THE EFFECT OF BIOCIDES ADDITION AGAINST MORPHOLOGY AND SIZE DISTRIBUTION OF BACTERIA CELLS IN INJECTION WATER

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ABSTRACT

Produced water is the water produced along with oil and gas. Produced water contains small to large quantities of suspended solids, such as corrosion material, scale, clay, wax, oil residues, and also bacteria and their metabolites. Produced water which is re-injected into the reservoir without having proper water treatment can lead to rock plugging and reservoir damage. One of the causes of reservoir damage is the occurrence of bacteria cells and their metabolites. Biocides are used to reduce the viability of bacteria cells in produced water to be applied as water injection. The aim of this study is to understand the effect of biocides on morphology and the size distribution of bacteria cells in injected water. Observations with scanning electron microscope (SEM) and laboratory tests showed that provision of biocides can cause damage of cytoplasmic membrane on bacteria cells or cells lyses and significantly reduced size distribution of bacteria cells.

Keywords: *biocide, injected water, morphology, size distribution, bacteria cells*

I. INTRODUCTION

Fluid that flows from well-head consisted of a mixture of gas, oil and water. Separator is used to separate the fluids into oil and gas, and the oil is transported to refinery for further processing into liquefied petroleum gas (LPG), gasoline, fuel oils, petrochemical, and others; whereas water derived from the separator, called as produced water is treated and utilized prior to disposal. Produced water quantities continue to increase as the oil and gas fields reach maturity. As oil and gas production in progresses in time, the produced water tends to significantly increase, while oil and gas production decreases.

Produced water usually contains contaminants such as suspended oil, minerals, chemical compound, solid including corrosion, scale, bacteria, wax and asphalts, and dissolved gas (Hansen and Davies, 1994). Apart from going to water disposal at the surface produced water is also used as injection water to to maintain reservoir pressure, and therefore extends production life time (Ekins *et al*., 2007). Injecting produced water into the reservoir is one of production technique used in oil exploitation (Gillette, 2008).

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 (Bader, 2007). 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 (Lu *et al.,* 2009).

Partially, the development of bacteria and their metabolites would affect the quality of water that will be injected into reservoirs. Accumulatively, the occurrence of bacteria and their metabolites in produced water would decrease the quality when the water is

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re-injected into the reservoir. Therefore, the development of bacteria and their metabolites should be minimized by adding antimicrobial compound or biocides. The objective of the study is to understand the effect of biocides addition against morphology and size distribution of bacteria cells in injection water.

II. MATERIAL AND METHODS

Materials used in this study include injection water taken from an oilfield in South Sumatra. The injection water was filtered using coarsed filter paper to clean it from dirt, then by using vacuum pump it was flowed through sterile cellulose membrane with pore size of 0.45 µm. The filtered injection water is put in a one liter bottle prior to laboratory test. The change of morphology of bacterial cells in the injection water was observed using scanning electron microscope (SEM), whereas the size distribution of bacteria cells was measured by particle size analyser.

A. Morphology of bacterial cells analysis

Bacterial cells morphology alteration was observed using SEM (JSM-639OLA), both before and after biocide addition. Preparation for bacterial cells was done prior to SEM examination. One hundred (100) mL of injected water was added with biocides with certain concentration and put into vacuum tube by flowing it through the sterile filter paper with pore size of 0.11 µm. After the injection water had all been filtered the membrane filter was taken out from tube and dried by putting it into Petri dish for 24 hours. The dried membrane filter was cut into circular pattern with diameter of 0.5 cm. The circular paper was then glued onto cylinder SEM sample holder of 1 cm in diameter and 1 cm in height. The samples were then dehydrated and thin-coated with gold for about 100 seconds before being analysed using SEM.

B. Bacterial cells size distribution analysis

Size distribution of bacterial cells is determined using *particle counter analyses* (Sysmex CDA-500) by putting 100 mL of injected water into the cell pack, which was then put into the sample chamber to be analysed. The output of the analysis includes bacterial cells distribution with 10%, 50%, and 90% spreads, and also the amounts and sizes of bacterial cells contained in the analysed injected water.

III. RESULTS AND DISCUSSION

A. The change of bacterial cells morphology

The effect of biocides addition against bacterial cells morphology were observed using SEM as depicted in Figure 1.

