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Inhibition of biogenic hydrogen sulfide produce by Sulfate Reducing Bacteria isolated from oil fields in Basra by nitrate based treatment

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Abstract:

The present study included using of Bio Competitive Exclusion BCX technology in treatment of biogenic production of hydrogen sulfide H_2S by Sulfate Reducing Bacteria SRB in batch cultures, injection of nitrate and nitrite promotes the growth of Nitrate Reducing Bacteria NRB which outcompete the Sulfate Reducing Bacteria SRB on carbon sources. Mix cultures of SRB and NRB were isolated from produce water of oil production facilities in Iraq including Nahran Omer and Al lahis oil fields. The activity of NRB and SRB were determined by measured the concentration of nitrate, nitrite and sulfide by using spectrophotometer and bacterial counts in three tube MPN technique. The results showed that NRB had a certain inhibitory effect on the growth of SRB and sulfide production under adding NRB nutrient nitrate and nitrite at all concentrations 200, 400, 600, 800 and 1000 mg/l, activity of NRB increased after treatment, all nitrate and nitrite consumption during 2 - 3 days of incubation. The highest inhibition of H_2S production was at concentration 1000 mg/l of nitrate where the results showed that there was significant decrease in sulfide level and number of bacteria 34 mg/l and 34.8 cell/ ml respectively after 10 days of treatment.

Introduction:

Anaerobic microbial sulfate reduction is a primary cause of oil reservoir souring [1], growth and metabolic activity of Sulfate Reducing Bacteria SRB can results in souring of oil reservoirs leading to various serious operational problems in aspects of environmental pollution [2], these bacteria are commonly considers the main culprits of Microbially Induced Corrosion MIC [3]. Also souring decreases the value of the crude oil and poses a health hazard to oil field workers [4]. The petroleum industry has begun implementing a nitrate-based microbial treatment to suppress reservoir souring in oil and gas fields [5], these innovative reservoir treatments recognize that detrimental sulfate-reducing bacteria SRB, which produce sulfide, can be replaced by a naturally occurring suite of beneficial microorganisms enhanced by the introduction of an inorganic nitrate-based formulation. This purposeful manipulation of the reservoir ecology has been termed Bio competitive Exclusion BCX technology which used to prevention and removal of sulfide from injection wells and produced waters, as well as surface facilities, pipe lines and gas storage reservoirs [4].

Souring is a phenomenon of great concern to oil producers, leading to implementation of various methods for its control. Injection of biocides into reservoirs to inhibit SRB is a common souring control method, however, this treatment is expensive, requiring frequent injection because it is effective only for a short duration or is ineffective because microbial biofilms develop resistance to the biocides [6]. The biocides can also be hazardous to human health and environment, therefore worker safety issues are also possible [7].

Addition of nitrate to oil fields to control souring is considered to be a potentially cost-effective and less toxic alternative to biocide treatment, particularly in offshore oil fields where injection water is high in sulfate, and also in continental oil fields [8, 9]. Nitrate injection can control souring by (i) direct sulfide removal by Sulfide-Oxidizing Nitrate Reducing Bacteria SO-NRB, (ii) formation of nitrite which inhibits SRB, the effects of nitrite is the inhibited Dissimulatory sulfite reductase Dsr, The enzyme catalyzing conversion of sulfite $SO3^{-2}$ to sulfide S^{-2} which is a terminal enzymatic step in the sulfate reduction reaction pathway of SRB [10, 11] (iii) chemical reaction of sulfide with nitrite, and (iv) biocompetitive exclusion of SRB

by heterotrophic Nitrate-Reducing Bacteria hNRB, which use nitrate to oxidize the same oil field organics as used by the SRB to reduce sulfate[8,12].

[13] Showed that the addition of nitrate can benefit Microbial Enhanced Oil Recovery MEOR by stimulating of fortuitous bio surfactant- producing bacteria. The aims of the current study was to isolated the mix culture of SRB and NRB from produced water of Nahran Omar and Al-lahis oil fields located in Basrah and assess the long term inhibition of nitrate and nitrite treatment on SRB activity and sulfide production.

