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Effect of Sodium Dodecyl Sulfate on the growth of sulfate -reducing bacteria isolated from water associated with oil production in the North Rumaila field, Basra Governorate – Iraq

Asaad F. Hamzah,[1] Wijdan H. Al-Tamimi, [2] Ahmed Abd-burghali[2]

[1]Technical institute of Basrah , Southern Technical University, Basrah, Iraq
[2]Departments of Biology, Science Collage, University of Basrah, Basrah, Iraq

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Corresponding author:
Asaad Faraj Hamzah, Ph. D
Technical institute of Basrah , Southern Technical University, Basrah, Iraq
E-mail: asaad.faraj@stu.edu.iq
Mobile: 0021891510281

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ABSTRACT

The current study included a study of the effect of chemical compounds as biocides on the effectiveness of sulfate -reducing bacteria that cause oil acidity in reservoirs. Samples of injection water used in extracting crude oil from the North Ramliyah oil wells were collected, and the number of bacteria present in that water was calculated. The effectiveness of Sodium Dodesyl Sulphate (SDS) on dormant bacteria by preparing active cultures and on wandering bacteria was used by placing wrought iron samples in active cultures for a week to form biofilms. The results showed a clear effectiveness of SDS on the growth of bacteria. The inhibitory concentration reached 30 parts per million (ppm) towards bacteria floating in the medium, while the inhibitory concentration reached 100 ppm Mtowards bacteria sheltered in the biological membrane.

Keywords:
Sulfate-reducing bacteria, Sodium Dodesyl Sulphate, Oil acidity, Petroleum microbiology.

1. Introduction

The scientist Bastin et al ,1926 considered the first to isolate sulfate -reducing bacteria (SRB) from oil production water (Ollivier, 2007). However, the use of diagnostic methods based on 16SrRNA revealed a great diversity of photosynthetic microorganisms in the complex ecosystem of oil reservoirs (Prajapat et al., 2019; Tiburcio et al., 2021).

For the purpose of improving oil production, gas, chemicals, or water are usually pumped into the wells for the purpose of increasing the pressure in the reservoirs and the oil rushing toward the production wells (Sen, 2008). The microbial communities present in the injection water contains different types of microorganisms that will enter oil reservoirs and modify the existing microbial communities (Ren et al., 2015; Pannekens et al., 2019). Sulfate -reducing bacteria (SRB) are considered one of the most dangerous types of bacteria found in oil reservoirs. These bacteria produce large quantities of hydrogen sulfide gas (H_2S) as a result of their metabolic activities which are driven by the consumption of organic sources such as lactate, acetate, fumarate benzoate, fatty acids, and others (Negm et al., 2022). Therefore, they cause many problems in Industrial facilities, especially petroleum ones, include microbial.

Corrosion occurs in injection and production wells, equipment handles, water pumping facilities, and crude oil transportation pipelines (Al-Abbas et al., 2013) and occurs as a result of cathodic depolarization through bacterial consumption of the hydrogen surrounding the metal surface or the emergence of acid differentiation cells (Yemashova et al., 2007; Kakooei et al., 2012; Tawfik et al., 2023). Liu et al. (2022) indicated that SRB is responsible for 80% of corrosion failures of operating

equipment in oil fields.

Sulfate -reducing bacteria cause the acidity of oil reservoirs due to their production of hydrogen sulfide gas, H_2S (Yang et al., 2014; Gao and Fan, 2023). This gas is formed as a result of the metabolic activities resulting from the consumption of these bacteria or aliphatic hydrocarbon compounds (Sahrani, 2008). This reduces the purchasing value of these products, and the combustion of acidified petroleum and gas products produces compounds that encourage corrosion, such as sulfate dioxide SO_2 (Battersby, 1985). Toxicity occurs to workers as a result of inhaling hydrogen sulfide gas (H_2S), the toxicity of which is equivalent to that of hydrogen cyanide (Kage et al., 2004). It may cause immediate death for humans in places where ventilation is not good, as the gas enters through the lungs and is transported with the blood to parts of the body and stops the respiratory control center in the brain, leading to a cessation of the disease. The process of breathing and then death (Gerasimon et al., 2007).

