Stimulation of Sulphate Reducing Activity at Salt - Saturation in the Salterns of Ribandar, Goa. India

S.Kerkar [1] and P.A.Loka Bharathi [2]

[1] Department of Biotechnology,Goa University

[2] National Institute of Oceanography,Goa

Abstract:

In order to address the question if Sulphate Reducing Activity (SRA) ever gets arrested at salt saturation, it was measured at 0-2, 2-5 and 5-10 cm depth in sediment cores from the salt pans of Ribandar (Goa) using ^{35}S – sulphate. The activity was related to the abundance of culturable Sulfate Reducing Bacteria (SRB) and the prevailing environmental conditions. The salinity in these ponds ranged from 10 to 350 psu during the salt producing season. The present study compares the rates of SRA under mesohaline (85 psu) and hypersaline conditions (330 psu) as are available in the salt pans during salt producing season. Variation in culturable abundance of SRB governed 98 and 75% of the variation in SRA at hypersaline and mesohaline conditions respectively.. Though SRA of 3.34 $nM.g^{-1}.hr^{-1}$ could be measured at hypersaline conditions of 330 psu, higher SRA of 116.03 $nM.g^{-1}.hr^{-1}$ could be measured in mesohaline conditions of 85 psu. These values seem to be higher than the rates reported for Black Sea or tropical mangrove forests. The present study shows that SRA is optimal between a salinity of 60 to 120 psu. Contrary to earlier reports (Oren, 1999), we hypothesize that SRA is not totally arrested at salt saturation but could rather get retarded. Further, our studies show that availability of an extraneous carbon source could serves to stimulate and thus counter the retardation. Our finding suggest that SRA in saturated salt pans is apparently more carbon than sulfate limited.

Keywords: Sulphate reducing activity, Sulphate reducing bacteria, Hypersaline, Saltpan.

INTRODUCTION

Sulphate Reducing Activity (SRA) i.e. anaerobic respiration with sulphate is the central component of the global Sulphur cycle and is exhibited exclusively by Prokaryotes (Rabus et al. 2000). This process can dominate anaerobic terminal oxidation of organic matter in high sulphate systems, degrading up to 50% of the organic matter in coastal marine sediments (Jørgensen,1982). In low sulphate sediments however it plays a minor yet an important role (Nedwell 1984; Takii & Fukui 1991). Dissimilatory sulphate reduction is carried out by a diverse group of strictly anaerobic Sulphate Reducing Bacteria (SRB) that share the ability to use sulphate as a terminal electron acceptor in the oxidation of organic matter, thus reducing sulphate stoichiometrically to sulphide. This activity is an important biogeochemical process that links the Sulphur and Carbon cycle. The reduction of sulphate to sulphide influences many other metabolic processes e.g. it has a profound effect on the availability of trace metals and directly influences the uptake by marine biota. Interaction of SRB with DMSP (Di-methyl sulphoniopropionate), an osmolyte of planktonic origin can have wide implications on the climate. It would be interesting to know the rates of SRA from salterns because these are an integral part of the communities living along the coast. Saltpans abound the estuarine zones of Goa, India and occupy a low lying area of 18,000 ha i.e. about 0.5% of the total area of Goa (3715 sq.km.). Given the expanse of the saltpans in this area and the importance of SRA itself in the geochemical processes of the marine and coastal ecosystem in particular, and the climate in general, it would be relevant to understand the contribution of SRA to the flux of carbon from these salterns.

SRB constitute a physiological and ecological assemblage of morphologically very different types of anaerobic bacteria (Le Gall and Postgate 1973). Electron donors include hydrogen and low molecular weight organic acids, such as lactate or acetate which are also utilized as carbon sources. Some species of SRB are nutritionally more versatile with ability to grow on high molecular weight fatty acids or simple aromatic compounds such as benzoate or phenol (Postgate1984; Widdel 1988). Moreover, spatial and temporal variability or the influence of temperature or electron donor concentration on the rates of sulphate reduction has been reported for marine sediments. Though many studies have dealt with ecological and eco-physiological aspects of sulphate reduction in hypersaline photosynthetic microbial mats,

relatively few studies have been concerned with SRA in hypersaline sediments where active sulphate reduction has been demonstrated at salinities up to 24-30% w/v NaCl (Klug et al.1985). Till now, microbial research in this area had been confined to the aerobic, halophilic microflora and relatively little work has been reported on the anaerobic halophiles or their ability to breakdown organic matter under anaerobic conditions in saltpans.

Man-made solar salterns consist of a series of linked pans where gradients of salinity occur due to evaporation of sea water: Brine gets concentrated sequentially, demarcating the saltern into primary, secondary, tertiary and finally the crystallizer pond. Salinity in these ponds range from 10 to 350 psu during the salt producing season. However, the present study limits the observations to two salinity levels under mesohaline (85 psu) and hypersaline conditions (330 psu) of the peak salt producing season.

