No.24- (9) 2019 Journal of Petroleum Research & Studies (JPR&S)

Determination of sulfide production by Reducing Bacteria isolated in the injection water of an Iraqi oil field

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Abstract:

Several oilfields undergo to reservoir souring, typically during water injection for secondary recovery, resulted in increasing concentrations of produced hydrogen sulfide (H_2S). The main reason for this is the mechanism of generating hydrogen sulfide are the sulfate reducing bacteria (SRB). These bacteria use sulfate (So_4) in the injection water as an electron acceptor and use organic acids which exist in formation water as a source of energy and carbon to generate H_2S. In addition to that, the issues of health and safety, the existence of H_2S decreases the worth of the produced hydrocarbon. The present study includes isolation and enumeration of sulfate reducing bacteria (SRB) from the injection and produced water of Ahdeb oilfield in Iraq by using Most Probable Number (MPN) technique. The Laboratory experimental work for production of sulfide with mix cultures of these bacteria was performed also with sodium lactate as an energy source. The experiments were carried out to determine the concentration of sulfide versus consumption of lactate in vitro. The concentration of sulfide is determined by using spectrophotometer method, whereas; the concentration of sodium lactate is calculated by using high performance liquid chromatography (HPLC) system. The experimental results demonstrates that the most numbers of bacteria in injection water are higher than the number in produced water samples. Whilst, the production of sulfide by SRB presents that inversely correlated to the concentration of sodium lactate. The growth experiments shows that the SRB concentration is increased in areas where the energy source and sulfate have high concentrations. Also, there is a direct relationship between SRB concentration and sulfide production. Therefore, the water injection from these bacteria

must be treated before the injection to the reservoir to provide all the condition of SRB growth.

Keywords: Reservoir souring, Sulfidogenesis, Sulfate Reducing Bacteria, Energy source.

الخلاصة:

تتعرض العديد من حقول النفط إلى حموضة المكامن، وتحدث عادةً أثناء حقن المياه من أجل الانعاش الثانوي، وهي تعني زيادة تركيز كبريتيد الهيدروجين (H_2S) في السوائل المنتجة، تعود آلية توليد كبريتيد الهيدروجين (H_2S) إلى وجود البكتريا المختزلة للكبريتات (SRB). تستخدم هذه البكتيريا الكبريتات المتوفره في ماء الحقن كمستقبل للالكترونات وتستخدم الاحماض العضوية المتوفرة في مياه التكوين كمصدر للكاربون والطاقة لتوليد H_2S، ان وجود H_2S يقلل من قيمة الهايدروكاربون المنتج بالاضافة الى قضايا الصحة والسلامة. تضمنت الدراسة الحالية على عزل وعد البكتيريا المختزلة للكبريتات (SRB) من مياه الحقن والتكوين من حقل الاحدب النفطي في العراق باستخدام طريقة العد الاكثر احتمالا (MPN) حيث تم إجراء تجربة مختبرية لانتاج الكبريتيد من خليط مزارع هذه البكتيريا واستخدام لاكتات الصوديوم كمصدر للطاقة. وقد أجريت التجارب من أجل تحديد كمية الكبريتيد المنتج مقابل استهلاك اللاكتات في المختبر. تم تحديد تركيز الكبريتيد باستخدام طريقة الطيف الضوئي و قياس تركيز لاكتات الصوديوم باستخدام نظام الفصل الكروماتوكرافي السائل عالي الاداء(HPLC).

أوضحت النتائج أن أعداد البكتيريا الموجودة في مياه الحقن أعلى من الاعداد الموجودة في عينات المياه المنتجة في حين أن نتيجة انتاج الكبريتيد بواسطة البكتريا المختزلة للكبريتات (SRB) أظهرت أن إنتاج الكبريتيد يرتبط عكسيا بتركيز لاكتات الصوديوم وكذالك أظهرت تجارب النمو أن تركيز البكتريا المختزلة للكبريتات (SRB) تزداد بالمناطق التي يتوفر بها وفرة من مصادر الطاقة و الكبريتات. كذالك توجد علاقة مباشرة بين تركيز H_2S وانتاج الكبريتيد لهذا يجب معالجة مياه الحقن من هذه البكتريا قبل حقنه بالمكمن الذي يوفر له جميع الظروف لنمو هذه البكتريا.

