

Factors Affecting Growth of Sulfate-Reducing Bacteria Isolated from Tropical Soil

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Sulfate-reducing bacteria (SRB) were isolated from soils around corroded pipelines and tanks. High numbers of the organisms occurred in areas closest to the corroded tanks and pipelines. Morphological types corresponding to rod, spirilloid, vibrioid and coccoid were encountered. All the organisms utilized lactate as carbon and energy source. None could grow at temperatures higher than 40 °C. All the isolates grew at 1% (w/v) NaCl while none could grow at 8% (w/v) NaCl. All the isolates grew at pH 7.0–7.5. Growth was not recorded at pH below 5.5 and above 8.0. These factors may be useful in manipulating tropical soil environments to reduce activities of SRB in corrosion of pipelines and tanks.

Introduction

The sulfate-reducing bacteria (SRB) are strict anaerobes. They perform anaerobic respiration by oxidizing certain organic compounds or hydrogen and often other reduce sulfur compounds to hydrogen sulfide. They play important role in the degradation of organic matter under anaerobic condition (Iverson and Olson, 1984). They use sulfate among other oxidized sulfur compounds as electron acceptors. The organisms are particularly important in shallow sediments of marine environments where neither organic matter nor sulfate is limiting (Johnson and Wood, 1993). Although SRB are obligate anaerobes, their presence and activity have been demonstrated in apparently oxidized environments (Hardy, 1981). The SRB were considered as the major bacterial group involved in the acidifying of oil fields and in microbially influenced corrosion (Hamilton, 1985). Their activities within the oil fields or within production facilities may cause severe damage (Tardy-Jacquenod *et al.*, 1996). Consequently, the knowledge of the dis-

tribution of SRB in oil production and refining set-up could be an indicator for possible bacterial corrosion.

Furthermore, there has been an increased interest in the ecology of SRB because of their implication in bio-corrosion of metals, fouling of crude oil as well as their role in the bio-geochemical cycling of molecules in sediments (Esiobu *et al.*, 1991).

It is generally accepted that in the absence of oxygen, corrosion of metals largely occur due to SRB activity. They are believed to influence the corrosion process either by consuming cathodic hydrogen or by producing sulfide which gives rise to cathodically active FeS.

Information on tropical soil isolates of SRB in association with bio-corroded pipelines and tanks are scanty. Since it is important to understand the physiology and ecology of microbes that are responsible for considerable economic losses, this study was undertaken to detect the presence of SRB around busted pipelines and tanks of African Petroleum, Lagos, Nigeria.

Materials and Methods

Soil samples

Soil samples were collected with sterile spatula from the African Petroleum Depot, Apapa, Lagos. Samples were collected at a depth of 0–10 cm into sterile Mc Cartney bottles. Samples were collected from the following points: A, near a corroded tank; B, 100m away from the corroded tank; C, near a corroded pipeline and D, 100m away from the corroded pipeline. A control sample (E) was obtained from the premises of Flour Mills of Nigeria Limited, Apapa, a distance of about 2 kilometres from the depot.

The Media

The basal medium used for isolation and cultivation of SRB was first described by Postgate (1984) but was modified to allow the isolation of wide range of SRB. The medium contained in 1 litre of distilled water; KH₂PO₄, 0.5 g; NH₄Cl₂, 1.0 g; Na₂SO₄, 1.0 g; CaCl₂·6H₂O, 0.1 g; MgSO₄·7H₂O, 2.0 g; yeast extract, 1.0 g; FeSO₄·7H₂O, 0.5; thioglycolic acid, 0.1 g; ascorbic acid, 0.1 g; sodium lac-

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tate, 0.35%(v/v), sodium acetate (0.1%, w/v) and Agar Bacteriological (Oxoid, Basingstoke, England), 15 g. The pH of the medium was adjusted to 7.6 prior to autoclaving at 121 °C for 15 min.

Isolation and partial identification of SRB

The SRB were isolated by plating diluted soil samples in quadruplicate on sterile medium. The inoculated plates were placed in an anaerobic jar after which a moistened pack of gas generating kit (Oxoid BR 38, Basingstoke, England) was placed inside. Furthermore, the anaerobic catalyst (Oxoid BR 42, Basingstoke, England) was used to produce a low oxygen atmosphere within the anaerobic jar. Incubation was carried out at room temperature ($30 \pm 5^\circ\text{C}$) for 14 days. Blackened colonies due to production of ferrous sulfide were counted. Colonies of SRB and other organisms which have merged on culture plates were purified by streaking on fresh sterile medium and incubated as described earlier. Total counts of bacteria in samples were determined by plating aliquots in quadruplicate on nutrient agar. The SRB were partly identified on the basis of cell morphology as described by Hardy (1981), Hamilton (1983) and Holt *et al.*, (1994).

Metabolic characteristics of the SRB isolates

The ability of the isolates to utilize a range of selected compounds as electron donors and acceptors was investigated in liquid media containing the test substrate. In all experiments, controls were set up which contained the complete basal medium.