Figure 1 shows the change of morphology of the bacterial cells after biocides addition as observed under SEM examination with 300x magnifications (B-1). It shows that the bacterial cells have deformed from their original shapes (stem). A-1 is the original shape of the bacterial cells, appearing as smooth stems; whereas B-2 clearly shows the deformation of bacterial cells due to biocides addition into the injection water (SEM magnification 4500x). Bacterial cells shrunk and become smaller or break into various portions. A-2 is a higher magnification SEM view of bacterial cells with no biocides addition showing no morphology deformation or separation.

The bacterial cells deformation is mainly caused by the biocides compound characteristic that destroyed wall components of cytoplasm membrane including lipid and protein. These two components are directly affected by the antibacterial compound treatment causing the liquid inside the cytoplasm such as potassium (K^+), anorganic phosphate (Pi), amino acid and larger molecules such as DNA and RNA that have been removed from the bacterial cells (lyses cells). This condition causes the bacterial cells to become fragile and damaged, and finally deceased due to the loss of the main supporting components of cells growths. Russel (2005) reported that various antibacterial compound such as ammonium-chloride, phenolic, organic acid, and ester may cause damage of cytoplasm membrane or morphologically may cause presence of lyses on bacterial cells.

Other components may also be affected by antimicrobial compound activity depending on the type of microbials and the active compound content in biocides. Aside from the cell wall and cytoplasm membrane, the function and protein structures (amino acids), DNA and RNA may also be easily targeted by biocides. Similarly, spore is also directly affected by biocides, although spore is more resistant due to its membrane, which is not easily penetrated by the antibacterial compounds (Russell, 2005).

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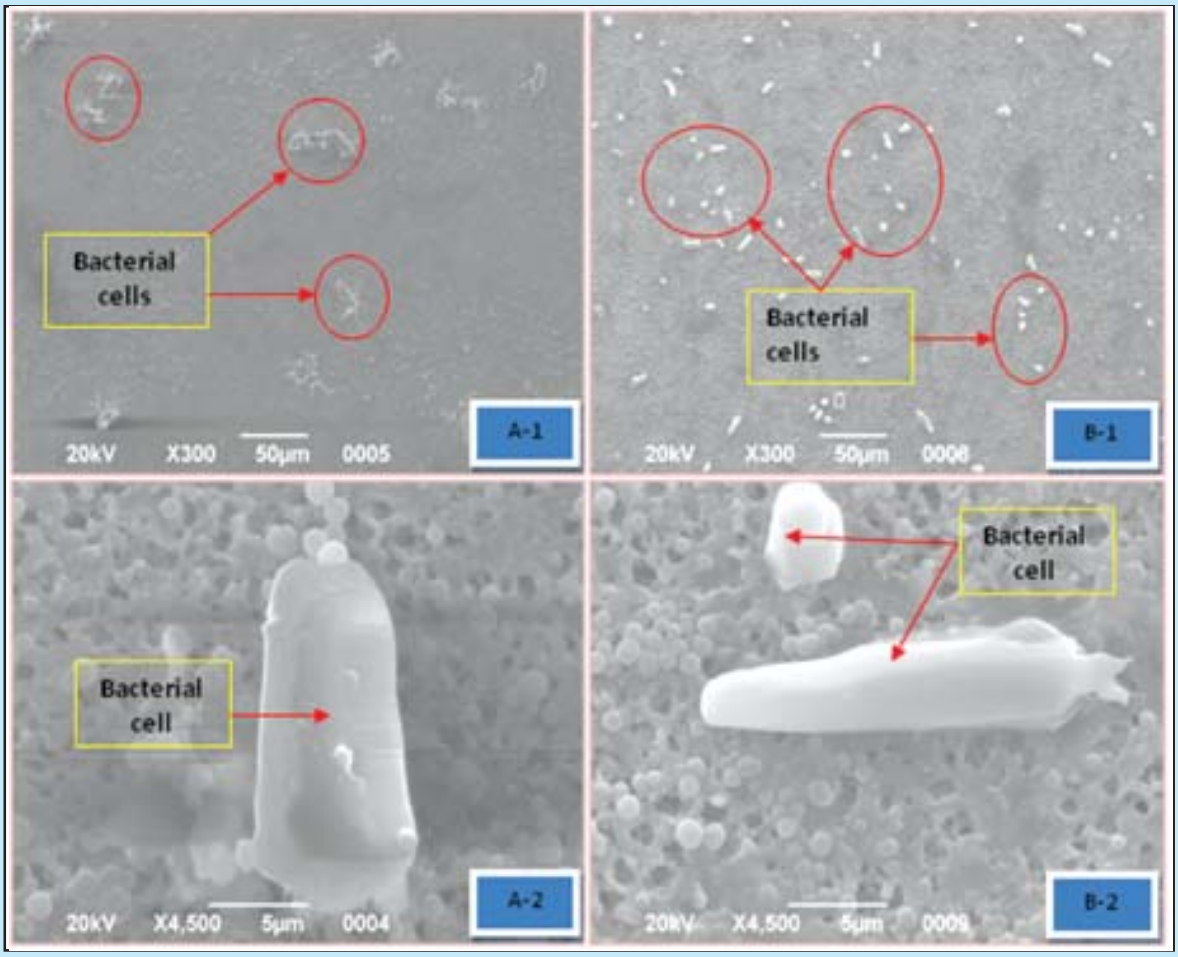