Introduction:

Reservoir souring is referred to increase H_2S concentration in the produced fluids. The widely accepted mechanism to produce H_2S is a microbial activity in the reservoir, which is mediated by sulfate reducing bacteria (SRB) [15, 22, 11, and 12]. These bacteria reduced sulfate (acceptor of electron) exit in the injection water for secondary recovery. Also, SRB can be used several syntheses as nutrients that are

available in the formation water [4]. Therefore, when sulfate is available in the reservoir, SRB can generate H2S by using nutrients.

Reservoir souring can be very detrimental because H_2S is a quite toxic compound and blazing gas, and it can cause toxicity to workers, the deadly concentration is 800 ppm for 50% of humans exposed for 5 minutes, poisoning numerous of systems in the body. Corrosion is another detrimental effect of hydrogen sulfide in oilfields. In the presence of moisture and at high partial pressures, H_2S can perform as a catalyst in the reduction of atomic hydrogen in steel, which causes sulfide stress cracking (SSC) in high solidity steels. Lastly, the reduction in the sale value of products is due to the pollution of hydrocarbon fluids with H_2S [10, 12].

Sulfate reducing bacteria can use different types of organic compounds such as lactate, acetate, propionate, n-alkanes, benzoate, benzene, toluene and phenol as a donor of electron and carbon sources available in oil reservoirs. *Desulfovibrio* Species and many SRB type can nurture on lactate. Incompletely oxidized which means oxidized lactate to acetate and CO_2 (Reaction 1) or oxidize completely to CO_2 (Reaction 2) and the electrons that produced from oxidizing are transported to enzymes of electron transport which exist in the cell membrane and then further to the SO_4 [24].

$$2 \text{ lactate} + SO4^{2-} + 2H^{+} \rightarrow 2 \text{ acetate} + 2CO_2 + HS^{-} + H^{+} + 2H_2O \quad (1)$$

$$\text{Acetate} + SO_4^{2-} + 3H^{+} \rightarrow H_2S + 2H_2O + 2CO_2 \quad (2)$$

Sulfate reducing bacteria is presented in apparent groundwater, marine environments, coastal sediments, marine hydrothermal vents associated with the volcanic or tectonic activity and hot springs. However, Bastin et al., (1926) presented the first indication of SRB activity in oil reservoirs [6]. The cell forms of SRB usually observed by light microscopy that are a rod, vibrio, filamentative, rounded and coccoid shaped. Various types of SRB tend to growth in clumps or cell aggregates and glued to surfaces [9].

The object of the current research is to compute the SRB in both produced and injection water of Ahdeb oilfield, to investigate the possibility of using the communities

of these bacteria for the production of sulfide and then, and to notify the sodium lactate consumption in laboratory system.

The Experiments Work:

Sample Collection:

Eight samples of produced water and injection water were collected in March 2017 from Ahdeb oilfield, which located in Wasit province in Iraq. The field is flooded with water consisted of produced water and Tigris river water after a treatment with a biocide. The samples of produced water were collected from separator tanks whereas the samples of injection water collected from tanks in sterilized plastic vial which completely filled to prevent react with air, the time between sample collections and microbiological analysis should not exceed 24 hours as maximum and kept cool [17]. The physical and chemical characteristics of produced and injection water which gets from Ahdeb oilfield were measured according to Page et al. (1982) because of their important role in the growth of Sulfate Reducing Bacteria (Table 1) [18].

Table (1) Physical and chemical characteristics of the produced and injection water samples of Ahdeb oil field.

Sample	Temp. ^{o}C	PH	Salinity (ds.m-1)	Ca	Mg	Cl	SO_4
				ppm			
Produced water	42	6.4	170.2	8293.3	2331	80849.7	624.6
Injection water	38	6.8	170.9	8877.5	2503	100224.3	669.9

Isolation of Sulfate Reducing Bacteria:

American Petroleum Institute (API) medium was prepared to isolate and enumerate of SRB [3]. The composition of this medium was given in the Table 2.

