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BIOCIDE SCREENING TEST TO PRODUCE WATER REINJECTION IN THE "X OIL FIELD"

PENGUJIAN SELEKSI BIOSIDA UNTUK REINJEKSI AIR TERPRODUKSI DI "LAPANGAN MINYAK X"

Zulkifliani
"LEMIGAS" Research and Development Centre for Oil and Gas Technology
Jl. Ciledug Raya Kav.109, Cipulir, Kebayoran Lama, P.O. Box 1089/JKT, Jakarta Selatan 12230 INDONESIA
Tromol Pos: 6022/KBYB-Jakarta 12120, Telephone: 62-21-7394422, ext. 1519, Fax: 62-21-7246150
E-mail:zulkifliani@lemigas.esdm.go.id

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ABSTRAK

Lapangan minyak menghasilkan air terproduksi dalam jumlah besar yang biasanya mengandung padatan, seperti korosi, kerak, bakteri, tanah liat, lilin, dan sisa minyak. Biosida digunakan untuk mengurangi jumlah sel-sel bakteri dalam air terproduksi yang diaplikasikan sebagai air injeksi ke dalam reservoir. Objek dari penelitian ini adalah untuk menguji aktivitas antibakteri dengan lima senyawa aktif biosida yang terdiri atas Glutaraldehide (Biosida-1), Aldehyde-Based and Surfactants (Biosida-2), Glutaraldehyde and Quartenary Ammonium Compounds (Biosida-3), Tetrakis Phosphonium Hydroxymethyl Sulfate (Biosida-4), and Amine Aldehide (Biosida-5) untuk mengurangi sel-sel bakteri pada pada air terproduksi. Hasil dari studi ini adalah Kelompok umum bakteri aerobic dikatagorikan sebagai kontaminan bakteri yang paling tinggi dalam air terproduksi. Bakteri yang teridentifikasi adalah Bacillus sp (2 jenis isolat) dan Pseudomonas alcaligenes. Jenis Biosida-2 dan Biosida-3 dapat mereduksi jumlah sel-sel bakteri dalam jumlah besar pada konsentrasi 200 ppm.

Kata Kunci: *biosida, air terproduksi, air injeksi, viabilitas*

ABSTRACT

Oilfield produced water with a high flow rate usually contains suspended solid, such as corrosion, scale, bacteria, clay, wax, and oil residue. Biocide is used to reduce viability of bacteria cell in produced water reused for produced water reinjection into oil reservoir. The objectives of this study is to examine anti bacteria activity of five active compound biocides i.e. Glutaraldehide (Biocide-1), Aldehyde-Based and Surfactants (Biocide-2), Glutaraldehyde, Quartenary Ammonium Compounds (Biocide-3), Tetrakis Phosphonium Hydroxymethyl Sulfate (Biocide-4), and Amine Aldehide (Biocide-5) for reduced bacteria cell in produced water in this field. Resulted in this study is general aerobic bacteria group is high contamination at the produced water reinjection. Bacteria isolates identified is *Bacillus* sp (2 types of isolates) and *Pseudomonas alcaligenes*. The type of Biocides-2 and Biocide-3 reduced the number of bacteria cells maximal at a concentration of 200 ppm.

Keyword: biocide, produced water reinjection, viability

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I. INTRODUCTION

Fluid flows from well-head consisted of a mixture of gas, oil and water. Separator is used to separate fluids into oil and gas, and the oil is transported to refinery for further processes into liquid petroleum gas (LPG), gasoline, fuel oils, petrochemical, and others; on thde other have water derived from separator is produced water. The quantity of produced water will increas at mature reservoirs. In addition to reduce the amount of produced water on the surface, the injection of produced water into the reservoir is also useful to maintain reservoir pressure, as a result the oil recovery can be achieved optimally and the oil production will be much longer.

Produced water usually contains contaminants such as suspended oil, minerals, chemical compound, solid including corrosion, scale, bacteria, wax and asphalts, and dissolved gas (Munirasu et al. 2016 and Fakharian et al. 2017). Apart of reducing water disposal at the surface produced water is also used as injection water to maintain reservoir pressure, therefore the production life time can be extended properly (Dastgheib et al. 2016,Lin et al. 2017). Injecting produced water into the reservoir is one of production technique used in oil exploitation (Manshad et al. 2017). The utilization of produced water for injection water must fulfill certain requirements of water quality standards. The important requirements include the content of total suspended solids (such as clay, silt, and sand), bacteria and their metabolites, the fraction of suspended oil, corrosion material, and scale. Generally, the specification of water quality that using as water injection consisting of <10 mg/L Total Suspended Solid (TSS) and <42 mg/L oil content (Ahmadun et al. 2009).