Figure 1
The change of bacterial cells morphology with no biocide addition (A-1 and A-2) and with biocide addition (B-1 and B-2)

B. Anti-bacterial cell sizes distribution analysis

Figure 2 shows injection water with no biocides addition (control) and containing bacterial cells of about 858 pcs/ mL. After biocides addition the amount of bacterial cells increased up to 1,565 pcs/ mL. This increment harmed and ruined the bacterial cells into smaller pieces through the antibacterial mechanism. The results are synergy with the morphology observation done by SEM (refer to Figure 1, B-1).

Bacterial cells size distribution analysis with diameter of ranging from 3.01 µm to 12 µm and 10%, 50%, and 90% spreads has reinforced the indication that biocides addition reduces diameter of bacterial cells. Significant shifting values occur between no biocides addition (control) and with biocides addition is 4.32 µm to 4.01 µm for 10% spread; 5.24 µm to 4.65 µm for 50% spread, and 9.17 µm to 7.16 µm for 90% spread. The diameters of bacteria cells have also decreased from 6.00 µm (control) to 5.18 µm (after biocide adding). Bacterial cells size distribution has shown that bacterial cells in injected water has been deformed significantly including their shapes, sizes and quantities under biocides addition.

Apart from the bacterial cells that has become viable, injection water also contains organic compound produced from bacterial cells metabolism, appearing as extraceluller that has negative effect on injection water quality as indicated by increase in total dissolved solid. Water with such quality when it is re-

