

## Sulfate Reducing Potential in an Estuarine Beach

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Sulfate reducing bacteria (SRB) and their activity (SRA) together with total anaerobic and aerobic bacterial flora were estimated during July 1982 - April 1983 and July - Aug. 1984 from 1, 3 and 5 cm depths using core samples. The average number (no.  $g^{-1}$ ) of SRB in these depths was  $1.27 \times 10^2$ ,  $2.01 \times 10^2$  and  $2.36 \times 10^2$  respectively. The averages of ratio of aerobic flora to SRB were 87.6, 61.4 and 27.6 and the anaerobic to the SRB were 3.1, 1.7 and 0.92 at 1, 3 and 5 cm depths respectively. SRA determined in liquid medium using serially diluted sediment ranged from 0.04 at 1 cm depth to  $1.02 \text{ mMS}^{-2} \cdot \text{g}^{-1} \cdot 24\text{h}^{-1}$  at 3 cm. SRA determined directly using sediment slurries showed values up to  $0.99 \mu\text{M} \cdot \text{g}^{-1} \cdot 24\text{h}^{-1}$  at 5 cm depth. Sodium molybdate at 20 mM conc. did not inhibit sulfate reduction.

Sulfate reducing bacteria constitute an ecophysiological assemblage of morphologically very dissimilar anaerobic bacteria which have in common the capacity to activate sulfate and reduce it to hydrogen sulfide in dissimilatory energy conserving reactions<sup>1,2</sup>. Sulfate reduction accounts for as much as 53% of total organic matter breakdown in coastal marine sediments<sup>3,4</sup>. In the present study an attempt has been made to quantify these bacteria in an estuarine beach and to estimate their sulfate reducing potential under laboratory conditions.

### Materials and Methods

Sediment samples were collected aseptically at low tide level from an estuarine beach at Dona Paula, Goa (long.  $73^\circ 48'E$  and lat.  $15^\circ 27'N$ ) using piston corer at monthly intervals from July 1982 to April, 1983 and during July-Aug. 1984. The cores were brought to the laboratory and sub-sampled at 1, 3 and 5 cm levels. Only the central portion of the core was used to minimise external contamination. Analyses were carried out within 30 min of sampling. The sub-samples were serially diluted in sterile 50% seawater blanks to which was added freshly autoclaved sodium thioglycolate (final conc. 0.03%).

Total aerobic heterotrophic bacteria (AB) was estimated on nutrient agar prepared with 50% seawater following serial dilution technique. Total anaerobic bacteria (ANB) was estimated with the same medium supplemented with 0.03% sodium thioglycolate just before inoculation. Agar surface was covered with sterile paraffin oil-paraffin wax (1 : 2 v/v) to a height of 2 cm to maintain anaerobic condition. The colonies were counted after 8-10 d incubation at room temperature ( $28 \pm 2^\circ\text{C}$ ).

Sulfate reducing bacteria (SRB) were enumerated in shake agar using modified medium of Hatchikian<sup>5</sup>.

The medium comprised sodium lactate, 50% - 15 ml;  $\text{NH}_4\text{Cl}$  - 2 g;  $\text{K}_2\text{HPO}_4$  - 0.2 g; yeast extract - 1 g; trace element solution - 5 ml; Agar - 8 g; and 50% artificial seawater<sup>6</sup> - 1000 ml. Freshly autoclaved sodium thioglycolate and filter sterilized  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  solution to give final concentration of 0.03 and 0.05% respectively were added just before use. Final pH was adjusted to 7-7.3 with sterile 0.5N NaOH. The sterilized medium was cooled to  $40^\circ\text{C}$  and dispensed into screw capped tubes containing inocula. The agar surface was covered with paraffin oil-paraffin wax mixture. Black colonies of SRB were counted after 4-6 d incubation at room temperature. Microscopic examination of stained cells of the colonies showed typical curved rods.