The use of water injection which does not eligible can be fatal to the reservoir and reduce the permeability of the rock core significantly. Some oil fields in China have been damaged by using water injection without treatment. Important parameters that need be noticed for injected water prior injection process are total suspended solid-TSS (silt, sand, bacteria and their metabolites, corrosion materials and scale) and suspended oil. Injected water should have TSS of < 10 mg/ L and oil content of < 42 mg/ L (Ahmadun et al. 2009, Fakharian et al. 2017). Injection water that does not meet the quality criteria could fatally damage the reservoir formation. Some production practices in China have damaged the reservoirs due to the use of untreated injected water (Liu et al. 2014).

Microbial problems in the oil production has a tendency increased over time (Tsesmetzis et al. 2016). At the beginning of exploitation, oil is obtained primarily due to internal pressure reservoir and only a few caoused by water content. After amount of oil in the reservoir decrease, the problem of microbes also increases. The bio corrosion and souring problems increase even after secondary production by water injection (Johnson et al. 2017). Bacteria activity can also form a crust, deposits, sludge or mud on distribution pipelines especially in areas of stagnant, wash tanks, oil and water storage tank, oil-water interface, filters, and equipment of well. Empty tank of offshore oil storage is usually filled sea water as a balance (ballast), where the sea water and the rest of the consortium of oil as a medium for bacteria growth. General aerobic bacteria and bacteria oil degrading will spend the next supply of oxygen and nutrients for suitable growing conditions for SRB. Water which is injected into the wells is often contaminated by SRB, iron bacteria and slime-forming bacteria (slime bacteria), although the water injection has been filtered with a 10 μm pore size filter is still contains a lot of bacteria that suppose to be passed as the size of bacteria cells smaller than 10 microns.

Partially, the development of bacteria and their metabolites would affect the quality of water that will be injected into reservoirs. The occurrence of bacteria and their metabolites in produced water would decrease the quality when the water is re-injected to the reservoir. Bacteria and its metabolites cause plug or scale in reservoir rocks, heat exchangers, and various filters. The problems caused by these bacteria activities produce in reduction of injection ability, low yield production, the down whole equipment damage, and reduction of efficiency in the heat exchanger. Therefore, the growth of bacteria cells in injection water should inhibited or eliminated by biocides treatment. Consequently of the produced water treatment system should be conducted in oerder to remove suspended solids material, particularly to minimize plugging (Fernando et al. 2016) and reservoir formation damage caused by bacteria cells by giving biocide (Lavania et al. 2011, Tsesmetzis et al. 2016).

Although the process of physical/mechanical (pigging) has contributed for minimizing the problem of bacteria, however the chemical water treatment using surfactants or biocides as inhibitors with right dose is a more effective way. Using of biocides as chemical inhibitor to control the activity of bacteria

in oil field water systems requires selection of the type and effective dose of biocides and compatibility to the water system at the field. The object of this study is to investigate the screening and effect of biocides on the viability of bacteria cells contained in produced water for application of water injection.

II. METHODOLOGY

The water sample used in this study was collected from the "X" Oilfield (outlet of Gathering Station). Microbiological parameter in produced water covering of total population of general aerobic bacteria group by Plate Count Method (Lee 2009) and anaerobic bacteria group, the type of SRB using Sani Check Kit (Biosan Laboratories, Inc.).

After determination of bacteria population, the next step is identification of the general aerobic bacteria is identified by biochemical analysis and isolates identification using Bergey's Manual of Determinative Bacteriology. And the next step was determination of screening biocide for produced water by RP-38 Method and Total Bacteria Population parameters.

A. Sampling Procedure

Water is sampled by sterile bottle (50 cc). Take the sample and give air space in the bottle. Close the bottle tightly and keep from cross contamination. Give sample code and other information if necessary and then put the samples in the safety box. Keep the bottle in< 4^{o}C, but don't be frozen.

B. Total Bacteria Count

The Quantitative Analyses is a microbial estimate method used to enumerate viablecell counts by diluting the microorganisms, followed by growing the diluted microorganisms inreplicate liquid medium dilution tubes. After optimal microbial growth incubation (3-5 days incubation),the positive and negative test results are based on the positive (visible turbidity) and negative (clear) replicate dilution tubes. The viability cells count in produced water is based on API RP-38 Method (America Petroleum Institute 1982).

