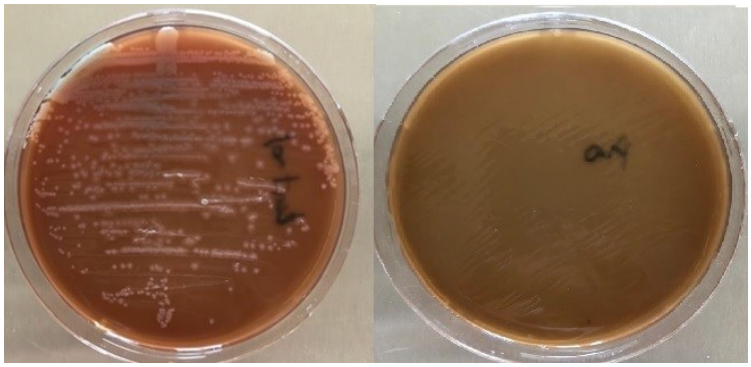

Figure 6: Before (left) and after (right) the treatment against *Escherichia sp by amine-based biocide (RF: 8).*

4.DISCUSSIONS AND CONCLUSIONS

- Results show that geothermal wells are open to bacterial contamination which might create potential problems. For the drilling operations with highly microbiological activity, this approach (RF of different biocides on certain bacteria types) can be used to optimize the efficiency.

- 5 of 16 bacteria species were gram-positive and examined gram-positive bacteria species were treated correctly by both biocides, which shows their sensitiveness to this treatment.
- 11 of 16 bacteria species were gram negative. Also, 6 of 8 highly contaminated bacteria species were gram negative which shows the main reason for the contamination is gram-negative bacteria existence.
- Amine-based biocides were much more effective compared to triazine-based ones on gram-negative type bacteria (on average: RF of amine-based was 4.2 higher than triazine-based biocides for the samples examined).
- No significant relation between oxygen demand and biocide treatment was observed. It was observed that amine-based biocide was more efficient on gram-negative and facultative anaerobe bacteria (with an average RF difference of 5.5). Treatment difference between two biocides on gram-negative and aerobe bacteria was closer (with an average RF difference of 2.5).

GLOSSARY

API = The analytical profile index
ATCC = American Type Culture Collection
DFT = Dynamic formation temperature
MSM = Mineral Salt Medium
PAC = Poly-anionic cellulose
PBS = Phosphate-buffered saline solution
RF = Reduction Factor

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