Sulfate reducing activity was determined using 1 ml inocula at  $10^{-2}$  dilution. Liquid medium of the above composition dispensed in screw capped tubes was used. In this case,  $2.5 \text{ ml} \cdot \text{l}^{-1}$  of freshly autoclaved 5% solution of  $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$  and  $5 \text{ ml} \cdot \text{l}^{-1}$  of 0.04% solution of  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  acidified with 0.2 ml of 4N  $\text{H}_2\text{SO}_4$  were added. Sixteen tubes were inoculated per sub-sample and 2 were analysed for sulfide content at 24 h interval<sup>7</sup>.

In order to determine SRA directly with the sediments, core samples were collected again during July-Aug. 1984. Sterile screw capped tubes were filled 3/4 th with unsterilized seawater, collected at the site of sampling, to which sediment samples were added as slurry to fill them. The seawater was supplemented with 10, 100 or 500  $\mu\text{M}$  of lactate and 0.0125%  $\text{Na}_2\text{S}$ . The tubes were capped, inverted once or twice to mix and then incubated at room temperature. About 10 tubes were inoculated per sample of 1, 3 and 5 cm depth. Tubes were removed at 3 h intervals and analysed for sulfide content. Dry weight of the

sediment in each tube was later determined, to estimate the activity per gram of dry sediment.

**Results**

AB population decreased with increasing depth. Total aerobic counts at 1 cm were marginally higher at 2 or 5 cm (Fig. 1).

SRB varied from undetectable level at 1 cm to  $6.7 \times 10^2 \text{ g}^{-1}$  sediment at 5 cm. Sediment at 5 cm depth always harboured a larger number of SRB than the regions above it. AB/SRB and ANB/SRB (Table 1) decreased with depth. Average SRA/SRB showed highest value at 3 cm.

SRA was determined for 8 d. The control tubes with no inoculum took 72 h to register 0 value for the added sulfide. Therefore 24 h values have been calculated by dividing the peak value by the number of days. Hence for comparison, in Fig. 1, 72 h value, peak value and the calculated 24 h value are given.

Activity determined after 72 h incubation was highest at 1 cm level throughout and ranged from 0.1 to  $2.2 \text{ mMS}^2 \cdot \text{g}^{-1}$ . Peak values for the period of

incubation however varied with depth. Thus at 1 cm the average activity was  $1.58 \text{ mMS} \cdot \text{g}^{-1}$  and the highest value was  $2.7 \text{ mMS} \cdot \text{g}^{-1}$ . The average peak values at 3 and 5 cm were 2.49 and  $2.19 \text{ mMS} \cdot \text{g}^{-1}$  respectively. The highest values recorded were 5.2 and  $5.6 \text{ mMS} \cdot \text{g}^{-1}$  at 3 and 5 cm respectively. This is equal to 18.81 and 21.46% of the sulfide that could be formed from the sulfate in the medium. When values for 24 h were computed from peak values the highest values recorded were 0.67, 1.02 and  $0.91 \text{ mMS} \cdot \text{g}^{-1}$  respectively at 1, 3 and 5 cm depth.

SRA when measured directly using low substrate concentration at 3 h intervals ranged from undetectable levels at 1 cm to  $0.99 \mu\text{M} \cdot \text{g}^{-1} \cdot 24 \text{ h}^{-1}$  at 5 cm level at the end of first 3 h period at  $10 \mu\text{M}$  lactate conc. (Table 2). With  $500 \mu\text{M}$  of acetate (not indicated in table) SRA was lower ( $0.74 \mu\text{M}$ ) than that recorded for lactate.