The microorganisms to be estimated are distributed randomly and evenly separatedwithin the samples tested in the liquid dilution tubes. The microorganisms to be estimated are alsoseparated individually and are not clustered together, nor do they repel each other in the liquiddilution tubes. In addition, the utilization of optimal growth medium, incubation temperature, andincubation period is needed to allow any single viable cell to grow and become quantifiable in theliquid dilution tubes used. Primary equipment and materials used for this method are serial dilutiontubes, dilution replicate tubes, pipettes, specific growth medium and reagents (if necessary), and incubator with appropriate optimal temperature setting or on the site samples temperature. The Total Bacterial Count is useful in the estimation of low microorganism counts where particulate matter or turbidity present in the sample matrix, such as in milk, food, water, and soil (Lee 2009).

SRB determination are described as follows: (1) Prepare sample flask or any other sample container is included with SRB media. (2) The vial should be filled with a freshly collected sample that has only a minimal oil layer on its surface. (3) Grip an applicator with the alligator clip and insert applicator into liquid sample. (4) Immerse for approximately 5 seconds, withdraw, and drain excess fluid by running applicator along the inner lip of vial or any other suitable sample container. (5) Holding applicator with clip, insert into tube of agar, taking care to center it as much as possible. (6) Push the applicator into the tube until it is about even with the surface of the tube. (7) Add 2-3 drops of mineral oil into the tube, and cap the tube. (8) For best result, place tube in any location where the temperature will be close (within 5 degrees) to that of the environment from which it was taken. (9) Observe the tube regularly for results and compare to the chart (Biosan Laboratories, Inc.).

C. Identification of Bacteria

After a number of bacteria cells were determine, the further test was the identification of bacteria. The bacteria identification procedure consists of several steps, which makes monoculture isolate were purified from bacteria colonies grown in a dish containing Nutrient Agar (media for bacteria cultivation). Mono-cultures were inoculated in the media in the test tube and then observed macroscopic, microscopic, and biochemical uses Bergey's Manual.

D. Biocide Screening Test

A high number of bacteria in the water injection is one serious problem. The use of untreated water injection will cause fatal damage to the reservoir. Treatment for this problem is the addition of biocides in accordance with the optimal concentration to reduce the number of bacteria cells. Therefore it is necessary to analyze bacteria problem solving which includes parameters of Biocide Screening Test.

In this study 5 biocides derived from the commercial formulation where used Biocide-1, Biocide-2, Biocide-3, Biocide-4, and Biocide-5. All these types of biocides are soluble in water. The first step is made stock solution of biocide with a concentration of 1.000 ppm. The stock solution will diluted to a final concentration of each biocide at 25, 50, 100, and 200 ppm in sample of water injection. The stock solution can be used and stored for a week at maximum temperature of 4°C in the dark to prevent photo degradation (Liu et al. 2014).

The Biocide Screening Test procedure described are as follows: (1) Insert a 0.5 ml sample of water injection that already added 0, 25, 50, 100, and 200 ppm of biocides into 4.5 ml of sterile Nutrient Broth tube, 0 ppm is a control. (2) Each concentration of biocide diluted with the same media from 10^{-1} to 10^{-5} and then incubated at 32°C for five days. (3) Furthermore, the bacteria growth was observed every single day. Bacteria growth is positive if there is a change of media turbidity and / or the formation of white precipitate at the bottom of the test tube (visually).

III. RESULTS AND DISCUSSION

A. Total Bacteria Count

Microbiological testing conducted on samples from the "X oilfield", covering the total amount of General Aerobic Bacteria. Based on observations of baseline showed that the common aerobic population of the bacteria in produced water is classified as a high contamination, at less than <100,000 cells/ml. Especially depend on the source of the sampling point (Table 1). The produced water sampling point is part of open system that has been comes in contact with atmospheric oxygen (DO average of more than 4.5 mg/l).

Based on SRB observation (Figure 1) shows that a small number of bacterial cells to the outlet Gathering Station classified generally insignificant contamination. In microbiology addition of biocide with the correct type and optimal concentration which can effectively reduce the bacterial cells that live in the water injection. Zulkifliani and Usman (2011) reported that the general aerobic bacteria population in water injection is more dominant than the SRB. This condition can occur because the surface facilities and allows the cells of general aerobic bacteria group can grow better than groups of anaerobic bacteria.

