REDUCTION OF BACTERIA CELLS VIABILITY IN INJECTION WATER BY USING AMMONIUM CHLORIDE

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ABSTRACT

Ammonium chloride is one of the biocides used to reduce viability of bacterial cells in produced water from oil wells that will be applied as injection water. The advantage of this biocide is having high epication penetrate the cell wall, causing death of bacteria cells (bacteriocidal). Tests for antibacterial activity was done by using the API RP-38 method by adding ammonium chloride into the produced water to obtain the final concentrations of 4, 8, 16, 32, 64, and 128 mg/L. Laboratory test results showed that giving ammonium chloride at a concentration of 64 mg/L can significantly reduced the viability of bacterial cells, including groups of aerobic and anaerobic bacteria.

Keywords*: biocide, injection water, viability, antibacterial, bacteriocidal, bacteria cell*

I. INTRODUCTION

Production of oil and gas is usually accompanied by the production of water. As exploited reservoirs mature the quantity of water produced increases. The produced water for water injection is one of the options taken by the oil and gas companies to disposes of the produced water. In addition to reduce the amount of produced water on the surface, the injection of produce water into the reservoir is also useful to maintain reservoir pressure, so that the oil recovery can be achieved optimally and oil production may take longer (Ekin *et al*. 2007).

Produced water as injection water must comply with certain requirements of water quality. Among the water quality that needs be seriously paid attention to is reducing the content of total suspended solids, such as clay, silt, sand, bacteria, their metabolites, the fraction of suspended oil, corrosion material, and scale (Lu *et al*. 2009).

Bacteria and their metabolites is one cause of damage to the equipments used in the petroleum industry, which can lead to blockage or to the process of scaling on the reservoir rocks, heat exchanger, and various filters. Problems caused by these bacteria resulted in decreased injectivity, low yield production, damage of downhole equipment, and decreased efficiency in the heat exchanger (HE). The bacteria from the type of Sulphate Reducing Bacteria (SRB) also increased the problem of corrosion in the well equipments and facilities that are made of metal surfaces. In addition, the SRB also produce hydrogen sulfide gas that causes a bad odor and damage the health (health hazard). In the production operations, it can reduce the efficiency of oil-water separation and causes the black powder in the gas transmission network (Davies and Scott, 2006).

Water produced from the oil wells before used as injection water, especially for waterflooding operation requires a water system treatment, because the produced water is often contaminated by SRB, iron bacteria, and slime bacteria, even though the injected water has been filtered by 10 μm filter pore size. A lot of bacteria can still pass through the filter since their size are less than 10 μm.

Chemical control of microbes in an oil field water system mainly depends on the chemical agent used. Although the process of physical/mechanical

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(such as pigging) has helped minimizing the problem of bacteria but chemical water treatment using surfactants or biocides as inhibitors with right dose is a more effective way. The use 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. Therefore, the growth of bacteria cells in injection water needs to be inhibited or eliminated by special treatment, namely by providing biocides. To overcome the problem of bacteria and their metabolites, the produced water need to be treated before used as injection fluid. The purpose of this study is to investigate the effect of biocides with the active ingredient of ammonium chloride on the viability of bacterial cells contained in injection water.

II. MATERIALS AND METHODS

The study of the viability reduction of bacteria cells in injection water, biocide with the active ingredient of ammonium chloride and the injection water from the oil field in South Sumatra were used. The stages of this study consist of: (a) microbiological analysis of water injection, (b) antibacterial activity in the injected water media, and (c) Killing Rate testing.

A. Microbiological Analysis

Microbiology observation in injection water covering a total population of aerobic bacteria group by Plate Count Method (Lee, 2009) and anaerobic bacteria group, the type of SRB using Sanicheck kit (Biosan Laboratories, Inc.) and continued with the identification of aerobic bacteria isolates in macroscopic, microscopic, and biochemical analysis with API test kit and identification guide by Bergeys Manual (Holt *et al*., 1994).