Biofouling, blockages occur in filters, valves, oil reservoirs and tanks, and injection wells as a result of the reaction of sulfate S=, with ferrous ions, Fe^{+2}, forming ferrous sulfide, FeS, which is a colloidal precipitate that combines with the cell masses of SRB, causing blockage (Lin et al., 2009; Enning et al., 2014). It also causes stabilizing oil water emulsions hinders the process of separating this emulsion (Sequeira, 1988, Meinerney and Sublette, 1997). These bacteria take shelter under what is called a biological membrane, as appropriate anaerobic conditions are available for their growth. The biological membrane consists of bacterial cells, polymerized extracellular materials, and organic materials. Lactobacillus, as well as inorganic deposits found in the environment (Beech, 2002). It is very important to control the growth and effectiveness of SRB in water systems and oil fields, and the most common method used to control them is the use of biocides, both organic and inorganic (Hurterent et la., 1992 and Kaur et al, 2009), as the effectiveness of these pesticides must be tested on wandering and attached bacteria. The current study included isolating SRB from oil well injection water and studying the effect of Sodium Dodecyl Sulfate (SDS) on the growth of these wandering and attached bacteria.

2. Materials and methods

Sample collection

The samples were collected from water associated with oil production in the North Rumaila fields and injection water from the water injection project in Abu Sakhir in Basra Governorate, using sterile and clean plastic bottles that were completely filled with water, tightly closed, and transported to the laboratory.

Count of SRB

Liquid (API) medium (API, 1975) was used as a selective medium. The medium was prepared by adding yeast extract 1 g, aqueous magnesium sulphate 0.2 g, and ammonia ferric sulphate 0.2g, sodium chloride 1g, dipotassium dihydrogen phosphate 0.01g, ascorbic acid 0.1g, and sodium lactate 2.24 g. The ingredients were dissolved in one liter of distilled water and the pH of the medium was adjusted to 7, then sterilized with an autoclave at a temperature of 121°C and a pressure of 1.5 pound/inch^2 for 20 minutes. Oxygen-reducing agents were added to it after sterilization 0.3 g/L of sodium dithionite $Na_2S_2O_3$ and 30 ml/L with an 18% concentration of sodium bicarbonate solution $NaHCO_3$, then the medium was saturated with nitrogen gas for ten minutes. As for the solid API medium, it was prepared by adding 15 g/L of agar (Rabus et al, 1996), followed by the most probable number (MPN) count method (APHA, 1985) is used to calculate the number of bacteria in 100 ml of the original sample. The liquid API medium was distributed in 25 ml tightly capped glass tubes, which were divided into three groups, each group consisting of 5 tubes. Added to the first group, 10 ml of the sample and 1 ml for the second group. As for the third group, 0.1 ml of the sample was added to it. The tubes were completely filled with the culture medium and closed tightly, then incubated at a temperature of 35 °C for 7 days. The positive result was recorded by the appearance of a black color, and the number of bacteria was calculated based on most likely counting tables.

Isolation of SRB

Mixture cultures were obtained by seeding 1 ml of sample on liquid API medium in glass tubes and then incubated at a temperature of 35◦C for seven days, to obtain isolated colonies spaced apart from each other, Rolling tubes method described by Hungate (1969) wsa used by taking 1 ml of the activated culture at 24-48 hours of age to prepare a series of ten dilutions using distilled water, then 0.1 ml of dilutions 10-7, 10-8 and 10-9 were taken and cultured in tubes containing API medium. Annealing at a temperature of 45◦C. The tubes were closed tightly, rotated between the palms of the hand, and placed in the refrigerator for a short period for the purpose of hardening the medium. Then they were transferred to the incubator at a temperature of 35◦C and incubated for 3 days. After the end of the incubation period, separate black colonies appeared that were purified by taking part of them with a pipette. Pasteur sterilized and transferred to API liquid medium.

Testing the effectiveness of SDS on the growth of wandering sulfur-reducing bacteria.

A stock solution of SDS at concentrations (5, 10, 15, 20, 30, 40, 50, and 100) ppm were prepared using the dilution law: N2 = V1 x N1 V2 x, and it was added to the culture medium. The API liquid was placed in 25 ml glass tubes, and the media was inoculated with 1 ml of a culture of an activated mixture of sulfur-reducing bacteria isolated from North Rumaila oil well injection water. The tubes were completely filled with the culture medium. They were tightly closed and incubated at a temperature of 35°C for 7 days (Hurterent et al., 1992). Control samples were prepared to compare the results.

Testing the effectiveness of SDS on the growth of sulfur-reducing bacteria attached to the metal surface Biofilm formation on the metal surface

To form the biofilm on the metal surface, the metal samples (wrought iron) were placed in 250 ml glass beakers containing API medium and inoculated with 3 ml of a culture of an activated mixture of sulfur-reducing bacteria and incubated at a temperature of 35°C for a week, which is sufficient time for the biofilm to form on the surface. Metal. To test the effectiveness of SDS on the growth of sulfur-reducing bacteria within the biofilm layer, the mineral samples over which the biofilm was formed were extracted from the flasks after incubating for a week, then placed in glass flasks containing liquid API medium. Concentrations of the substance 50-150 parts per million were added and then incubated. At a temperature of 35°C for 24 hours, control samples were prepared to compare the results.