Material and method:

Isolation of Sulfate Reducing Bacteria and Nitrate Reducing Bacteria media

SRB isolated by using API medium [14]. The API was modified by adding 0.4 g Sodium acetate, 0.1 g sodium benzoate, Sodium propionate 0.9 g, Ethanol 0.1%v/v and refers as API-M. NRB isolated by using Coleville Synthetic Brine medium CSB [15, 16] after modified by adding 10 g of NaCl, The medium to study the consortium between SRB and NRB in the presence of nitrate or nitrite was API medium which also modified and refers as API-S medium (unpublished). The PH of all media adjusted to between 7-7.3. After autoclaving, they are flushed with a mixture of N_2:CO_2 90:10% for 20 min during the flushing add to the media of API-M and API-S sodium bicarbonate solution, selenite-tungestate solution, vitamin B_{12} solution [17], sodium sulfide solution [18], trace elements solution[19] and mix vitamin solution [20] for CSB medium all these solutions were added excepted selenite-tungestate solution.

Sample collection:

samples of produce water were collected from the separator tanks of two non water flooded oil fields in Basrah , Nahran Omer, oil reservoirs are located at a depth of ~2000 – 3000 m below ground and the in situ temperature~50-70 °C and Al-lahis oil fields, the deep of reservoir were about~3700 - 3900 m, temperature in situ was about ~50-70°C samples collected in sterile glass bottles which were filled completely to prevent contact with air, they were transport to the laboratory and flushed with %10 N_2.

Isolation of SRB and NRB

The mix cultures of SRB and NRB were obtain from produce water by inoculum 5-10 ml of subsample into the flushed N_2 screw cap contain liquid modified API-M medium for isolation of SRB and liquid CSB medium for isolation of NRB, the screw cap completely filled up with medium and closed tightly, left in the incubator at 37 °C for 30 - 35 days. Positive result of SRB determined by the color changing to blacking while Positive result of NRB determined by the turbidity and examination by phase contrast microscopy. From some sample of produced water bacteria were isolated as mentioned previously after enrichment in microcosms [21].

Nitrate reduction by NRB: The ability to reduce nitrate was determine by the presence of nitrite and nitrate in the cultures [22].

Chemical analysis: Sulfide, nitrite, nitrate, concentration were determined spectrophotometrically [23, 24] by using UV-visible Sco-Tech Germany spectrophotometer.

Bacterial count in cultures: To estimate the bacterial count of SRB in the cultures dependent three -tube MPN technique [25].

Inhibition of hydrogen sulfide by nitrate, nitrite and NRB treatment

to study the effect of nitrate, nitrite and NRB on inhibition of hydrogen sulfide production by consortium of SRB in batch culture, 5%v/v of two days old enrichment mix cultures of SRB consortium and NRB were inoculated into the 100 ml sterile serum bottles flushed with nitrogen, contained anaerobic liquid modified API-S medium, nitrate and nitrite were added from an anoxic sterile stock solution in the following concentration 200, 400, 600, 800 and 1000 mg/l the serum bottles sealed with butyl rubber stoppers and surrounding with parafilm. All bottles were incubated for 10 days at 37°C. Microbial activity for SRB was monitored as the change in the concentration of sulfide, and bacterial count, NRB activity was determined by the measuring concentration nitrate and nitrite at zero time and every day along the period of incubation, all batch experiment were carried out in duplicate. Control runs in duplicate were conducted under similar conditions without injection of nitrate or nitrite [26].

Statistical analysis

Results were analysis statistically by statistical package for social science program SPSS version 20 statistic program by ANOVA analysis model procedure where comparisons between means were made using significant differences.

Results

Isolation and cultivation of SRB and NRB

The enrichment cultures of SRB and NRB by using modified API-M medium and CSB medium under anaerobic condition showed that both groups of bacteria were present in the community of produced water, SRB growth was indicated by a black precipitate after 25 days of incubation whereas NRB growth was indicated by turbidity and phase contrast microscopy after 30 - 35 days of incubation as shown in figure (1). Direct cultured of some produced water samples showed no growth of SRB and NRB but the microcosms was more appropriate for bacterial groups and showed growth of bacteria after 14 days of incubation.