B. Antibacterial Activity Test

Antibacterial test in injection water is based on API RP-38 method (American Petroleum Institute, 1982). This method is used to determine the efficacy of biocides tested with a certain concentration of bacteria in injection water. The procedure is to insert the injection of 0.5 mL water sample into a tube containing 4.5 mL of Nutrient Broth sterile is already containing ammonium chloride with the final concentration of 4, 8, 16, 32, 64, and 128 mg/L and 0 mg/L for the control tube respectively. All tubes were incubated at 33 ^{o}C for 24 hours and then each dilution tube made up to 10^{-5} and observed until the fifth day. If visually there is changes of turbidity and a white sediment in the bottom of test tube, it means a positive growth of bacteria and if no such change is seen is, it means no bacteria growth.

Killing Rate Testing is a test to determine the speed of the lethal activity of biocides against bacteria cells (bacteriocidal impact) contained in injection water during a certain period of time and concentration. Killing Rate Testing conducted as follows. Bacteria cells are suspended in an isotonic solution (Gram Negative or Gram Positive inoculating fluid) to contain 10^{6} cells/mL. 1 mL suspension is added into vials containing 4 mL of ammonium chloride and the selected concentration dilution in sterile distilled water. Take 0.5 mL at minute 5, 30, 60, 120, and 180, then put in 4.5 mL of Nutrient Broth medium, then diluted to 10^{-5} (Wang *et al*. 2009).

III. RESULTS AND DISCUSSION

A. Microbiological Analysis

Based on the microbiology observations, it was found two groups of bacteria in the injection water that can live. They are the aerobic bacteria with a population of 3.2 x 10^{5} cells/mL and anaerobic bacteria (the SRB) with a total population of 10^{2} cells/mL. Observation of bacteria cell population showed that the total of aerobic bacteria group are more dominant than the anaerobic group. This condition can occur because of the water has been on outside the reservoir formation or at the facilities located on the

Figure 1
Analysis of SRB bacteria from oil field water.

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Table 1
Results of the aerob bacteria identification in injection water

No.	Biochemical Test	*B. subtilis*	*B. cereus*	*B. brevis*	*B. sphaericus*	*Bacillus* sp.
1	Shape of cell	rod	rod	rod	rod	rod
2	Gram Stain	+	+	+	+	+
3	Spora Stain	+	+	+	+	+
4	Katalase	+	+	+	+	+
5	Starch Hydrolysis	+	+	-	+	+
6	NaCl 7%	+	+	-	+	+
7	Nitrate	+	+	-	-	+
8	Lisine	-	-	-	-	-
9	Ornitine	-	-	-	-	-
10	H_2S	-	-	-	-	-
11	Glucose	+	+	-	-	-
12	Manitole	+	-	-	-	-
13	Xylose	-	-	-	-	-
14	ONPG	+	+	+	-	-
15	Indole	-	-	-	-	-
16	Urease	+	-	-	-	+
17	VP	+	+	-	-	+
18	Sitrate	+	+	+	-	+
19	TDA	-	-	-	-	-
20	Gelatine	+	+	+	+	+
21	Malonate	-	-	-	-	-
22	Inositole	-	-	-	-	-
23	Sorbitole	-	-	-	-	+
24	Ramnose	-	-	-	-	-
25	Sucrose	+	+	-	-	+
26	Lactose	-	-	-	-	-
27	Arabinose	-	-	-	-	-
28	Adonitole	-	-	-	-	-
29	Rafinose	-	-	-	-	-
30	Salicine	+	-	-	-	-
31	Arginine	+	+	+	+	+

surface and has been contacts with oxygen on the surface, allowing the cells of an aerobic bacteria group to grow better than the anaerobic bacteria group. In comparison, the the total population of anaerobic bacteria in the formation water from the same oil field is around 10^4 cells/mL since the water is still in the reservoir under anaerobic conditions. (Figure 1).