Results and Discussion

After 14 days of anaerobic incubation, colonies were observed showing the characteristic dark pigment of SRB on agar plates as a result of deposition of ferrous Sulfide due to H_2S formation. Contamination by non SRB occurred and extensive purification had to be carried out. Although SRB are obligate anaerobes, the SRB are rather oxygen-tolerant (Iverson and Olson, 1984). Hardy (1981) also found that cells of marine strains of SRB survived well in aerobic and anaerobic sea water. The result of the quantification of the SRB from the different soil samples is as presented in

Table I. The enumeration technique yielded fairly high numbers of SRB. More SRB were found near the corroded pipelines and tanks than locations farther away from the corroded metals. The fact that the least number of SRB were found in the control soil situated far away from the location indicated a high probability of the involvement of the SRB in metal corrosion. The SRB population values were higher than what was reported for ground water samples (Johnson and Wood, 1993). However, the SRB population values were lower than what was reported by Jørgensen and Bak (1991) and Dalsgaard and Bak (1994) using the most probable number technique. Soils generally contain higher numbers of SRB than waters due to presence of high levels of organic matter (Esiobu *et al.*, 1991).

The population of SRB at the various sample site is indicative of the rate of sulfate reduction at each sample point. Samples collected from points beside the corroded pipelines and tanks gave the highest SRB number due to the presence of carbon sources which aided their growth. Samples from the depot premises were higher than the value of the control sample because of constant pollution of the area after pipe or tank burst. Though there is no evidence that SRB can utilize hydrocarbons for growth, their proliferation in presence of assimilable organic substances provided by hydrocarbon utilizing aerobes.

This physiological attribute obviously aid their activities in metal corrosion especially in surface pipes and tanks. Five distinct morphological types were encountered. The first designated as Sp were rod shaped, motile and had spores (Table II). It could be related to the genus *Desulfotomaculum*. Others include the ellipsoidal cell with pointed ends and rod shaped cells in filament which probably are related to the genera *Desulfobulbus* and *Desulfonema*, respectively. The coccoid cells and the curved rods are probably related to *Desulfococcus* and *Desulfovibrio* respectively. The detailed characterization and phylogenetic analysis of these isolates are in progress to determine their real taxonomic status.

All the isolates grew optimally by using lactate as sole carbon and energy source. Acetate was utilized by only Sp and Sf while glycerol was not utilized by any of the isolates. All the isolates utilized

Table I. Population of bacteria in the soil samples.

Sample	Total bacterial counts (cfu/g)	SRB (cfu/g)	% SRB
A	$2.71 \times 10^7 \pm 1.16 \times 10^6$	$5.00 \times 10^4 \pm 1.23 \times 10^4$	0.185
B	$2.84 \times 10^7 \pm 3.27 \times 10^6$	$3.05 \times 10^4 \pm 3.02 \times 10^3$	0.107
C	$3.42 \times 10^7 \pm 3.61 \times 10^6$	$6.50 \times 10^4 \pm 4.49 \times 10^3$	0.190
D	$4.13 \times 10^7 \pm 5.08 \times 10^6$	$4.00 \times 10^4 \pm 1.28 \times 10^3$	0.097
E	$2.19 \times 10^7 \pm 3.76 \times 10^6$	$0.25 \times 10^4 \pm 0.29 \times 10^3$	0.011

* Values are mean of four replicates.

Table II. Morphological types of SRB isolated from tropical soil.

Isolates	Shape	Gram reaction	Motility	Spores	Probable genus
Sp	Rod	–	+	+	<i>Desulfotomaculum</i>
Se	Sphere	–	–	–	<i>Desulfobulbus</i>
Sf	Rod	–	+	–	<i>Desulfonema</i>
Sc	Cocci	–	–	–	<i>Desulfococcus</i>
Sv	Curved Rod	–	–	+	<i>Desulfovibrio</i>

sulphate as electron acceptor whereas none utilized nitrate as electron acceptor. Postgate (1984) reported that some species utilize nitrate or fumarate or even selenate instead of sulfate, and others even can grow fermentatively on pyruvate. All the isolates utilized sulfite and thiosulfate as electron acceptor except the isolate designated as Sp.

The abundance and distribution of SRB are influenced by pH, temperature and salinity (Gibson, 1990). The range of pH for growth was close to the value (6–7.4) reported by Esiobu *et al.*, (1991). Because of the upper limit of pH required for growth by these organisms, soil liming around the pipelines and tanks would be a good means of achieving considerable reduction in growth of the SRB and consequently reduction in bio-corrosion of pipelines and tanks. At neutral pH, H₂S in form of HS[–] forms a film on surface of iron which prevents corrosion. However, at low pH values and anaerobic conditions, H₂S is highly corrosive to iron (Iverson and Olson, 1984). $\text{H}_2\text{S} + \text{Fe} \rightarrow \text{FeS} + 2\text{H}^+$.

All the isolates were mesophiles. The ambient temperature in the depot most times of the year is

$33 \pm 2^\circ\text{C}$ which favours growth and activities of the SRB. A wide range of salt tolerance has also been reported for SRB. Hardy (1981) showed that the maximum NaCl concentration permitting growth was 7% (w/v), Voordouw *et al* (1992) reported SRB that exhibited optimum growth at 5% (w/v) of NaCl. In the present study however none of the isolates could grow at NaCl concentration above 5% (w/v). This is expected of soil isolates. So far, the halophilic *Desulfovibrio* strains tolerating high salinities are represented by only one species *Desulfovibrio halophilus* (Tardy–Jacquenod *et al.*, 1996). Consequently periodic flushing of the pipeline and tank areas with waters of 7% (w/v) NaCl concentration could be effective in reducing the growth of the SRB.

Other control measures that could be employed include the use of metal alloys that are resistant to corrosion by SRB, use of protective coating on the pipes and tanks, spraying the soil area with potent biocides and cathodic protection of the pipelines and tanks.

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