**Discussion**

Using sediment density value<sup>8</sup> of  $1.2 \text{ g} \cdot \text{cm}^{-3}$  (this figure was found to be the same for the beach sediment

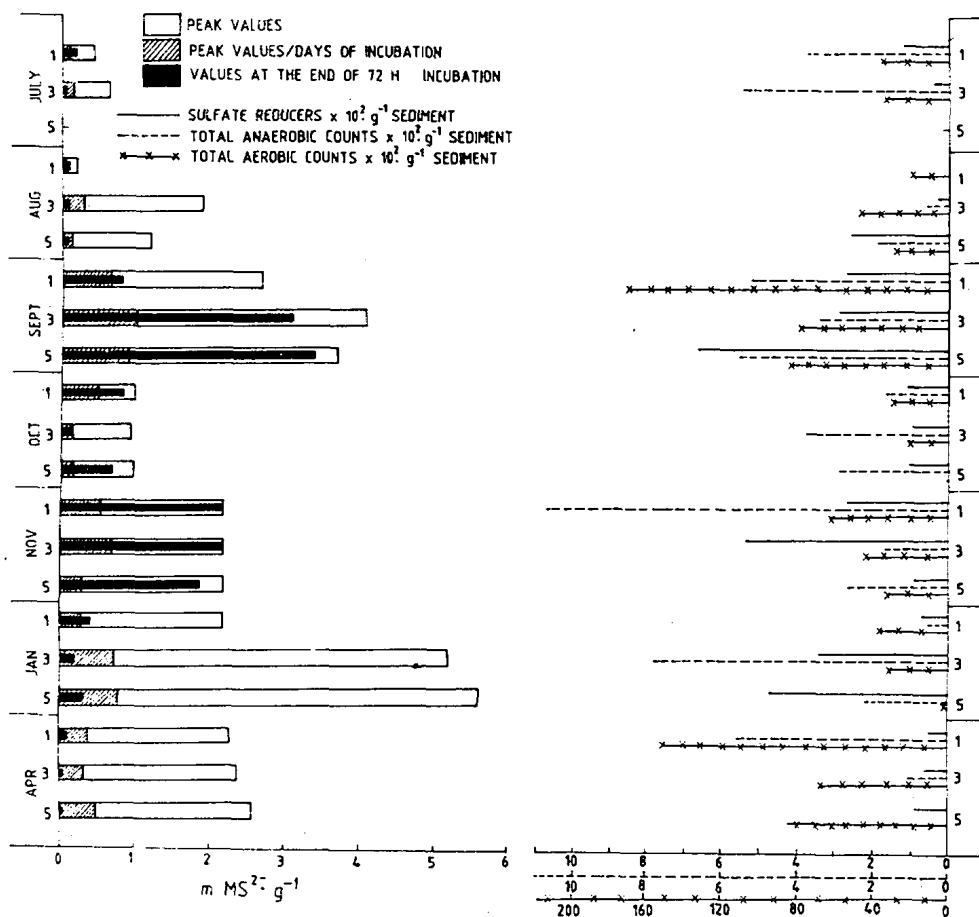


Fig. 1 -- Vertical distribution of AB, ANB, SRB and SRA ( $\text{mMS}^2 \cdot \text{g}^{-1}$ ) at 1, 3 and 5 cm depths of the sediment

Table 1—Ratio of AB, ANB, SRB and SRA at 1, 3 and 5 cm Depths

	AB/SRB			ANB/SRB			SRA/SRB*		
	1	3	5	1	3	5	1	3	5
1982									
July	31.5	97.3	—	2.1	14.1	—	7.5	33.0	—
Aug.	—	155.9	11.7	—	2.0	0.7	—	103.0	6.0
Sept.	64.3	27.4	12.8	2.3	1.2	0.8	25.0	35.0	14.0
Oct.	29.7	23.3	2.7	1.5	4.0	3.0	45.0	14.0	13.0
Nov.	20.0	8.4	42.2	4.0	0.3	3.0	21.0	13.0	31.0
1983									
Jan.	56.5	9.1	0.3	0.7	2.2	0.5	45.0	21.0	18.0
April	323.7	108.7	95.7	11.9	2.0	—	83.0	63.0	58.0
Av.	87.6	61.4	27.6	3.1	1.7	0.9	32.0	40.3	23.3