Although some type of aerobic bacteria groups symbiosis by providing an important contribution to the growth of anaerobic bacteria groups. As reported by Davies and Scott (2006) that the metabolites such as slime from several genera of aerobic bacteria will be a medium for the groups of anaerobic bacteria to grow and expand when water is re-injected into the reservoir that under anaerobic condition.

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Based on identification conducted to isolate aerob bacteria found in the injection water and fitted with the Bergeys Manual, it was obtained five species, all belonging to the genera of *Bacillus*. They are *Bacillus subtilis*, *Bacillus cereus*, *Bacillus brevis*, *Bacillus sphaericus*, and *Bacillus* sp. as depicted in Table 1.

The genera of *Bacillus* is a commonly found in the formation water in oil environment although with the extreme environmental conditions such as temperature, pH, oxygen levels, and extreme salinity. Holt *et al*. (1994) mentioned that the genera of *Bacillus* have a characteristic rod-shaped with a length ranging from 0.5 to 2.5 μm and a diameter ranging from 1.2 to 10 μm. Generally included in the group of Gram negative bacteria, moving with flagella, has a spora, resistant to heat, and can grow in aerobic or anaerobic facultative conditions. Added by Davies and Scott (2006) that the physical environmental conditions such as heat spectrum that reaches 100°C, pH value of 1 to 13, oxygen concentrations ranging from anoxic conditions (without oxygen) to the water with saturated oxygen, flow rate of stagnant to the pressure pump, and salinity up to 30% are the factors that limit bacteria growth universally. But certain genera such as *Pseudomonas*, *Bacillus*, *Aerobacter*, and *Flavobacterium*, can still grow and develop properly in these conditions if there is water.

B. Antibacteria Activity Test

The results of antibacteria test contain with active ingredients of ammonium chloride revealed that these biocides have a significant influence on the decrease in cell viability of bacteria of the genus *Bacillus* (Gram positive). According to Cords *et al.* (2005) the active ingredient of ammonium chloride included in the synthetic compounds with ability as an antibacteria compound in the group of Gram positive and Gram negative. Provision of ammonium chloride at a concentration of 4, 8, 16, and 128 mg/L does not have a significant influence on the decrease in cell viability of aerobic bacteria group. But at a concentration of 64 mg/L there is indication that the reduction of viability bacteria cells occurred from of 10^5 cells/mL (control or without biocide) to ≤ 10 cells/mL (see Table 2) and there is no change of turbidity or sediment formed on the bottom of the test tube (Figure 2).

Table 2
Antibacterial test with ingredient ammonium chloride against the bacteria contained in water injection

Treatment Code	Concentration (mg/L)	Tube Dilution Test at						MPP (cell/mL)
		0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	
B	128	+	-	-	-	-	-	01-Okt
B	64	+	-	-	-	-	-	01-Okt
B	32	+	+	+	-	-	-	10^2 - 10^3
B	16	+	+	+	+	-	-	10^3 - 10^4
B	8	+	+	+	+	-	-	10^3 - 10^4
B	4	+	+	+	+	+	-	10^4 - 10^5
Kontrol	0	+	+	+	+	+	-	10^4 - 10^5

Description:
MPP = Most Probable Population
\+ = Medium is cloudy or white sediment in bottom of test tube
\- = Media remained clear and there was no white sediment