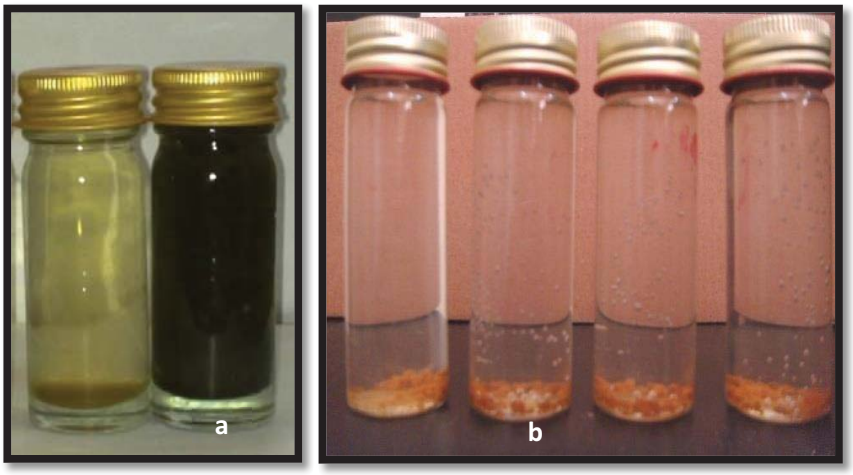

Fig. (1) (a) Mix culture of SRB, (b) Mix culture of NRB.

Nitrate reduction by NRB

Nitrate Reduction test showed that mix cultures with vigorous growth have a strong nitrate reduction where the cultures reduced nitrate completely to N_2 or NH_3, they give no change in color when a sulfanilamide/ naphthylethylenediamine solution and zinc powder is added.

Inhibition of hydrogen sulfide by nitrate, nitrite and NRB treatment

The results of treatment by nitrate, nitrite and NRB on inhibition of sulfide production by SRB revealed significant inhibition in all concentration 200, 400, 600, 800 and 1000. Figures (2a & 3a) showed that growth of SRB and sulfide production was well inhibited after 2 days of treatment where there was no blackening observed in treatment bottles. Significant $P \leq 0.02$ inhibition of sulfide production was observed with treatment of nitrate in comparison with control, where the highest levels of sulfide was 137 mg/l. Production of hydrogen sulfide was largely inhibited in all treatment of nitrate and most obvious effect in concentration 1000 mg/l which has the longest inhibition time during 10 days of treatment with significantly decrease of other treatment $P \leq 0.003$, the highest sulfide levels was 34 mg/l in concentration of 1000 mg/l as shown in figure (4).

In respect to treatment with nitrite results showed also significant inhibition of sulfide production in contrast with control $P \leq 0.001$ the highest levels of sulfide was 68 mg/l in concentration of 1000 mg/l during 10 days of treatment as observed in figure (5), there was no significant differences in sulfide levels between nitrate and nitrite but generally the sulfide levels in nitrite was lowest than in nitrate.

Measuring concentration of nitrate or nitrite at different treatment in the presence of NRB revealed high activity of NRB in cultures, where all the media that treated with nitrate or nitrite showed large decline in concentrations during 2 - 3 days of treatment, the lowest decreasing was 1.4 mg/l for nitrate and 0 for nitrite as shown in figures (6 & 7). After 10 days of incubation all nitrate and nitrite were consumption by NRB the SRB were grow and produced H_2S in cultures as shown in figures (2b & 3b).