Sulphate reduction rates were measured in undisturbed sediment cores or in sediment slurries using radio-labeled sulphate. It is envisaged that with increasing salinity, the SRB community would respond by increased SRA and the study examines the limits of salinity that establishes maximum SRA. Hypersaline conditions could cause stress to certain physiological groups which could respond by increased rates of respiration. The present paper attempts to address sulphate reduction in the evaporative saltpans of Ribandar, Goa, India. It re-examines the hypothesis of Oren (1999) that sulphate reduction is eliminated at salt saturation. We however append here that, 1) sulphate reduction is not totally eliminated at saturation 2) rather it could be stimulated to a certain extent on addition of a suitable carbon source to counter carbon limitation in the system.

MATERIALS AND METHODS

The traditional salt industry has been existing in Goa since 500 A.D. Goan salt was considered to be of the best quality and was exported to several African and Arabian countries during the post Medieval period. Goa has over 200 salt pans, still existing in 13 villages of 4 Talukas of Pernem, Bardez, Tiswadi and Salcette and produces 35,000 metric tones of salt annually.

Sampling site :

The study site was the Ribandar saltpans (15 ^{0} 30.166 N and 73 ^{0} 51.245 E) Goa, India and is situated along the Mandovi estuary in Tiswadi taluka. The total area of this saltpan is 12,329.12 m^2 (Fig 1 A and B). This area is under the influence of semidiurnal tides and the surrounding marshy land supports a rich mangrove vegetation. The site is subjected to heavy annual rains (125 cm) during the monsoons. The prevailing climate is characterized by three distinct seasons viz. Pre-monsoon: from February to May; Monsoons: from June to September and Post Monsoon: from October to January. The climate on an average is generally warm and humid, fluctuating from a minimum of 20^{0}C in the month of December to 42 ^{0}C in May .The salt producing season is normally during the non monsoon months. Salt pans are located in areas where the ground level is held below the low tide level. Pans are cleared of muck subsequent to the monsoon and leveled by stamping to make the bed flat and hard. It takes approximately 20 days to clean up and prepare a salt pan. Each pans is surrounded by 'bandhs' or mud borders on all four sides to prevent siltation and help regulate the flow of water. In the Ribandar saltern, sea water from the Mandovi estuary enters the creek through a sluice gate at high tide and during low tide, water from the creek is drained out into the estuary. Feed water is drawn from this creek and is allowed to flow from the first bed into the second and ultimately to the crystallizer pan. The flow of water is regulated by using straw-mud plugs in the bandhs. Water gets heated up evaporates in the sun, forming frothy layer at saturation. To speed up crystallization, the pan is seeded with crude salt. In the peak salt producing season, salt is harvested and collected daily.

Sample Collection / Analyses:

Sediment cores (0-10 cm) from the salt pans of Ribandar were collected in triplicates using an 1.5 inch diameter, graduated PVC corers. The corers were sealed at both ends with sterile core caps to prevent direct contact with air and transported to the laboratory in an icebox for further physico-chemical and microbiological analysis. All sample collections at 0-2, 2-5 and 5-10 cm were carried out at a fixed time i.e. 10:30 a.m. regardless of the tide for analyses. Overlying saltpan water was collected in sterile glass bottles. Samples were analysed within a few hours of collection.

Physico-Chemical Parameters:

Portions of the sediments were analyzed for temperature recorded on site. Eh and pH were measured by Thermo Orion model 420A, USA as described in Orion instruction manual. Sulphide was analysed by Pachmayr's method (1960) as cited by Schlegel et al. (1964). About one gram sediment samples were fixed in 2% zinc acetate for sulphide estimate. Subsequently sediment dry weight was determined. Total organic carbon (TOC) was estimated by titrimetry with the wet oxidation method of Allen et al(1976). Similarly 1ml water samples from salterns were collected at different salinities and fixed for sulphide. Dissolved oxygen was also measured by Winkler's titrimetric method (Carpenter,1965).

Pore water was collected by centrifugation and analyzed for salinity using a hand refractometer (S/MillE, ATAGO, Co.Ltd, Japan) and for sulphate by turbidometry (Clesceri et al.1998) respectively. Salinity was expressed as psu (percentile salinity units) or g/l. Particle size analyses at three depths was carried out by Wet sieving method for sand and the Pipette method for silt and clay analyses (Day,1965;Carver,1971). All measurements were carried out in triplicates.

Enumeration of SRB .

Agar shake tube method was used for enumeration of SRB. Hatchikian's medium, (1972) was modified using seawater and by supplementing with 6.2% NaCl to give a final concentration of approximately 10%. The carbon sources used were sodium lactate (0.75% v/v) and sodium acetate (0.2% w/v). Black colony forming units were counted following incubation of 15-20 days at room temperature (28 ± 2 ^{0}C) and expressed as average number of triplicate tubes at countable dilutions. Bacterial counts were also carried out using the MPN (Most probable number) method with the same liquid media supplemented with 0.5 mM lactate and 0.5 mM acetate as carbon sources .The medium was prepared anaerobically and dispensed in screw cap tubes with butyl rubber stoppers. Samples were counted in three fold MPN dilutions series, which were carried out with 1gm sediment samples from three depths that were subsequently diluted in eight 1:10 dilution steps.