4. Results

The results of counting sulfur-reducing bacteria using the most probable counting method (Fig. 1) showed that the number of these bacteria in the water associated with production was 23 cells/100 ml of sample, while their number in the injection water was <2. The method of isolation and purification under anaerobic conditions using nitrogen gas with. Adding oxygen-reducing agents has high efficiency in isolating sulfur-reducing bacteria, as black colonies indicating the growth of these bacteria showed growth after 3 days at 35 degrees using solid API medium, (Fig. 2), while the results of the test showed the effectiveness of SDS against the growth of bacteria. For the suspended sulfur reductases in the liquid API medium, the minimum inhibitory concentration for the growth of these bacteria was 30 parts per million. As for the adherent bacteria, the minimum inhibitory concentration was 100 parts per million, as shown in Table (1).

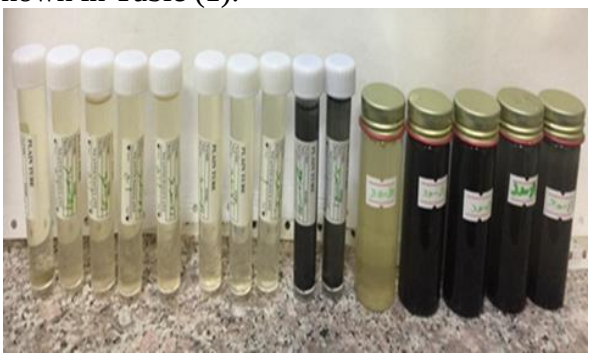

Fig. 1. Most probable counting method to SRB counting

Fig. 2. Single colonies of sulfur-reducing bacteria using the rolling tube method

Table. 1. The effect of different concentrations of SDS on the growth of floating and adherent SRB.

Conc. (ppm)	10	20	30	40	50	60	70	80	90	100
SRB roaming culture	+	+	-	-	-	-	-	-	-	-
SRB adherent cultures	+	+	+	+	+	+	+	-	-	-
SRB control culture	+	+	+	+	+	+	+	+	+	+

+: The appearance of bacterial growth; -: No bacterial growth.

5. Discussion

Isolation results showed the presence of sulfur-reducing bacteria in the water associated with oil production, as appropriate conditions are available for the growth of these bacteria in terms of lack of aeration, abundance of nutrients, and high concentration of sulfate, and this encourages their growth. These results agreed with what was reported by (Sequeira, 1988). It was indicated that low concentration of oxygen and the availability of carbon sources, sulfates, and secondary nutrients in oil production systems are factors that encourage the growth of sulfur-reducing bacteria. Studies have also indicated that conditions are suitable for the growth of these bacteria in oil production systems because they use hydrocarbon compounds as organic sources of energy (Balk, 2007; Dos Santos et al., 2015). The crude oil containing simple compounds such as nitrogen, phosphate, manganese, water, and other microelements encourages the growth of these bacteria (Gaylarde et al., 1999). The results of isolating sulfur-reducing bacteria showed the appearance of black colonies within a period of 3 days, as the use of medium (API) supplemented with oxygen-reducing agents and saturated with nitrogen gases and carbon dioxide reduces the incubation period of this bacteria, and the medium contains iron, which combines with sulfide resulting from sulfate reduction, leading to the formation of black ferrous sulfide, which is an indicator of the growth of sulfur reductases (Widdel, 1988).

The injection water was treated with biocides such as chlorine, so the numbers of bacteria in it were low compared to their numbers in the water accompanying production. Lisa et al. (2011) indicated that the efficiency and effectiveness of pesticides decreases and their concentration decreases after a period of use and until they reach the oil reservoirs, and the pesticides do not have any effect on the layers of biofilm, which is a source of bacteria in the water associated with production. The results of testing the effectiveness of SDS showed that it is effective against sulfur-reducing bacteria floating and attached to the surface of the metal, as this substance is effective in destroying the plasma membrane of bacterial cells. This is consistent with Maillard (2002) who indicated that biocides are chemical compounds that contain. Active groups may interact with components of the outer cell wall, with the cell membrane, or with components of the cytoplasm, causing bacterial cell death. High concentrations of SDS have been used against bacteria adhered to the surface of the metal. This is consistent with that of Battresby et al (1985) who indicated that concentrations of pesticides ant the antibiotics used against attached bacteria are higher than those used against wandering bacteria because the attached bacteria take refuge under the biofilm layer and this layer acts as a barrier between the bacteria and biocides. This result also coincided with Gardner and Stewart (2002), who indicated that biocides are less effective against bacteria attached to biofilm layers.

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