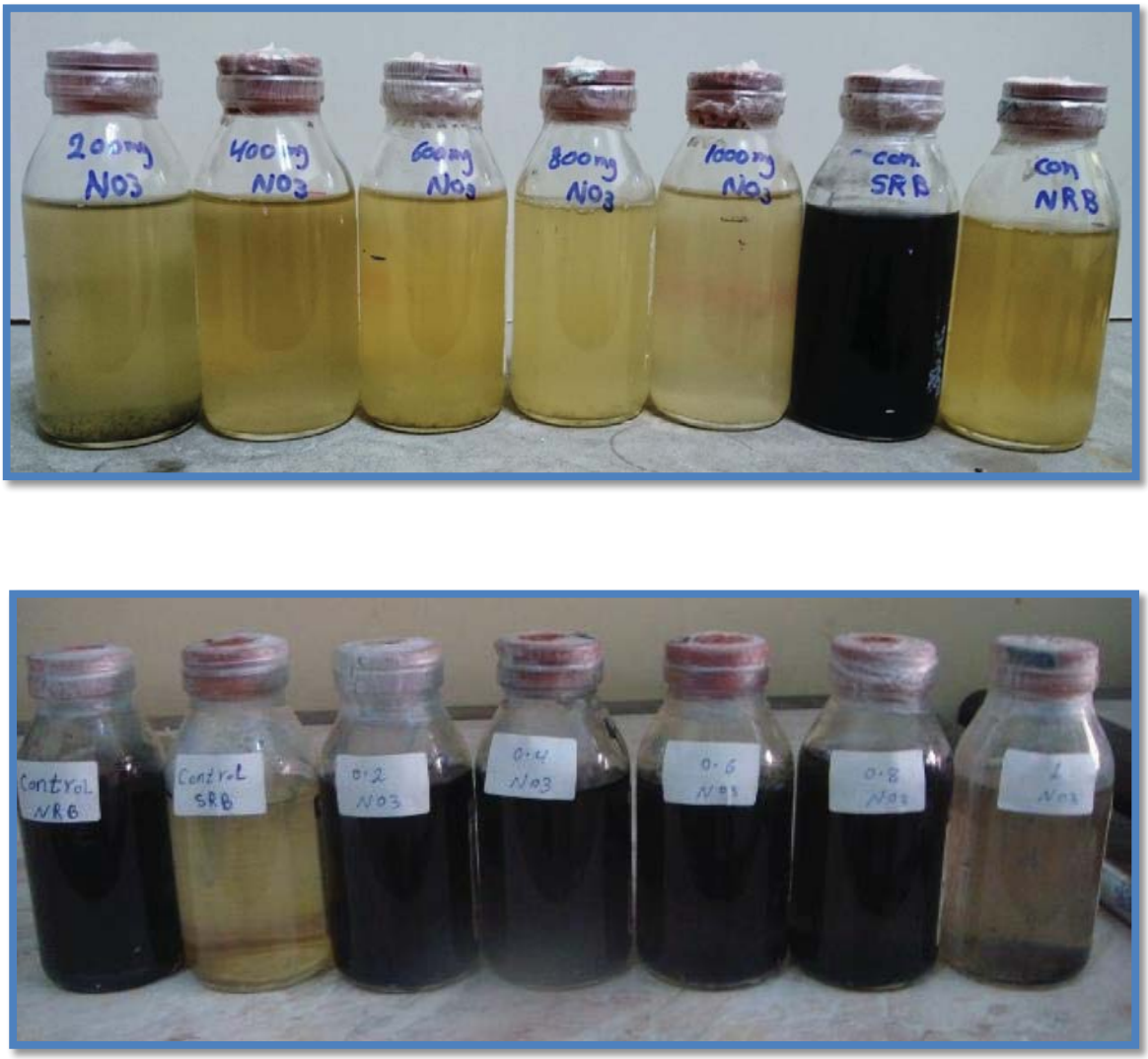

Fig.(2) effect of different concentrations of nitrate and NRB on SRB activity and sulfide production after (a) 2 days and (b) 10 days of treatment.

Fig. (3) Effect of different concentrations of nitrite and NRB on SRB activity and sulfide production after (a) 2 days and (b) 10 days of treatment.