Figure 1
Sulfate Reducing bacteria observation.

Table 1
General aerobic bacteria group from the "X" Oilfield

Time Sampling	Produced Water	Remarks
H-1	10,000-99,999	Interpretation of Quantitative Results
H-3	10,000-99,999	> 100,000 cell/ml = excessive
H-5	10,000-99,999	10,000-99,999 cell/ml = high
H-7	10,000-99,999	1,000-9,999 cell/ml = moderate
H-9	10,000-99,999	100 - 999 cell/ml = low
H-11	10,000-99,999	10 - 99 cell/ml = very low
		< 10 cell/ml = Generally Insignificant

B. Identification of Bacteria (Bacteria Type)

After determination of the number of general aerobic bacteria present in the injection water from all the sampling points, followed by purification bacteria successfully grown and then performed biochemical identification to obtain the species name (binomial system). Based on the identification of isolates carried the bacteria found in the samples of produced water has three isolates of *Bacillus* sp. (2*Bacillus*genus but maybe 2 of the same or different species) and *Pseudomonas alcaligenes* (Figure 2).

The genus *Bacillus* is a genus commonly found in water in the oil industry despite extreme environmental conditions such as high temperature, pH, low oxygen levels and high salinity. The genus *Bacillus* is rod-shaped and straight with dimensions of 0.5 to 2.5 x 1.2 to 10 μm, and often arranged in pairs and chains, with rounded or square ends. Gram staining was positive and motility of cell by peritrichous flagella. They have ability to make dormant endospores in unfavorable growth conditions. Endospores are oval or round and sometimes cylindrical and highly resistant to extreme conditions. Actually genus *Bacillus* has Aerobic or facultative anaerobes, with diversity ability to heat, pH, and salinity. Found in a variety of habitats, some species are pathogenic to vertebrates or invertebrates. The genus can be grown in the laboratory in a nutrient broth with 6.5% NaCl and temperature 42°C. It has Fermentation of carbohydrates and their derivatives such as glucose, maltose, mannitole, and xylose (Holt et al. 1994). Added by Davies & Scott (2006) that the genus *Bacillus* can survive at physical environmental conditions such as heat to a temperature of 100°C, conditions of pH 1 to pH 13, the concentration of oxygen from anoxic to saturated, stagnant flow rate until the pressure, and can live well until the salinity reached 30%.

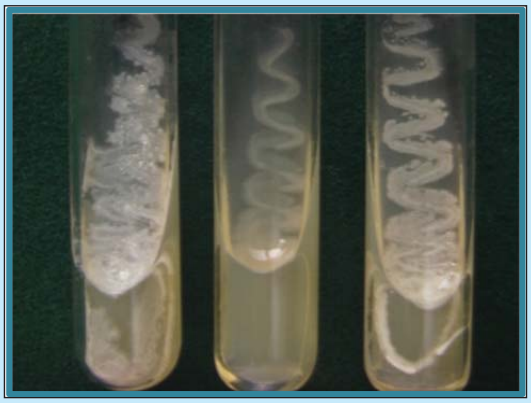

Figure 2
Bacteria Isolated from Produced Water:
(1) *Bacillus* sp., (2) *Pseudomonas alcaligenes*,
(3) *Bacillus* sp.

Members of the genus *Pseudomonas* are very general in nature and can be isolated from a variety of natural materials (widely distributed nature). Form the basis of morphological features generally straight or slightly curved but not helical rod with dimensions of 0.5-1.0 x 1.5-5.0 μm and the presence of one or more polar flagella. No spores are produced, and a negative Gram stain. The genus *Pseudomonas* is aerobic and able to use the H_2 and CO for energy resources (Matassa et al. 2014)

C. Biocide Screening Test

Based on the results of the microbiological testing lab that the number of General Aerobic Bacteria range from 10,000 to 99,999 cells/ml and were classificated of high contaminated. Problems presence of bacteria in produced water need to be addressed by the provision of appropriate biocides and the optimum concentration to be able to reduce the number of general aerobic bacteria significantly, so if used as a water injection does not cause damage to the reservoir. To solve the problem testing biocide screening using five types of biocides that are sold commercially. Types of biocides are used to contain the active ingredient, namely Glutaraldehyde (Biocide-1), Aldehyde-Based and Surfactant (Biocide-2), Glutaraldehyde and Quartenary Ammonium Compound (Biocide-3), Tetrakis Hydroxymethyl Phosphonium Sulfate (Biocide-4), and Amine Aldehyde (Biocide-5). Biocides are added to the water that comes from the Flotator Outlet each with a concentration of 25, 50, 100, 200 ppm and without the addition of biocide (control).