Statistical analysis was carried out using MS Excel version 2000 to relate physicochemical and bacteriological parameters.

Sulphate Reducing Activity:

The sulphate reducing activity is described in three parts.

Part1: Unammended sediment from 3 depths incubated for 24 and 48hrs:

Triplicate mini cores from the above intact cores (85 and 330 psu) were prepared corresponding to the three depths by removing the luer tips from plastic disposable syringes (2ml) before drawing in sediment sample. Each sample was homogenized anaerobically by stirring under a nitrogen atmosphere in air tight plastic bags and immediately used for experiments (King 2001). Sodium sulphate 10 µl (74 KBq) from BARC, Mumbai was injected into the homogenized section to distribute the label evenly and the activity was arrested at the end of 24 and 48 hrs. Further series of experiments were restricted to 0-2 cm as SRA was maximum at this level.

Part2:Unammended sediments from 0-2cm incubated for short period (hours) for 85psu and prolonged period (days) for 330psu

Unamended mini-cores from 0-2 cm were processed as in Part1 and incubated anaerobically for four different incubation periods viz. 12, 24, 48 and 60 hrs for 85 psu However, for 330 psu cores, the incubation time was prolonged to 1,2,4,8,12 &16 days .

Part3 :Ammended sediment slurry from 0-2cm incubated for 24 to 48 hrs.

Surface sediments 0-2 cm depth from both salinities were amended with 10 mM concentrations of each of the following substrates viz .acetate, formate, butyrate, lactate and benzoate and mixed in 5 different glass vials using autoclaved sea water to give a total slurry of 2 ml. Samples were incubated with radiolabel ^{35}S for 24 and 48hrs.

Radiotracer assay in sediments was carried out using single step chromium reduction assay (King 2001). Perkin Elmer Wallac 1409 DSA liquid scintillation counter was used for measuring the radioactivity. Sulphate reduction rate was calculated using the formula:

$$(H_2\,{}^{35}S/{}^{35}SO_4^{-2}) \times {}^{32}SO_4^{-2} \times IDF/T \text{ dpm}$$

Where IDF = isotopic discrimination factor taken as 1.06

T = incubation time.

SRA was expressed as $nM.g^{-1}.d^{-1}$ in sediments and $\mu M.d^{-1}$ in water. Experimental controls for slurry without labeled sulfate were always run parallely to check for background contamination from ^{35}S aerosols. These readings were generally low and used for correcting experimental readings. Autoclaved sediment slurries during the initial standardization were used for correcting the experimental values. These readings ranged from 0.0037 and 0.00438 $nM.g^{-1}.d^{-1}$

Carbon flux

Carbon mineralisation has been calculated based on Redfield's stoichiometry for organic matter oxidation by sulphate reduction C_{org}: SO_4^{-2} = 2.1 (Volkov et al, 1998) and on the basis that on an average 2 moles of organic carbon (C) are oxidized for every mole of sulphate reduced (Jorgensen, 1977). For comparison, sulphate reduction or carbon mineralization rates expressed by different authors, have been converted to a common unit of $g.C.m^{-2}.d^{-1}$(Table 4)

RESULTS

Physico-chemical Parameters:

Temperature measured in the hypersaline ponds was generally higher at least by 5 ^{0}C in the surface. Like wise salinity and sulphate in the hypersaline sediments were 3-4 times more than in mesohaline ponds. However, the mesohaline salterns were more alkaline especially at the surface. Thus sulfide values were nearly 4 times higher in mesohaline ponds and increased with depth. These values were matched with lower redox values, almost three times lower at the surface of the mesohaline pond.

The sediment was dark brown in the top 2 cm and dark black below. These vertical changes were also reflected by geochemical and biological parameters. The sediment at 0-2 cm was dominated by 45 % silt; 46 % sand and 45 % clay at 2-5 cm and 49 % sand at 5-10 cm.

Counts

SRB from the mesohaline pond sediments grew homogeneously and at a faster rate in the medium than those from hypersaline ponds which formed flocs and aggregates. Low MPN ranging between 0.53 X $10^3 g^{-1}$ to 0.93 X $10^4 g^{-1}$ were noted at 330 psu, with highest cell numbers at 2-5cm. The agar counts however were one order lower in the surficial sediments and decreased by two orders of magnitude in the deeper section. In the mesohaline pond, the MPN counts were 1.13 X $10^5 g^{-1}$ at the surface and decreased by one order with depth (Table 2).