\*Expressed as  $M \times 10^{-7}$  sulfide formed by one colony forming unit.  $d^{-1}$

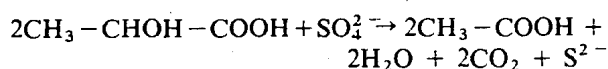
 Table 2—SRA ( $\mu M.g^{-1}.24 h^{-1}$ ) Determined Directly with Sediment Slurry and Supplemented Seawater at 1, 3 and 5 cm Depths

Incubation (h)	Lactate (10 $\mu M$ )			Lactate (100 $\mu M$ )			Lactate (500 $\mu M$ )			Lactate (500 $\mu M$ ) + Na <sub>2</sub> MoO <sub>4</sub> (20 $\mu M$ )		
	1	3	5	1	3	5	1	3	5	1	3	5
	3	ND	0.40	0.99	2.06	0.16	1.36	1.24	1.01	0.86	—	—
6	ND	ND	ND	903	ND	ND	ND	ND	1.83	—	—	0.18
9	0.04	0.08	0.91	0.23	0.07	0.20	1.04	0.49	ND	—	—	1.22
12	ND	ND	ND	0.61	ND	ND	0.11	ND	ND	—	—	0.60

ND = not detected; — not determined

sample used in the present study) the SRB counts (no.cm<sup>-3</sup>) computed would be  $1.52 \times 10^2$ ,  $2.41 \times 10^2$  and  $2.76 \times 10^2$  respectively at 1, 3 and 5 cm depth. The values are hundred fold less compared to Jorgensen<sup>3</sup>. Lyons *et al.*<sup>9</sup> reported SRB numbers ranging from 50 to 660 .g<sup>-1</sup> at Jeffreys and Wilkinson basins. ANB and AB were more than SRB by a magnitude of 10<sup>1</sup> or 10<sup>2</sup>. The present findings show that only AB were higher in number by one order of magnitude throughout the period and rarely by 10<sup>2</sup> (Fig. 1).

Sulfate reducing activity (SRA) or sulfate respiration may be represented as follows:



SRA is measured in terms of sulfide produced rather than sulfate utilized. The closed culture tubes make it possible to estimate the sulfide produced. The peak values of SRA (Fig. 1) ranging from 0.2 mM at the surface (1 cm depth) to 5.6 mM at 5 cm depth give an

idea of the rates and amount of sulfur being turned over in these sediments. A minimum period of 72 h is necessary to record any measurable activity when working with serially diluted sediment as the inoculum. The 24 h values calculated from these figures (Table 3) are less than those calculated from the peak values but the range is still in  $\mu MS^{-2}.g^{-1}.24 h^{-1}$ .

SRA determined directly with undiluted sediment slurry and seawater supplemented with low concentrations of substrate enables measurement of sulfide at shorter intervals (Table 2). Thus at 10  $\mu M$  conc. of lactate (substrate concentrations available per gram is  $30 nM \pm 5nM$ ), SRA ranges from undetectable levels to  $0.99 \mu M.g^{-1}.24 h^{-1}$ . With an increase in lactate concentration the activity recorded only a marginal increase. When values are calculated for 1  $\mu M$  substrate conc. for comparison, the tubes with 10  $\mu M$  substrate conc. gave higher values than those with 100 or 500  $\mu M$ . With lower concentration (10  $\mu M$ ), 5 cm depth gave much higher values than 3 or

Table 3—Sulfate Reducing Activity ( $\mu M.g^{-1}.24 h^{-1}$ ) Computed from 72 h Values Determined with Diluted Sediment and Culture Medium at 1, 3 and 5 cm Depths

Months	Depths		
	1	3	5
1982			
July	67	13	—
Aug.	30	30	30
Sept.	260	1030	1130
Oct.	280	50	223
Nov.	730	730	630
1983			
Jan.	130	67	100
April	36	13	13

1 cm, but with 500  $\mu M$  conc. the trend is similar to that measured in culture medium and diluted sediment. This suggests that SRB at deeper regions use low concentrations of substrates more efficiently than their counterparts in shallow regions. Short term experiments carried out with acetate at 500  $\mu M$  conc. at 1 cm level showed SRA of 0.74  $\mu M$  which is lower than that recorded for lactate.