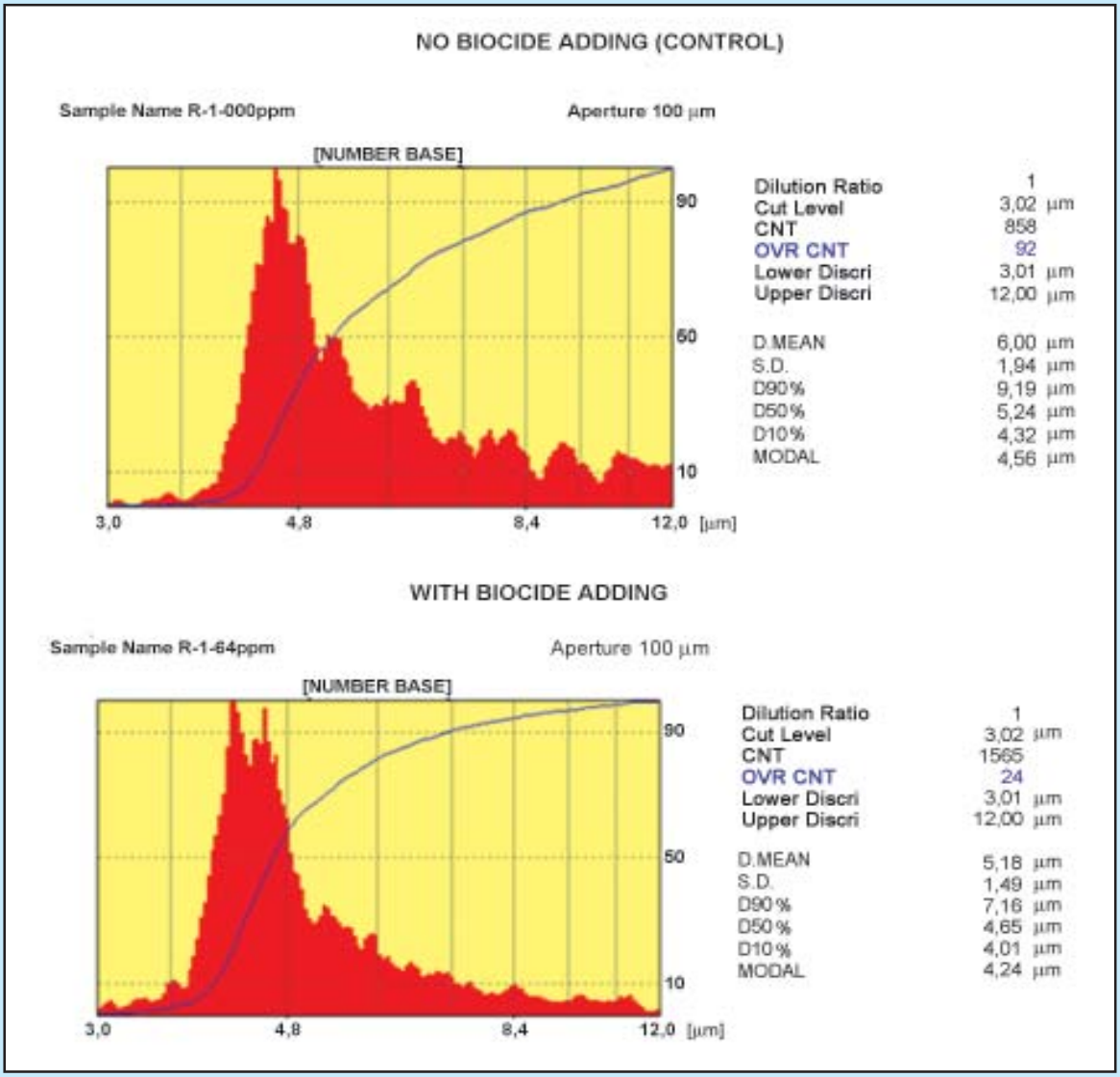

Figure 2
Bacterial cells size distribution in injected water with no biocides addition (control) and with biocides addition

injected into reservoir could seriously damage the reservoir rocks. Visual observation shows that extracellular produced from bacterial cells using injection water media is thin, elastic and apperas in bright color (Figure 3). Thullner (2009) reported that the growths of microbes in porous media or rocks would produce microbial biomass (viable cells and *extracellular polymeric substances* or EPS).

Bacterial cell populations and their products (EPS) will increase biomass aggregate stability and if accumulated in a long period of time could cause plugging of rock's pore-throat. The addition of biocides in in-

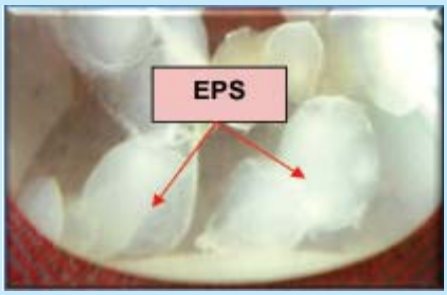

Figure 3
The appearance of extracellular polymeric substances (EPS)

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jection water treatment, therefore, is strongly needed to increase the quality of water by reducing the bacteria cells viability and their products.

IV. CONCLUSIONS AND RECOMMENDATION

The addition of biocides in injection water significantly damage morphology and also reduce the viability of the bacterial cells (lyses cells) effectively. The diameters of bacterial cells decrease in all spreads (10%, 50% and 90%) with average decrease from 6.00 µm (control) to 5.18 µm (after biocide addition). Further study is needed to understand the effect of bacterial cells and their products in relation with bioplugging and reservoir permeability.

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