Sulphate Reducing Activity:

Maximum SRA was generally restricted to the black sediments below the brown on top. The rates were also affected by salinity which generally enhanced the SRA up to 80 psu and then decreased drastically up to 125 psu (Fig 2). Maximum recorded SRA with ^{35}S at 330 psu was 3.34 $nM.g^{-1}.hr.^{-1}$ On the other hand in mesohaline ponds the rate increased by 30 times to 116.02 $nM.g^{-1}.hr.^{-1}$ following 24 hr incubation period (Fig 3A). Short incubation times of 24 and 48 hrs gave consistent results at 85 and also at 330 psu whereas longer incubation times gave erratic results. Amendment with 5 different substrates to 330 psu core, showed an enhancement in activity for all substrates added after 24 hr. However, there was no significant difference in activity for different substrates. Lactate amendment after 48 hr, showed a maximum SRA of about 10.9 $nM.g^{-1}.hr.^{-1}$ which is 23% more than that of control. Thus the substrate value gradation was Lactate > Benzoate > Acetate > Formate against depthwise gradation of 0-2 > 2-5 > 5-10 cms. (Fig 4). Amendment to 85 psu sample made no difference in SRA as such at 24 hr incubation, where the activity ranged from 86.62 to 86.71 $nM^{-1}.g^{-1}.hr$ as compared to the control which was 86.6$\pm$ 0.1 nM g^{-1}. hr^{-1} (not shown in figure).

DISCUSSION

Sulphate dominates the dissolved electron acceptors in marine sediments in concentrations up to 28 mM, which is much greater than all other electron acceptors i.e. O_2-NO_3 combined (King, 2001). Sulphate reducing activity (SRA) in marine ecosystems, especially the coastal regions contributes as much as 50% organic carbon turnover (Jorgensen,1982). Sulphate accounts for 70 - 90% of total respiration in salt marsh sediments, where total sediment respiration rates are 2.5 –

5.5 g.Cm^{-2}.day^{-1}. The role of SRB could also be equally important in the hypersaline salt pans of Goa. Besides, being hypersaline environments, the marine saltpans are high in sulphate concentration up to 12.6g/L or 131 mM and low in oxygen (minimum value of < 0.045 mM) (Kerkar,2004). Thus with decreasing oxygen level, dissimilatory sulphate reduction becomes more prominent.

SRA measured at 85 psu was nearly thirty times the value measured at 330psu. This activity is constrained not only by the microbes responsible for it but also by a suite of physico chemical parameters. At 330 psu, temperature, besides salinity and sulphate concentration all probably have greater influence on SRA than at 85 psu. At 330 psu, the correlation coefficient of the relation between sulphate and salinity is 0.81 thus suggesting the influence of variation in one affects the other up to 65%. SRA is also dictated by temperature. It has a higher effect at higher salinity (330 psu) at a r value of 0.82 indicating an influence of 67%. The measured sulphide is also dictated by SRA to a statistically significant level ($r = 0.96$; $p>0.001$). Increased SRA increases sulphide content. This in turn increases the pH. Therefore in sulfate reducing sediments, salinity relates to pH positively. Hence pH is more influenced by salinity at 85 psu because of increased SRA as compared to the activity at 330 psu. "

SRB numbers were low at 330 psu. However, SRB abundance has a stronger influence on SRA at this salinity as compared to 85 psu. Besides, the bacterial community like TDLO (*Thiobacillus denitrificans* like Organisms) that are responsible for using the sulphide produced, also influence the SRA (Loka Bharathi et al.1997) as TDLO can be retrieved both anaerobically in agar shake tubes and aerobically on plates. Paradoxically, TDLO retrieved under aerobic condition relate strongly to SRA at 330 psu at an r value of 0.99 when the solubility of oxygen is relatively low. Those retrieved under anaerobic conditions have a stronger influence on SRA at 85 psu at an r value of 0.745 when the solubility of oxygen is higher. It is noted that TDLO at 330 psu are 1-2 orders higher than at 85 psu. These observations suggest that TDLO have an optimal level of oxygen requirement which allows them to function either under oxic or anoxic conditions (Kerkar 2004). The measure of sulphide in an environment is a net concentration of production minus utilization or sulphide production and sulphide oxidation. In this study the TDLO which are responsible for oxidizing sulphide and reducing nitrate have been enumerated to account for sulphide oxidation if any, in these sediments. Incidentally, the amount of NO_3

measured in the overlying waters of these sediments was 4.3- 4.5 µM and nitrite ranged from 0.84 to 0.9 µM and in these conditions the dissolved oxygen varied from 0.152 to 0.161mM O_2. These electron acceptors can be used by Thiobacillus-like group to oxidize sulphide. Though they have not been experimentally tested, field observations show that they bear a strong relationship to SRB (Kerkar ,2004). The abundance and distributive patterns of SRB suggest that 330 psu could be relatively more conducive for higher specific activity of SRA as MPN of SRB was 10 to 50 fold lower than that at 85 psu .