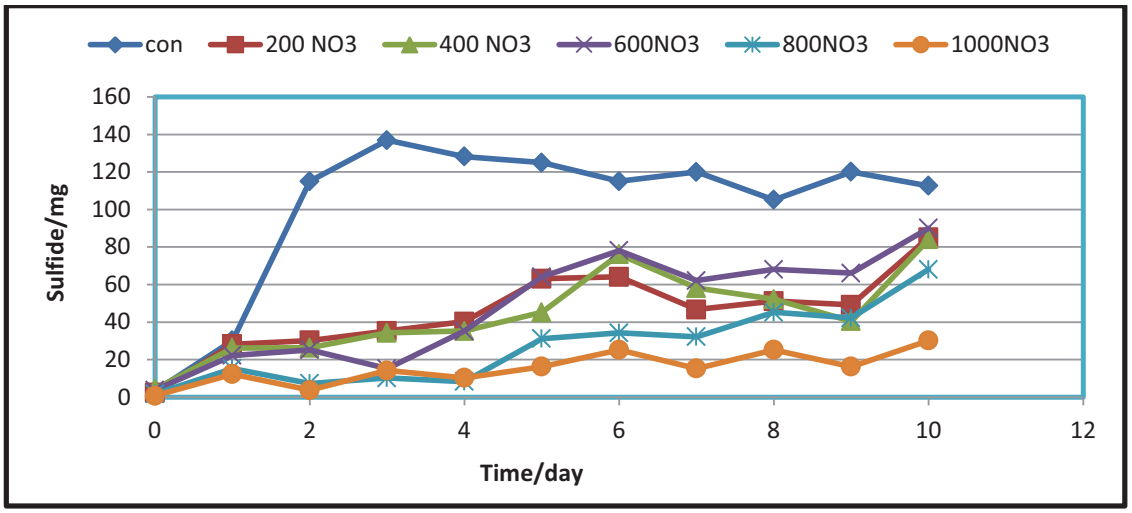

Fig. (4) Effect of treatments nitrate and NRB on sulfide production capacity of SRB comparison with control.

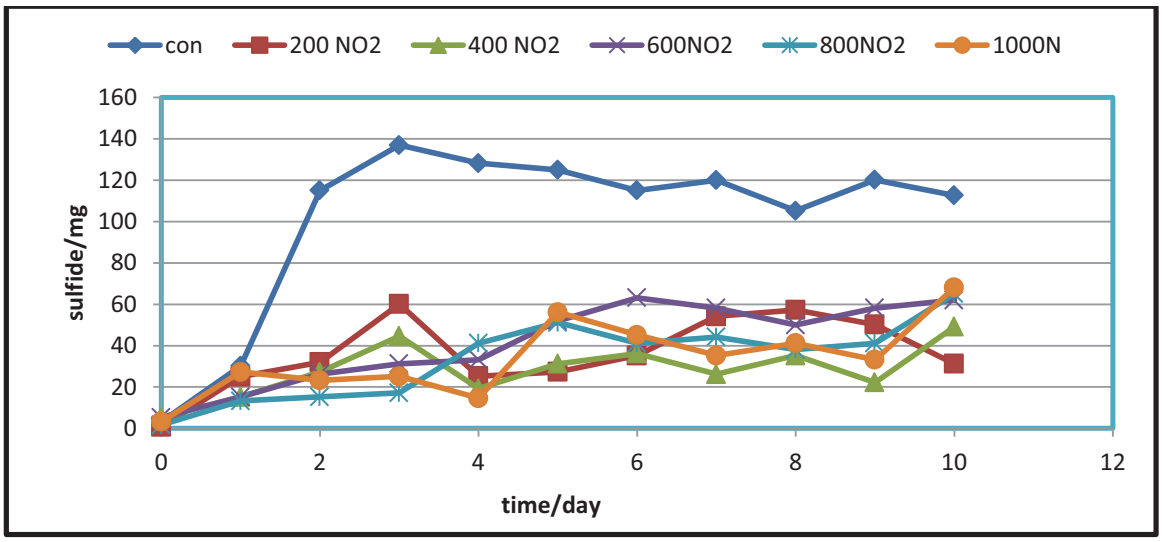

Fig.(5) Effect of treatments l nitrite and NRB on sulfide production capacity of SRB compared with control.