Table (2) the chemical components of the API medium for grow of SRB (API, 1975).

Chemical component	Amount (g/L)
yeast extract	1
hydrous magnesium sulfate	0.2
hydrous Ferric ammonium sulfate	0.2
sodium chloride	10
dipotassium hydrogen phosphate	0.01
Sodium lactate	0.15
Ascorbic acid	0.1

The volume of distilled water was full up to 1000 ml. The medium pH was adjusted to 7 and after the autoclaved it was flushed for 20 min with a mixture of N_2:CO_2 90:10% during the flushing, add 30 ml/l from the solution of bicarbonate [23], 1 ml/l from the solution of Selenite –Tungstate [23], 1 ml/l from the solution of vitamin B_{12} [23], 1ml from the solution of Trace element [25] 1ml from the solution of mix vitamin [1] and 1ml from the solution of sodium sulfide [26]. The mix cultures of SRB were obtained from water by inoculums 5 - 10 ml into the flushed N_2 screw cap contains liquid API medium and the screw cap was completely filled up with the medium, closed tightly and left in the incubator at 38°C for 7 days. The isolation of SRB under anaerobic conditions was performed in a selective API medium containing sodium lactate as a sole carbon source with reducing and enrichments agents which gives the growth of these bacteria after 3 days of incubation. The sodium lactate is classic carbon source which grows of approximately 80% of SRB [5] where indicated of SRB growth was by a black precipitate as shown in Figure (1). The iron that present in the medium interacted with sulfide resulting from the reduction of sulfates and results in the black ferrous sulfide which was an indicator of the growth of SRB [16].

Fig. (1) (a) Control and (b) Growth of mix culture SRB change color to black.

To ensure the bacterial growth in cultures and observation the shape of cell, phase contrast microscopy was made after gram staining. Gram staining consists of four steps: Crystal violet which is first stain, Mordant (Gram's Iodine), Decolourizer (ethyl alcohol) and the safranin which is the stain of Counter. Every component has stayed nearly one minute on a glass slide that smear with bacteria and between a step and other, the slide was washed with water [7]. The result of the gram stain presented a diverse community of SRB associated with H_2S generation as shown in Figure (2).

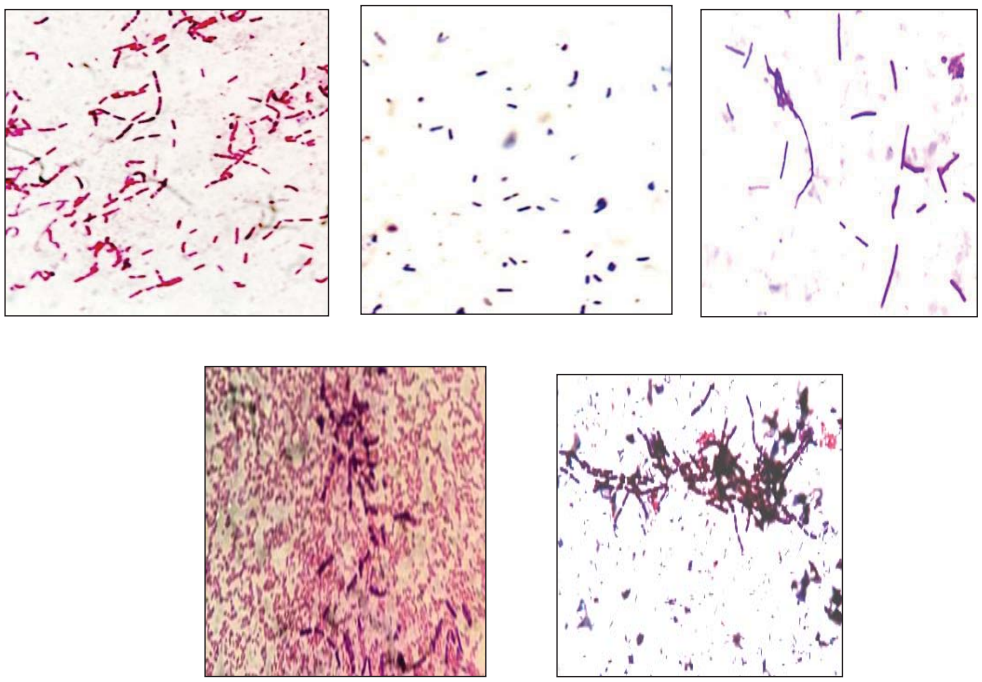

Fig. (2) Gram staining of mix cultures of SRB.