The total number of SRB and the rate of SRA are both higher at 85psu than at 330psu. Though SRA was measured at all the three depths, the measurements were restricted to surface layer of 0-2 cm as SRA was consistently highest at this layer irrespective of salinity. SRA measured at 330 psu was nearly thirty times lower than value measured at 85 psu at the end of 24 hrs. We presumed that longer incubation times would be conducive for higher salinities, so 330psu sediments were incubated for longer periods. However, SRA measured at 0-2 cm at the end of 4 days was only 3.34 $nM.g^{-1}.hr^{-1}$ which is only 37% of the values obtained by short incubation period of 24 hr. Hence, we preferred to be consistent with short incubation periods of 24 to 48 hr for measuring the activity for both salinities. However, as the SRA was low especially at 330 psu, substrate amendment was tried. Fatty acid amended sediments at 85 psu did not show any significant change in the SRA which varied between 86.6 and 86.7 $nM.g^{-1}.hr^{-1}$ as compared to the control which was 86.5 $ng.g^{-1}.hr^{-1}$. However, the 330 psu amended sediment showed an SRA which ranged from 5.94 to 6.53 $nM.g^{-1}.hr^{-1}$ compared to control without amendments that gave 5.31 $nM.g^{-1}.hr^{-1}$ thereby indicating that the amendment caused an increase of 19 to 39 % in SRA. Similarly, at 48 hr lactate amendments showed a maximum SRA of about 10.9 $nM.g^{-1}.hr.^{-1}$which is 23% more than that of control. The gradation of the influence of substrates was Lactate > Benzoate > Acetate > Formate (Fig 4). These observations with amended sediments suggest that longer incubation periods up to 48 hr increased the SRA at 330psu.

The SRA value of 2050 $nM.cm^{-3}.d^{-1}$ obtained by tracer technique at 85 psu at the surface layer of the sediments was 4 orders more than the rates reported by Li et al (1999) which was 0.5 $nM.ml^{-1}.day^{-1}$ for sediment slurries. These authors have reported a seasonal variation with a maximum value of 13 $nM.ml^{-1}.day^{-1}$ (Table 4 A & B). These changes in the rates of

sulphate reduction have been linked to the sulphate content by Li et al (1996). They reported highest values after a flood and the increased activity was attributed to the stimulation by both sulphate and organic carbon in the flood water. In these salterns unlike the limnetic conditions, there is very little sulphate limitation and availability of organic carbon could be less. TOC values have been measured for surface sediments and found to be as low as 2.4%. Hence, higher activity in the salt producing season may not be due to TOC or sulphate alone but rather to the overall increase in salinity. This would lead to higher respiration rates, although such positive influence of salinity on SRA was evident only from 80 to115 psu. Ollivier et al. (1994) state that, as there is no evidence of oxidation of volatile fatty acids (VFA) such as acetate by sulphate reducers at high salinities, the ambient strains seem to be incomplete oxidizers, resulting in accumulation of acetate in sediments. Oren (1998) suggests that salinity could have a role in limiting the decomposition of VFA i.e. increasing salt concentrations up to 300 psu resulted in the unusual accumulation of levels up to 800 mM of acetate. Further he reported that at salinities above 150 psu, sulphate reduction decreases. This threshold is quite higher than the threshold seen in the present study .It is thus possible that there could be differences in the threshold values in different marine salterns.

SRA ranged from 150 $nM.g^{-1}.day^{-1}$ to 2790 $nM.g^{-1}.day^{-1}$ in the salt pan sediments (0-10 cm) at 85 psu. These values are much above those reported for Lake Elisa in South Australia where the average values are 55 ± 14 $nM.g^{-1}.day^{-1}$ (Ollivier et al. 1994). The range of the activity in the present study on volume basis, ranged from 13.1 $\mu g.cm^{-3}.day^{-1}$ at 5-10 cm to 65.6$\mu g.cm^{-3}.day^{-1}$ at 0-2 cm at 85 psu. These values are 408 $nM.cm^{-3}.day^{-1}$ to 2050 $nM\ cm^{-3}.day^{-1}$at 85 psu and 8-60 $pM.cm^{-3}.day^{-1}$at 330 psu. This range is much wider and the maximum values are much higher than that reported for Great Salt Lake at Utah by Brandt et al. (1999). These authors reported values of 363 ± 103 $nM.cm^{-3}.day^{-1}$ and 6131 ± 835 $nM.cm^{-3}.day^{-1}$ at 120 and 50 – 60 psu respectively (Table 4A). Still higher rates have been reported by Caumette et al. (1994) with values of 8200 $nM\ .cm^{-3}.d^{-1}$ in the hypersaline ponds of Salins-de-Giraud, France.

Jorgensen and Bak (1991) have emphasized that when SRA is higher at the surface than at the sub-oxic or anoxic zones below, the actual SRA could be much higher than what is measured and therefore could be underestimated. The sharp zonation and restriction of SRA to the upper few centimeters are different in many marine sediments. Sulphate can penetrate much deeper

into the sediments and thus the zone of SRA can reach even up to a distance of 10-20 cm or more (Teske et al. 1998;Jorgensen et al. 1990). The relatively higher SRA observed at the shallower depths thus appears to be quite common in marine environments.