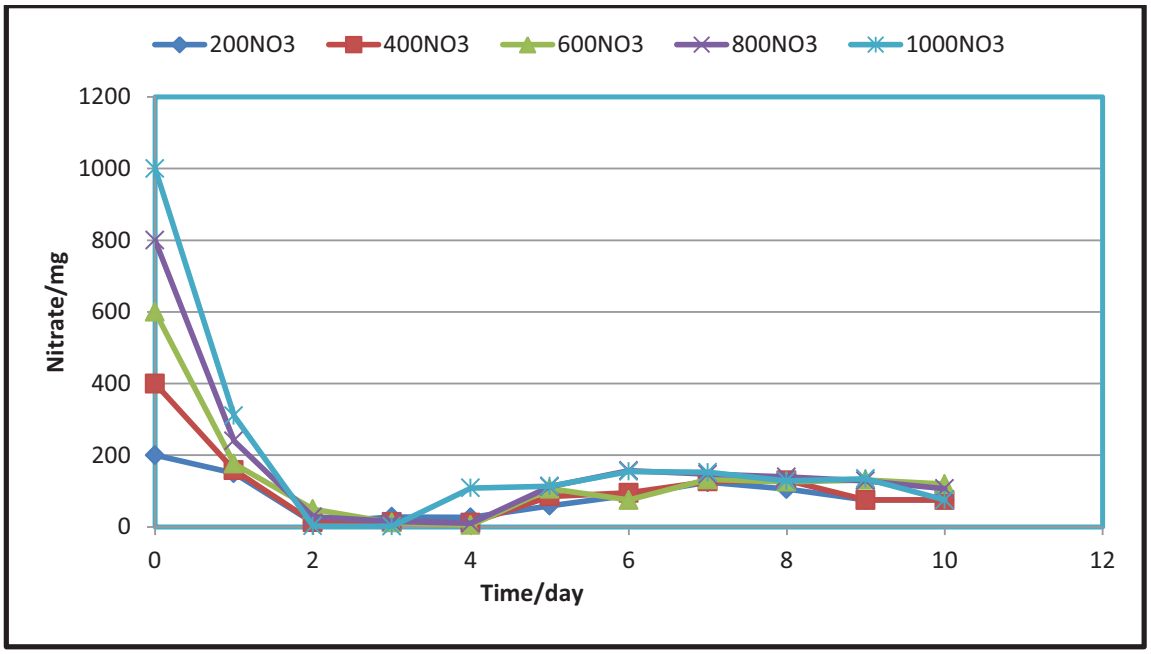

Fig. (6) Change of nitrate concentrations in bottles treated with NRB and nitrate.

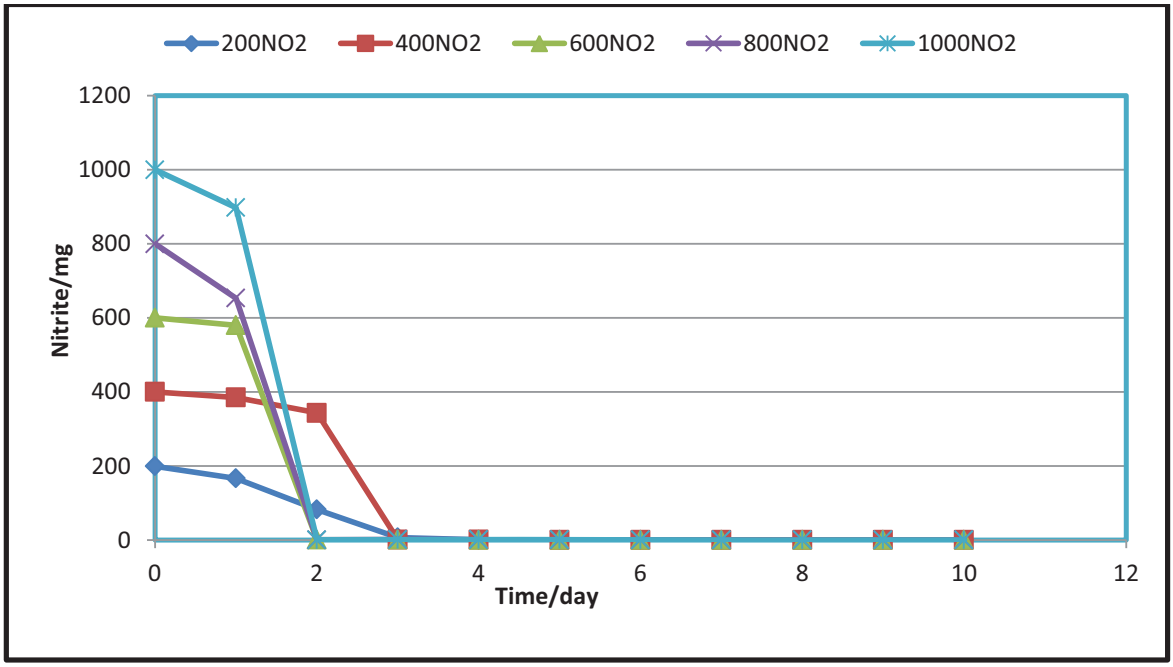

Fig. (7) Change in nitrite concentrations in treated bottles with NRB and nitrite.