Enumeration of Sulfate Reducing Bacteria:

Viable count of SRB was estimated by using five-tube Most Probable Number (MPN) technique. The medium of API was dispensed into three groups of a screw cup and each group consisted of 5 tubes where the first collection has received 10 ml of the sample, the second collection received 1 ml of the sample while the third collection received 0.1 ml of the sample. These prepared groups were incubated at 38°C for 7 days. Determine of positive result by change the color to black. The numbers of bacteria were estimated by using MPN tables [2]. The result of MPN analysis has detected the presence of SRB in water samples of Ahdeb oilfields where the number in produced water was about 31 cell/100 ml, while, in injection water it was 130 cell/100 ml these results were in agreement with previous studies [19, 14]. The number of SRB in injection water was higher than that in produced water and that may be to the reservoirs at the primary production stage being an aggressive environment to microbial activities. Ruseska et al, (1982) have reported that it was not possible to find a larger increase in the numbers of SRB in the produced water [20], also the results compatible with Al-Tamimi (2015), which showed that number of bacteria was low in produced water of Nuhran Omer oilfields [1]. More suitable environment for growth of SRB in Tigris river which is used as a source of injection water due to availability of sulfate, nutrients, in addition of substantial levels of fatty acids, which are available from the formation water which can be used directly by SRB, thus the number of SRB was high in injection water. Although the injection water was treated with biocide, there was a high number of SRB. This is due to inactivity of biocide used in this field.

Determination of dissolved sulfides in cultures:

The concentration of sulfide was measured by mixing 50 µl of culture with 950 µl of 5 mM copper sulfate and 50 mM hydrochloric acid, the optical density was measured at 480 nm and the number obtained were compared with the standard curve [8].

Determination concentration of carbon source in cultures:

The determined concentration of sodium lactate was measured by using high performance liquid chromatography (HPLC) system (Safi et al., 2013). The concentration of sodium lactate was estimated in cultures with standard sodium lactate through the following form:

$$\textbf{Sodium lactate con.} = \frac{\textbf{peak area of sample}}{\textbf{peak area of stanard}} \times \textbf{Standard concentration}$$

Determination of sulfide production in culture:

The sulfide production experiment was performed with the established anaerobic growth by adding 0.05 v/v of two days old enrichment of SRB mix cultures consortium into the sterile serum vials containing 100 ml anaerobic liquid API medium was flushed with nitrogen. The serum vials were sealed with butyl rubber stoppers and aluminum crimp seals. All vials were incubated for 7 days at 38°C. The activity of SRB for H_2S production was determined by measuring the sulfide concentration and the sodium lactate concentration in the culture at zero time and at every day along the period of incubation. All batch experiments were made in duplicate.

These previous experiments were done in order to measure the sulfide production during the mix cultures growth of SRB with sodium lactate as energy sources in vitro. The results showed that the concentration of sulfide was clearly increased led to blackening the medium until it reached to maximum value of 66.4 ppm compared with the control treatment 9.5 ppm, where no changes in sulfide after 4 days of incubation (Figure 3) versus consuming of sodium lactate that was gradually decrease from 5 to 2.6 ppm in comparison with control 6.9 to 5.2 ppm at the same period time of incubation as depicted in Figure 4. Our result compatible with Hallbeck, (2014) that showed that production of H_2S is dependent on the concentration and type of energy source lactate works as energy source and as carbon source for the biomass production [13].