The oxic conditions in these layers do not seem to inhibit the activity of SRB. Dissolved oxygen measurements have been made for overlying water which range from 44μM to 157 μM O_2 where the prevailing Eh was -26 to 340 mV. However, for the sediments the measured Eh ranged from -75.33 to -316 mV, suggesting the O_2 values were below detection limits ~ 7μM O_2. Pure culture studies with *Desulfovibrio* sp. have demonstrated that sulphate reducers are able to survive exposure to dissolved air for hours under sulphide free conditions (Cypionka et al.1985). However, these authors also state that oxygen is toxic only if H_2S was also present. Under such conditions it is still intriguing how SRA can be maximum at the surface where paradoxically sulphide or oxygen can co-exist.

As expected the redox potential decreased with depth. The decrease in redox potential with depth is neither due to increased SRA or SRB with depth but due to decreased oxidation of sulphide in the depths below as compared to that on the surface. This trend prevailed throughout the sampling season and is indicative of sediment layers being comparatively less disturbed by bioturbation. Routine disturbance like salt harvest or allowing inlet of water does not greatly seem to affect this trend.

The distribution and numbers of the SRB were retrieved maximally at 0-2 cm together with highest SRA recorded. This could be attributed to the physical texture of the silt and clay fraction which amounts to 85% in the surficial sediments. Albrechtsen and Winding (1992) state that the total number of bacteria and potential for mineralization are influenced by sediment texture with increasing content of clay and silt and decreasing content of sand. These authors noted that the total counts increased but the mineralization rate declined. The nature of the sediment also governs the redox potential to some extent. Smart and Barco (1978) found higher Eh values in sand than in silt and clay sediments. However, in our studies the grain size influenced the depth of the sediment core where the silt and clay fractions dominated the top layers and the sandy fraction increased with depth. Decreased potential anaerobic activities were more prevalent in

sandy zones than in silty and clay zones which is not because of lower concentration of organic matter but also due to increased probability of aeration.

Integrated ^{35}S sulphate reduction measurement in sediments at 330 psu was only 0.7 X 10^3 $mM.m^{-2}.y^{-1}$. This was 30 times lower than the values measured at 85 psu (21.4 X 10^3 $mM.m^{-2}.y^{-1}$.) salinity. This decrease in activity could be explained on the basis of the limits of salinity within which SRA is optimal. The present study shows that SRA is optimal between a salinity of 60 to 120 psu.

Conclusion:

The culturable fraction of SRB alone could contribute to an important variability in SRA under mesohaline and hypersaline conditions. SRA on different substrates depict the metabolic versatility of SRB and suggest a more general participation in the flow of carbon and electrons in these hypersaline sediments. The present study shows that SRA is optimal between a salinity of 60 to 120 psu. At 250 psu when salt saturation begins, SRA is not arrested but gets retarded till 350 psu after which it is almost negligible. This ecosystem could contribute to an SRA of 0.066 $M.m^2.d^{-1}$ (24 $M.m^2.yr^{-1}$) during the peak salt producing season and nearly 30 times more under mesohaline conditions corresponding to a carbon flux rate of 131 $mM.m^{2}.d^{-1}$ These values seem to be higher than the rates reported for Black Sea or tropical mangrove forests. At 330 psu after 48 hours' incubation, lactate amendment showed a maximum SRA of about 10.9 $nM.g^{-1}.hr.^{-1}$ showing 23% increase as compared to un-amended control. Thus SRA in hypersaline salt pans is apparently more carbon limited than sulfate limited. The study lends support to our hypothesis that SRA is not totally arrested at salt saturation but gets rather retarded. Salinity threshold of SRA lies between 80-115 psu. Further supplementation with an extraneous source of carbon serves to stimulate SRA and counter the retardation. Further experiments with amendments could indicate the limits of threshold for SRA at different substrate concentration at different salinities.

REFERENCES

Albrechtsen H. J. and A. Winding (1992). Microbial biomass and activity in sub-surface sediments from Vejen, Denmark. Microb. Ecol. 23:303-317.

Allen S.E.,H.M.Grimshaw, J.A.Parkinsan,C.Quarmby and J.D.Roberts (1976).Chemical analysis in methods in plant ecology.S.B. Chapuon(ed). Blackwell Scientific publicatios, Oxford,ChapterB,411-466.

Brandt K. K. B. K. C. Patel and K. Ingvorsen (1999). *Desulfocella halophila* gen nov, sp nov, a halophilic fatty acid-oxidizing sulphate reducing bacterium isolated from sediments of the Great Salt Lake. Int J Syst Bacteriol. 49:193-200.

Carpenter J. H. (1965). The Chesapeake bay institute technique for the Winkler dissolved oxygen method. Limnol. Oceanogr. 10:141-143.

Carver R. E. (ed) (1971). Procedures in sedimentary petrology. Wiley- Interscience, A division of John Wiley and Sons Inc, New York, London, Tokyo 653.