Effect of different treatments on number of SRB

Tables (1and 2) revealed reduced in number of SRB at second day of treatment at all of nitrate and nitrite treatments in comparison with control the viable count of SRB reached a maximum number of >1100 cell/ml, then the numbers were rise at all concentrations except of 1000 mg/l nitrate where the growth was completely inhibited and the highest number of cells was 34.8 cell/ml.

Table (1) Number of SRB according to MPN index at different treatments of nitrate

Period time/ days	Number of cells/ml					
	Control	200mg	400mg	600mg	800mg	1000mg
2	>1100	9.44	21	3	7.3	<3
4	240	>1100	240	9.4	7.2	<3
6	149	>1100	215	3.57	3.57	<3
8	149	1100	240	3.5	240	9.18
10	115	1100	462	462	462	34.8

Table (2) Numbers of SRB according to MPN index at different treatments of nitrite.

Period time/ days	Number of cells/ml					
	Control	200mg	400mg	600mg	800mg	1000mg
2	>1100	<3	26.8	<3	<3	7.23
4	240	93.3	23.1	93.3	23.1	9.4
6	149	>1100	3.57	34.8	3.57	6.19
8	149	>1100	240	240	292	240
10	115	1100	>1100	>1100	>1100	>1100

Discussion:

SRB participate in Microbially Induced Corrosion MIC of equipment and H_2S driven reservoir souring in oil field sites [27]. In recent years, some papers have examined the microbial community and souring activity of oil fields [2, 28]. It would be necessary to study the indigenous microbial community of SRB that utilize naturally occurring Volatile fatty acid VFA and other bacteria that outcompete it such as NRB in reservoirs to initiate some database of information because there were no studies concerning the isolation and identification of these bacteria and their ability to controlling souring by nitrate and nitrite injection in Iraq. These bacteria could be selected because it can be proliferation with low concentrations of nitrate and nitrite and use the VFA preferentially, thereby denying the SRB this required nutrient [4].

The Present study revealed the possibility of using BCX technology in controlling of biogenic formation of hydrogen sulfide in oil reservoirs in Iraq which suffered serious operational problems**.** The bacteria of SRB and NRB of some produced water samples were isolated by using anaerobic microcosms because these bacteria found in very low numbers, this result was similar to [21] who showed the inability to detected these bacteria by molecular methods in water samples due to low numbers but in microcosms bacteria increased from undetectable levels to 5- 20% abundance. Microcosms is serum bottles containing reservoir fluid as an inoculums permit the testing of many parameters related to souring and its mitigation in a controlled environment, such microcosms can serve as a starting point for enrichment cultures and isolation of SRP or other reservoir associated cells [21, 25].

Inhibition of hydrogen sulfide by nitrate, nitrite and NRB treatment

The application of nitrate and nitrite injection on the microbial community in oil fields as an alternative to biocide addition for controlling sulfide production has never been studied in Iraq to our knowledge, in the present study used mix cultures of SRB and NRB originated from the produce water were used because these bacteria found in reservoir environment as a consortium, level of nitrate and nitrite dependent on the composition of SRB, this was in

agreement with [13] in used the indigenous microbial consortium collected from the produced water as inoculums and [30] who used a mixed culture enriched from the produced water of Coleville oil field in Canada in control of souring in oil reservoirs and in treatment of gas and liquid contaminated with sulfide and nitrate.

The results of the present study showed that NRB had a certain inhibitory effect on the growth of SRB and sulfide production, under the condition of adding NRB nutrient nitrate or nitrite at different concentration. Nitrate has the inhibition effect on sulfate reducers. [31] Used a *Desulfovibrio vulgaris* as a model sulfate reducing bacterium and found from physiological analysis that nitrate has the direct inhibition on sulfate reducers because of osmotic and nitrite stress which involvement in inhibitory mechanisms. Also addition of nitrate inhibited sulfide production because its promotes the growth and activity of NRB which increased until third day of incubation at low and high concentrations, the NRB activity was indicated by the consumption of nitrate and nitrite as shown in figure (6 & 7). Increase the growth and NRB activity population led to out competing SRB population for electron donors, so the activity of SRB was very low sulfide levels and numbers of cells did not increased in contrast with control, figure (4) and table (1) This was in agreement with [26] who investigated the response of SRB to nitrate and nitrite in the presence of NRB in batch culture and found that dosing NRB with a certain degree of nitrate or nitrite at the same time had a certain inhibitory effect on the growth of SRB and sulfide production, and compatible with [15] who reported that addition of nitrate and an NR-SOB culture to a growing SRB consortium inhibited the production of sulfide by this consortium immediately.