The biocides on laboratory screening test results obtained (Table 2 and Fig. 2) that the biocide active ingredient of Glutaraldehyde (Biocide-1) at concentrations of 25 and 50 ppm do not show the effects of the reduction in the number of bacteria because it is still the same as the treatment without biocide (control), which amounted to 10,000-99,999 cells/ml (a high contamination). Then after biocide concentration to 100 ppm level shown a decrease in the number of bacteria cells significantly into 100-999 cells/ml (a low contamination) and when the concentration increased to 200 ppm, the reduction occurred the number of cells that become increasingly significant bacteria 10-99 cells/ml (a very low contamination).

Further testing using the biocide ingredients of Aldehyde-Based Surfactant (Biocide-2) showed better results its ability to reduce the number of bacteria cells as compared to the first biocide (Biocide-1). At a concentration of 25 ppm can reduce the number of bacteria cells to be 1.000 to 9.999 cells/ml (a moderate contamination) and at a concentration of 50 ppm give effect to decrease the number of bacteria cells by 50%, ie. 100-999 cells/ml (a low contamination). If the biocide concentration was increased to 100 ppm, the number of bacteria cells decreased more significantly to 10-99 cells/ml (a very low contamination) and when the concentration was increased to 200 ppm on produced water have not found that bacteria cells live (generally insignificant).

At the next step is the addition of biocide treatment with the active ingredient of Glutaraldehyde and Quartenary Ammonium Compound (Biocide-3). The addition of these biocides at concentrations of 25 ppm has been a decline of control, ie. 1,000 to 99,000 cells/ml (a moderate contamination) but at concentrations of 50 and 100 ppm showed the same result, namely a 100-999 cells/ml (a low contamination). Then the concentration of 200 ppm biocide showed similar results with biocide active ingredient of Aldehyde-Based and Surfactant (Biocide-2), which is no longer found bacteria cells that live (generally insignificant).

Giving the biocide active ingredient of Tetrakis Hydroxymethyl Phosphonium Sulfate (Biocide-4), at

Table 2
Biocide screening test with injection water

Biocide	Concentration (ppm)	Test Tube at Dilution					Bacteria Population (cell/ml)
		10^1	10^2	10^3	10^4	10^5	
Biocide-1	25	+	+	+	+	-	10,000 – 99,999 (high)
(Glutaraldehyde)	50	+	+	+	+	-	10,000-99,999 (high)
	100	+	+	-	-	-	100 - 999 (low)
	200	+	-	-	-	-	10 - 99 (very low)
Biocide-2	25	+	+	+	-	-	1,000 – 9,999 (moderate)
(Aldehyde-Based and	50	+	+	-	-	-	100 - 999 (low)
Surfactant)	100	+	-	-	-	-	10 - 99 (very low)
	200	-	-	-	-	-	< 10 (Generally Insignificant)
Biocide-3	25	+	+	+	-	-	1,000 – 9,999 (moderate)
(Glutaraldehyde and	50	+	+	-	-	-	100 - 999 (low)
QAC)	100	+	+	-	-	-	100 - 999 (low)
	200	-	-	-	-	-	< 10 (Generally Insignificant)
Biocide-4	25	+	+	+	+	-	10,000 – 99,999 (high)
(Tetrakis Hydroxymethyl	50	+	+	+	+	-	10,000 – 99,999 (high)
Phosphonium Sulphate)	100	+	+	-	-	-	100 - 999 (low)
	200	+	-	-	-	-	10 - 99 (very low)
Biocide-5	25	+	+	+	+	-	10,000 – 99,999 (high)
(Amine Aldehyde)	50	+	+	+	-	-	1,000 – 9,999 (moderate)
	100	+	+	-	-	-	100 - 999 (low)
	200	+	-	-	-	-	10 - 99 (very low)
CONTROL	0	+	+	+	+	-	10,000 – 99,999 (high)