Caumette P., R. Matheron, N. Raymond and J. C. Relexans (1994). Microbial mats in the hypersaline ponds of Mediterranean salterns (Salins-de- Giraud, France). FEMS Microbiol. Ecol. 13:273-286.

Clesceri L. S., A, E, Greenberg and A. D. Eaton. (1998). Standard methods for the examination of waste and waste water.20th edition, American public health Clostridium acetobutylicum. J. Gen. Microbiol. 68:307-318.

Cypionka H, F. Widdel and N. Pfennig (1985). Survival of sulfate-reducing bacteria after oxygen stress and growth in sulfate-free oxygen-sulfide gradients. FEMS Microbiol. Ecol. 31:39-45.

Day P. R. (1965). Particle fractionation and particle-size analysis in Black, C. A. ed. Methods of soil analysis, Part 1: American Society of Agronomy, Inc., Madison, Wisconsin, 545-567.

Hatchikian, E.C. (1972). Mechanism d'oxido-reduction chez les bacteries sulfato-reductrices. Thesis Marseilles.

Jorgensen B. B. (1977). Bacterial sulphate reduction within reduced microniches of oxidized marine sediments. Mar. Biol. 41:7-17.

Jorgensen B. B (1982). Mineralization of organic matter in the sea bed. The role of sulphate Reduction. Nature. 296, 643-645.

Jorgensen B. B., M. Bang and T. H. Blackburn (1990). Anaerobic mineralisation in marine sediments from the Baltic Sea-North Sea transition. Mar. Ecol. Prog. Ser.59:39-54.

Jorgensen B. B. and F. Bak (1991). Pathways and microbiology of thiosulfate transformations and sulfate reduction in a marine sediment (Kattegat, Denmark). Appl. Environ. Microbiol. 57, 3:847-856.

Kamat N. (1977). Santa Cruz: The ecological mysteries of a Goan village. http://www.geocities.com//Athens/Forum/1503/5-Cruz. html.

Kerkar S. (2004) Ph.D. in Marine Sciences.Thesis entitiled: "Studies on bacteria of the disssimilatory reductive prosecces of the supphur cycle from the salt pans of Goa".

King G.M. (2001). Radiotracer Assay (^{35}S) of sulphate-reduction rates in marine and freshwater sediments. Chap. 24, Methods in Microbiol. vol. 30, Acad. Press Ltd.

Klug M., P. Boston, R. Francois, R. Gyure, B. Javor, G. Tribble and A. Vairavamurty (1985). Sulphur reduction in marine and evaporate environment. D. Sagen (ed). The global sulphur cycle N.A.S.A. technical memorandum, 87570, Washington DC. 128-157.

Le Gall J. and J. R. Postgate (1973). The physiology of sulphate reducing bacteria. Toyent D.W. (eds). Adv. Microb. Physiol 10: 81-133.

Li, Jian-Hua, S. Takii, R. Kotakemori, H. Hayashi (1996). Sulfate reduction in profundal sediments in Lake Kizaki, Japan. Hydrobiologica. 333: 201-208.

Li Jian-Hua, K.J. Purdy, S. Takii and H. Hayashi (1999). Seasonal changes in ribosomal RNA of sulphate-reducing bacteria and sulphate reducing activity in a freshwater lake sediment. FEMS Microb. Ecol. 28:31-39.

Loka Bharati P. A., S. Nair and D. Chandramohan (1997).Anaerobic Sulphide oxidation inmarine Thiobacillus like organisms.Jour.Mar.Biotech.5:172-175.

Nedwell DB (1984) The inpuy and minerlization of organic carbon in anaerobic aquatic sediments. Adv. Microbial Ecol. 7: 93-131.

Ollivier B., P. Caumette, J. L. Garcia and R. A. Mah (1994). Anaerobic bacteria from hypersaline environments. Microbiol. Rev. 58(1):27-38.

Oren A. (1998). Anaerobic degradation of organic compounds at high salt concentrations. Antonie V.Leeuwenhoek . 54:267-277.

Pachmayr F. (1960): Vorkommen & Bestimmung von Schwefelvenbindungen in Mineral wasser. Thesis, Univ. of Munich.

Postgate J. R. (1984). The sulphate reducing bacteria, 2nd ed. Cambridge University Press.

Rabus R., T. Hansen and F. Widdel (2000). Dissimilatory sulfate-and sulphur-reducing prokaryotes. M. Dworkin, S. Falkow, E. Rosenberg (eds)

Smart, R.M and J.W. Barko (1978). Influence of sediment salinity and nutrients on the physiological ecology of selected salt marsh plants. Estuarine coastal Mar.Sci.7:487-495.

Schlegel H. G., Truper H.G (1964).Sulphur metabolism in Thiorhodaceae. I Quantitative measurements on growing cells of *Chromatium okenii.* Antonie van Leeuwenhoek. J. Microbial. Serol. 302,225-238.