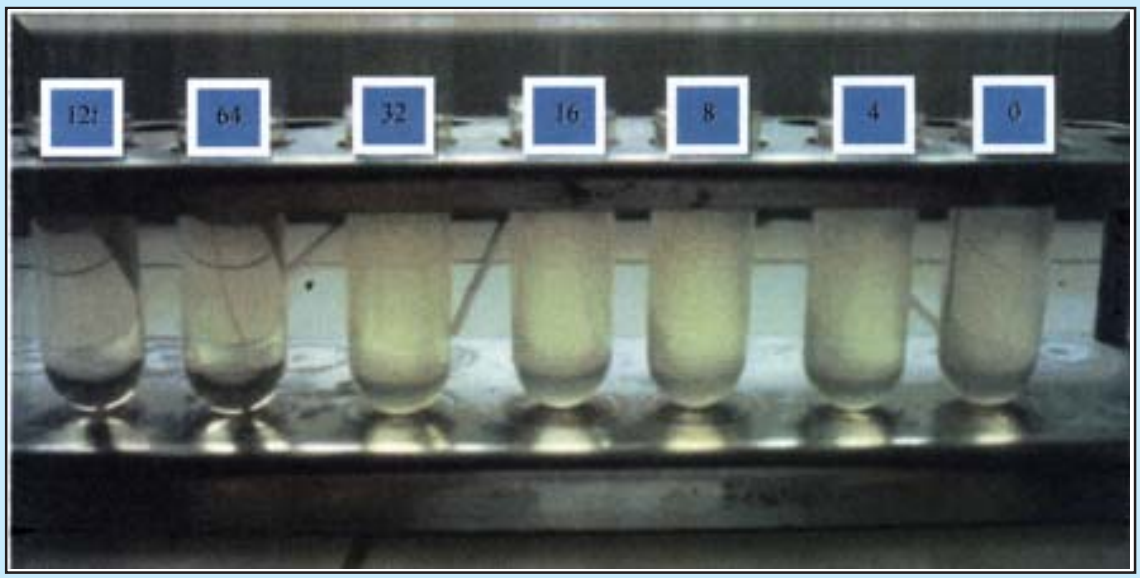

Figure 2
Effect of ammonium chloride on the viability of bacteria cell for five days incubation at a temperature of 33°C

It can be seen in Figure 2 that test tube with ammonium chloride at concentration of 128 and 64 mg/L is still clear, the growth of bacteria cells is not significant (≤ 10 cells/mL). Whereas at concentrations 32, 16, 8, 4 mg L, and control (0 mg/L) the test media becomes turbid, as an indicator of the growth of bacteria cells in the test media. The results of this test illustrates that administration of ammonium chloride at a concentration of 128 and 64 mg/L give lethal effect to bacteria cells contained in the injection water (bacteriocidal impact). On the other hand, physicochemical analysis results showed that the water injection tends to alkaline pH of 8.54. This condition can stimulate the performance of biocides that contain active ingredient of ammonium chloride to be at a maximum as an antibacteria compound that can reduce the viability of bacteria cells in injection water significantly. Reported by Davies and Scott (2006) that the antibacteria compound of this type of ammonium chloride has the maximum efficiency at an alkaline pH and is likely resistant to acidic condition.

Based on the results of antibacteria activity test, the effective concentrations of biocide to reduce the viability of bacteria cells are 128 and 64 mg/L. But in terms of economy point of view and for further testing as well as field applications the concentration used is 64 mg/L. The selected concentrations were used for further testing, such as determination of SRB and Killing Rate Testing. Figures 3 and 4 reveal the test results.

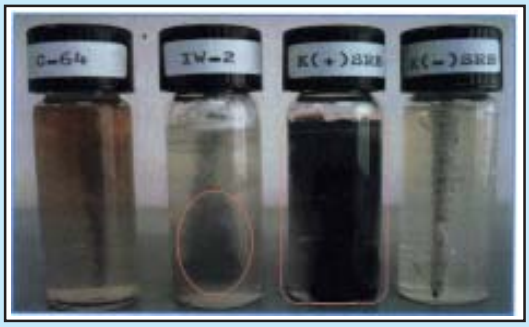

Figure 3
Analysis of SRB using ammonium chloride at concentration of 64 mg/L

Figure 3 shows that by adding of ammonium chloride at a concentration of 64 mg/L there is reduced viability of bacteria SRB from 10^2 cells/mL (code on IW-2) to <10 cells/mL (code on C-64). This indicates

that addition of the biocide with active ingredients of ammonium chloride reduced effectively the viability of aerobic bacteria cell in injection water as well as inhibited effectively the growth of anaerobic bacteria (SRB) which can caused damage the facilities in the field (corrosion) and produce a bad odor (souring). Sayegh *et al.* (2001) reported that the anti bacteria compounds such as ammonium chloride, amine etoxilate, alkylpyridium salts, and isopropanol in an appropriate concentration are recommended for use as a corrosion inhibitor in water treatment.