Sodium molybdate is known to be an inhibitor of sulfate reduction<sup>10-12</sup>. However, when it was used in control tubes, sulfide was always detected indicating that it did not have any inhibitory effect. The observations suggest that the physiology of SRB in these tropical sediments may be slightly different from those occurring in temperate waters.

Sulfate reduction in waters and sediments have been measured using radio tracer techniques and values up to 1000 nM  $SO_4^{2-}.cm^3.d^{-1}$  have been recorded in summer months<sup>3</sup>. Nedwell and Abram<sup>13</sup> reported rates between 1 and  $50 \times 10^3 mg-at.SO_4 - S cm^{-2}.d^{-1}$  above 20 cm column in Colne point salt marsh in June-July when *in situ* temperature was between 18° and 24°C. In the present study, SRA determined directly with the sediment is  $10^3$  fold lower than that determined in culture medium (Tables 2 and 3) but is only  $10^1$  fold higher than values given by Jorgensen<sup>3</sup> and nearly the same as that recorded by Nedwell and Abram<sup>13</sup>. With diluted sediment samples, both SRA and SRB could be estimated from the same sample. A certain trend could be observed—the turnover of sulfur through sulfate respiration was low and the rate was fast at 1 cm whereas it was opposite at 3 and 5 cm levels.

There is a significant relationship between the numbers of SRB and the activity for 24 h from peak values ( $r = 0.74$  at 0.1% level). The population is also proportional to the peak values ( $r = 0.6$  at 1% level)

and to the 72 h incubation value ( $r = 0.64$  at 1% level). This correlation between the activity and the number of colony forming units is in agreement with the observations made by Jorgensen<sup>8</sup>.

The averages of the ratio AB/SRB and ANB/SRB (Table 1) clearly indicate the abundance of total aerobic and anaerobic heterotrophs with respect to SRB. The values decrease with depth, showing that SRB increases with depth while the general aerobic and anaerobic flora decrease with depths. SRA/SRB gives the specific activity of bacteria (Table 1). The activities calculated are  $10^5$  fold higher than that reported by Jorgensen<sup>8</sup>. If lactate concentration is assumed to be in  $\mu M.g^{-1}$  sediment then the concentration of lactate available per gram sediment under experimental conditions is  $10^6$  fold higher. Hence, theoretically, the specific activity could be calculated to be  $10^6$  fold less. The specific activity would therefore range from  $60 M \times 10^{-12}.colony^{-1}.d^{-1}$  at 5 cm in Aug. to  $1030 M \times 10^{-12}.colony^{-1}.d^{-1}$  at 3 cm in the same month. This is still  $10^1 - 10^2$  fold higher than the values obtained by Jorgensen<sup>8</sup> which is  $1.5 M \times 10^{-12}.colony^{-1}.d^{-1}$ .

Higher numbers of AB and ANB at 1 and 3 cm levels indicate a higher aerobic/anaerobic turnover of carbon in these depths when compared to the 5 cm depths where sulfate reducers have a more important role than the other two. Although only lactate dependant sulfate reduction and sulfate reducers have been estimated in the present study, it has been found that the distribution of lactate utilizing sulfate reducers in marine sediments was the same as that for acetate and propionate utilising species<sup>14</sup>. However, Balba and Nedwell<sup>15</sup> showed that carbon flow through acetate pool was far greater than that through either propionate or butyrate in Colnepoint salt marsh. Hence further studies on the activity dependent on other fermentation products needs to be pursued to throw more light on the substrate versatility or specificity and also on the variations in the sulfate-reducing activity of these microorganisms in the tropical sediments.

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