Sulfate and nitrate are terminal electron acceptors for these different group of bacteria and competition based on thermodynamics, kinetics and redox potential is established when both anion are present. microbial reduction of nitrate to nitrogen or ammonia provide more energy than sulfate, approximately three time more energy than reduction of sulfate therefore in the presence of both nitrate and sulfate, NRB tend to grow faster and dominate to outcompete SRB for available electron donors [4] and suppress the growth of SRB by various mechanisms resulting in a gradual sweetening of the reservoirs [32]. The Results showed that the growth of

SRB was resumed once all nitrate and nitrite were consumed in treatment bottles the liquid phase of cultures were turned from yellowish color to black figure (2b&3b) , sulfide concentration and numbers of cells increased gradually as shown in figures (4 &5) and table (1&2) This was similar with results of [26] who showed that dosing nitrate or nitrite with NRB had a significant inhibitory effect in 72 hours but after that the effect began to decline.

Treatment with nitrite also showed strong inhibition of sulfide production and number of bacteria figure (5) and table (2) the significant differences in sulfide concentrations between nitrite and control was higher than nitrate, this indicated that nitrite had direct inhibition on SRB because nitrite inhibits dissimilatory sulfite reductase (Dsr), the enzyme that catalyzes the reduction of sulfite to sulfide. Dsr has a strong affinity for nitrite [11]. These results was in agreement with [26] who found that input of NRB and sodium nitrite had better inhibitory effect on SRB activity than the role of nitrate, also the results were correlated with other studies conducted by [33] who showed that nitrite is more effective in preventing some high temperature oil field acidizing, because of its direct reaction with the sulfide and is proved to be an effective inhibitor of sulfate reducing long term,

The concentration of 1000 mg/l nitrate had the longest inhibition time because of the Conversion of nitrate to nitrite offered poor environment for SRB growth, sulfide level and number of cells increased little until the 10^{th} day, As shown in figure (4) and table (1), this is due to formation of nitrite as a results of nitrate reduction which was not reduced because there were nutritional limitation in culture further causing complete inhibition of sulfide production, this was in agreement with [2] that the competitive reduction of nitrate by NRB and nitrite produced were responsible for the suppression of SRB also this results was in accordance with other studies who showed that initiation of nitrite stress could be of particular ecological significance in the persistence of SRB in the environment with elevated level of nitrate which has been shown to effectively inhibit SRB populations [34, 35]. Nitrite accumulation caused inhibition of sulfide because it will probable transient due to further reduction to nitric oxide and nitrous oxide and finally nitrogen which are considered potential inhibitory agent of SRB in addition to an overall increase in redox potential [36].

Feasibility

Biocompetitive exclusion technology could be suitable strategy for SRB control in oil wells. Nitrate and nitrite are less expensive and an environmentally friendly treatment it has replaced toxic and expensive of biocides in controlling sulfide level and eliminate microbially induced souring, corrosion and other problems associated with H_2S formation in many oil fields of Canada, South America, middle east and North America, also indigenous NRB enhanced oil recovery through increase oil production by producing surfactant, solvents and organic acids so in most cases these microbes increased oil production. This study suggests the possibility of using the technology of BCX in treatment of biogenic H_2S production in oil fields in Iraq.

Conclusion:

1- Addition of nitrate and nitrite with NRB has a strong inhibitory effect on the production of H_2S and growth of SRB.
2- Sulfide levels and number of bacteria significantly decreased at concentration of 1000 mg/l nitrate.
3- NRB activity increased after injection of nitrate and nitrite.
4- H_2S production in treatment with nitrite was lower than with nitrate.

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