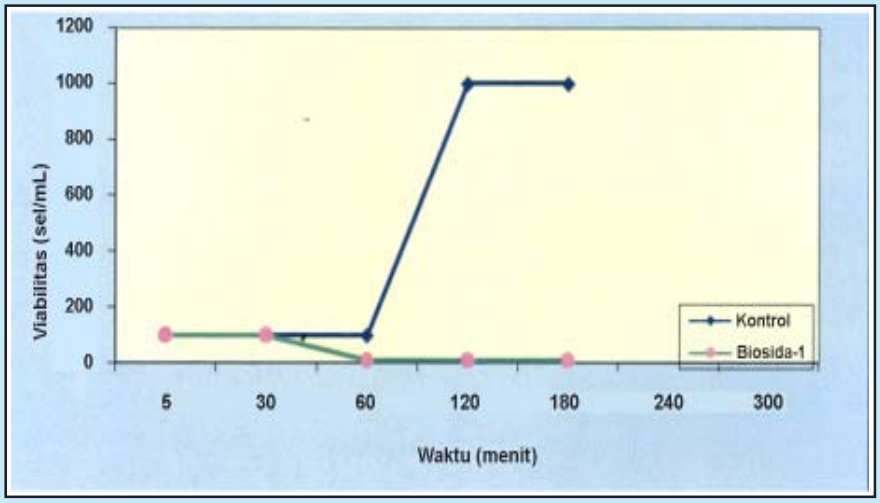

Figure 4
Killing Rate Testing using ammonium chloride at concentration of 64 mg/L in water injection

After analysis of antibacteria activity, it was obtained that biocide concentrations of 64 mg/L is effective to reduce the viability of bacteria cells. The concentration was then used in the Killing Rate Testing aimed to determine the speed of kill (bacteriocidal impact) to the population of bacteria in the injection water during the period of time (Figure 4).

Killing Rate Testing results as presented in Figure 4, showed that the antibacteria activity with the active ingredient of ammonium chloride in the 5th and the 30th minutes have not seen any lethal effect (bacteriocidal impact) to bacteria cells or still the same as in the control treatment. After the 60th minutes, the antibacterial activity started to cause the reduction of bacteria cell viability of 10^2 cells/mL to <10 cells/mL. The antibacterial activity remains effective until 120th and the 180th minutes. In the controls, the number of population increased according to the accretion time of incubation, i.e. after the 60th minutes. This condition indicates that the effect of ammonium chloride was effective as an antibacteria (bacteriocidal impact) starting at 30 minutes after the contact of ammonium chloride with bacteria cells in the injection water. Killing Rate Testing results can be used as a technical reference for the application of biocides with the active ingredient of ammonium chloride in the field, so that the use of biocides can effectively reduce the viability from of bacteria cells in injection water and efficient its economic point of view.

IV. CONCLUSIONS AND SUGGESTIONS

A. Conclusions

1. Antibacteria activity with the active ingredient of ammonium chloride at a concentration of 64 mg/L showed more effective in reducing bacteria cells viability in injection water.
2. Provision of biocides in injection water had a significant influence on decreased of aerobic group bacteria cells viability from100.000 cells/mL to ≤ 10 cells/mL and anaerobic bacteria groups from 10^2 of SRB cells to <10 cells/mL.
3. Lethal effects of biocides (bacteriocidal impact) began to appear after 60 minutes of contact between the biocide and bacteria cells.

B. Suggestion

Careful screening test is needed before using biocides for injection water due to the fact that oil fields have a specific characteristic.

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