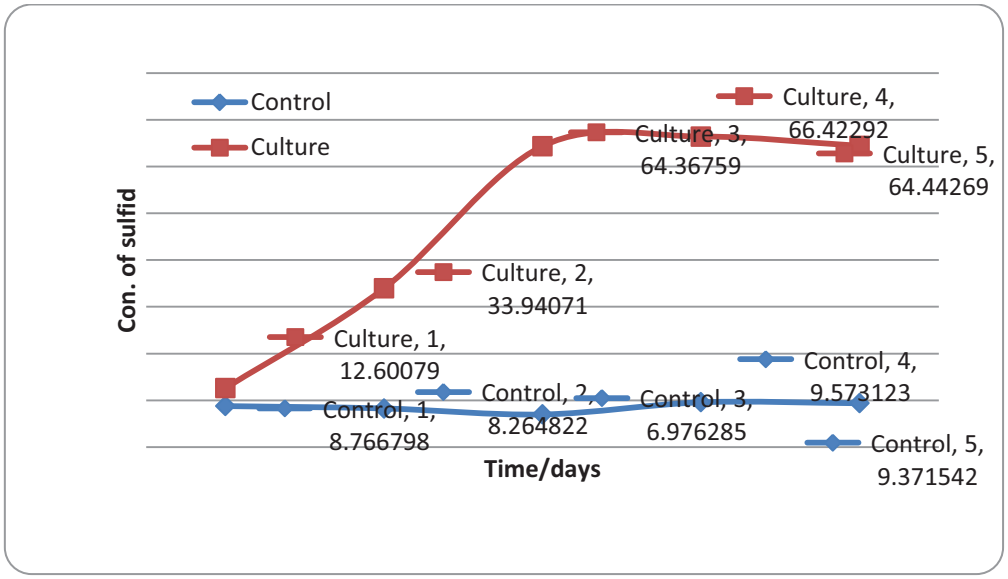

Fig. (3) Sulfide production in SRB culture.

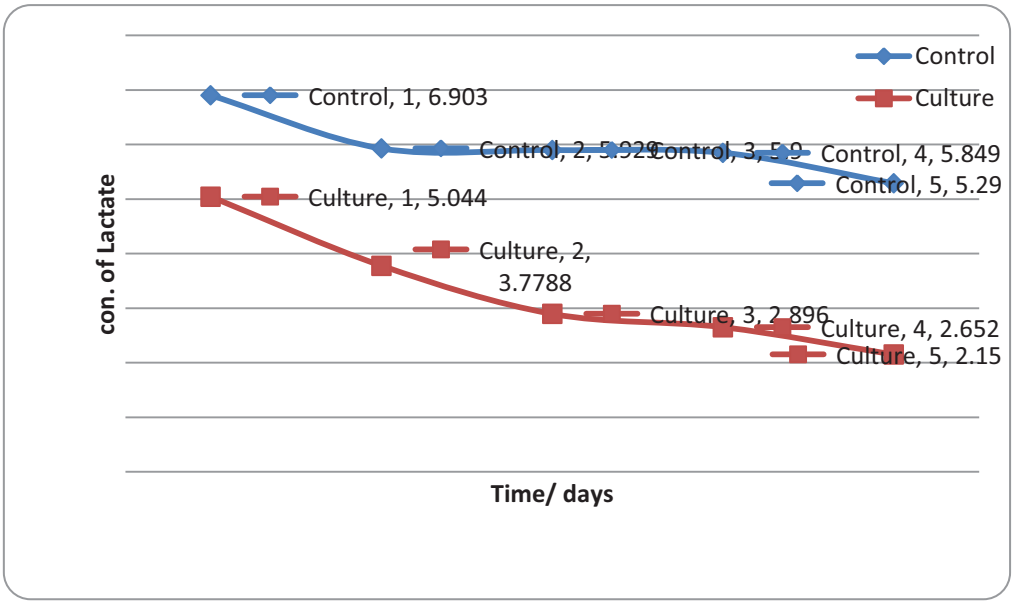

Fig. (4) Utilization of lactate in SRB culture.

Conclusion:

- The numbers of SRB in injection water of Ahdeb oilfield were relatively high indicated the inactivity of biocide that in the use of the treatment of biogenic generation of H_2S in this field.
- A continue relationship was detected between the concentration of SRB and sulfide production.
- The SRB concentration increased in areas where the sulfate and energy source had high concentrations.

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