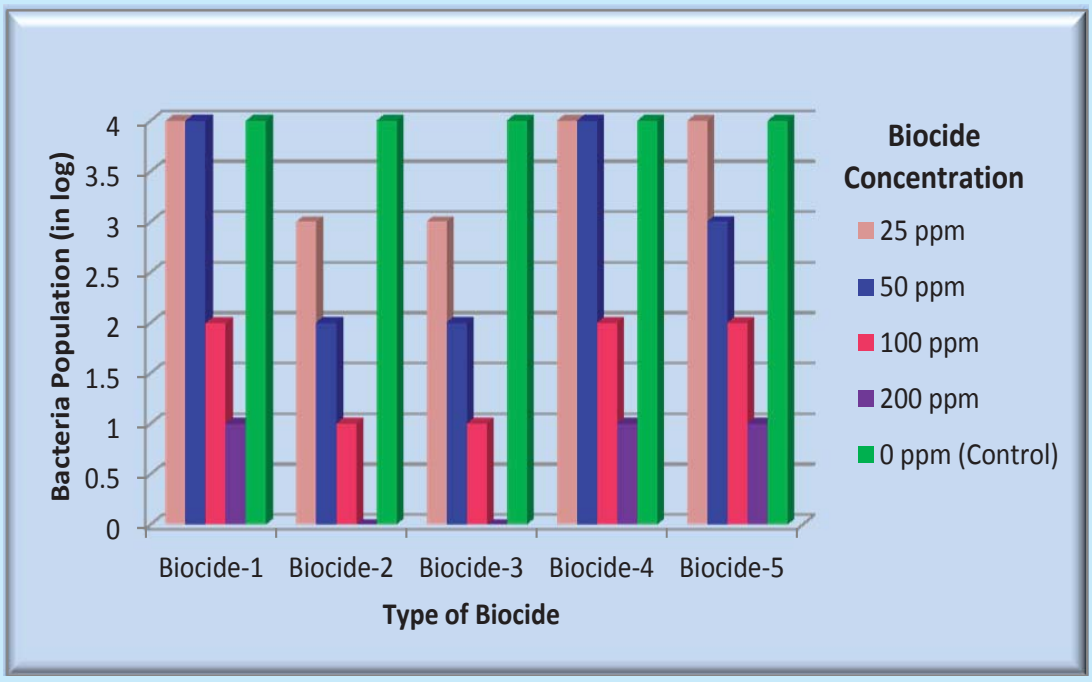

Figure 3
Biocide screening test with produced water.

concentrations of 25 and 50 ppm showed similar results with made active biocide Glutaraldehyde (Biocide-1), which have not shown an influence on the reduction of the number of bacteria cells because it is still the same as the treatment without biocide (control), which amounted to 10,000 to 99,999 cells/ml (high contamination). The effect of this biocide seen at 100 ppm concentration which is 100-999 cells/ml (low contamination) and when the concentration was increased to 200 ppm, some bacteria still can survive, although the number is small, ie 10-99 (very low contamination).

At a concentration of 25 ppm with the active ingredient of Aldehydes (Biocide-5) the number of bacteria cells is the same as the control (10,000 to 99,999 cells / ml). After increasing the concentration to 50 ppm resulted in a decline in the number of bacteria cells to be 1,000 to 9,999 cells/ml (moderate contamination). And the number of bacteria cells continued to decline in the concentration of biocide 100 and 200 ppm, which respectively are 100-999 cells/ml (low contamination) and 10-99 cells/ml (very low contamination).

Overall testing five types of biocides provide positive effect on the reduction of the number of bacteria cells that live in injection water. Bacterial cells is lysis by exposure to biocide, Zulkifliani and Nita (2011) reported that the change of morphology of the bacterial cells after biocides addition. The deformation of bacteria cells due to biocides addition into the injection water.

Based on the test results can be reported that the order type of biocide efficacy in reducing the bacteria cells are Biocide-2 (Aldehyde-Based and Surfactant), Biocide-3 (Glutaraldehyde and Quartenary Ammonium Compound), and Biocide-4 (Tetrakis Hydroxymethyl Phosphonium Sulphate). Zulkifliani and Usman (2011) reported that the performance of biocides containing the active ingredient ammonium compounds can reduce cell viability of bacteria in the water injection significantly.

IV. CONCLUSIONS

General aerobic bacteria group contaminating produced water. They belonged to *Bacillus* sp (2 types of isolates) and *Pseudomonas alcaligenes*.

Based on the biocide screening test that the Biocides-2 (Aldehyde-Based and Surfactants) and Biocide-3 (Glutaraldehyde and Quartenary Ammonium Compounds) at the concentration of 200 ppm has highest bacteria inhibitor.

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