Takii S. & Fukui M. (1991). Relative importance of methanogenesis, sulfate reduction and denitrification in sediments of the lower Tama river. Bull. Jap. Soc. Micro. Ecol. 6: 1-8

Teske A., N. B. Ramsing, K. Habicht, M. Fukui, J. Kuver, B. B. Jorgensen and Y. Cohen (1998). Sulfate-reducing bacteria and their activities in Cyanobacterial mats of Solar Lake (Sinai, Egypt). Appl. Environ. Microbiol. 64, 8:2943-2951.

Volkov I. I., D. Dyrssen and A. G. Rozanov (1998). Water alkalinity and anaerobic mineralisation of organic matter in the Sea. Geokhimiya. 36:69-78.

Widdel F. (1988). Microbiology and ecology of sulphate and sulphur-reducing bacteria. Biology of anaerobic micro-organisms. A. J. B. Zehnder (ed). New York, John Wiley and Sons, Inc. 469-585.

Acknowledgement

Director, National Institute of Oceanography, Dona Paula Goa and Head, Department of Biotechnology, Goa University for the facilities provided.
Address correspondence to – P.A. Loka Bharathi, Microbiology Laboratory, National Institute of Oceanography, Dona Paula, Goa, 403 404, India. E-mail: Loka@darya.nio.org.

Legends to Tables

Table 1: Comparison of Physico-Chemical Parameters of Hypersaline and Mesohaline salterns.

Table 2: Comparison of SRB counts (g-1) of Hypersaline and Mesohaline salterns.

Table 3: (A) Sulphate Reducing Activity: Comparative values.

(B) Sulphate Reducing Activity: Comparative values (contd).

Table 4: Carbon Flux

Legends to Figures

Fig 1: (A) Location of the sampling site, 'Ribandar salt pan'.

(B) Shading represents the position of the salt pan.

Fig. 2: Effect of salinity on sulphate reduction activity in overlying saltpan water.

Fig 3: SRA in unammended sediments : at 85 psu (A) and 330 psu (B).

Fig 4: SRA in 0-2 cm sediment at 330 psu amended with different substrates at 24 and 48 hours.

TABLE 1

Comparison of Physico-Chemical Parameters of Hypersaline and Mesohaline salterns

Depth (cm)	Temp (^{0}C)	Salinity** (psu)	$SO4^{-2}$** ($\mu M.ml^{-1}$)	Redox (mV)	pH	S^{-2} ($\mu M.g^{-1}$)
Hypersaline saltern						
0-2	40.50	323.33	184.17	-75.33	8.25	0.43
	(0.50)*	(11.55)	(2.6)	(12.68)*	(0.35)	(0.04)
2-5	38.50	188.00	47.50	-80.85	8.28	0.33
	(0.50)	(10.82)	(0.83)	(14.15)	(0.48)	(0.05)
5-10	36.50	200.00	44.38	-89.50	7.93	0.27
	(0.50)	(10.00)	(0.42)	(18.00)	(0.73)	(0.02)
Mesohaline saltern						
Depth (cm)	Temp (^{0}C)	Salinity** (psu)	$SO4^{-2}$** ($\mu M.ml^{-1}$)	Redox (mV)	pH	S^{-2} ($\mu M.g^{-1}$)
0-2	35.50	84.67	49.48	-179.50	8.63	1.68
	(0.50)	(0.58)	(6.77)	(4.50)	(0.33)	(0.36)
2-5	33.75	64.33	36.67	-316.60	8.41	1.84
	(0.25)	(1.16)	(7.08)	(58.40)	(0.41)	(0.09)
5-10	32.50	80.33	39.69	-224.00	8.32	0.93
	(0.50)	(1.53)	(10.31)	(26.00)	(0.62)	(0.32)

* $\pm$ sd. standard deviation, ** pore water measurements, n=3.

TABLE2
Comparison of SRB counts (g^{-1}) of Hypersaline and Mesohaline salterns

	Hypersaline Pond 330psu		Mesohaline Pond 85psu	
Depth(cm)	MPNx10^3 Counts	CFU x 10^3 Agar counts	MPN x 10^3 Counts	CFU x 10^3 Agar counts
0-2	4.6	0.87	112.5	13.48
	(1.01)*	(0.36)	(2.5)	(10.25)
2-5	0.93	0.03	52.82	10.15
	(0.32)	(0.03)	(0.27)	(2.85)
5-10	0.53	0.03	12.8	4.6
	(0.02)	(0.04)	(0.24)	(4.12)

* $\pm$ sd. = standard deviation, n=3

Table 3 A:

Sulphate Reducing Activity: Comparative values

Reference	Year	Place	SRA ($nM.cm.^{-3}.d^{-1}$)
Skyring	1988	Lake Elisa S. Australia	44±14
Marty et al	1990	Berre Lagoon sediment France	160-400
Jorgensen & Bak	1991	Marine sediments of Kattegat (Denmark)	500-3100
Canfield & De Marais	1991	Microbial mats Baja - California	2000-10000
Visscher et al	1992	surface littoral sediment Texel - Netherlands	567
Canfield & De Marais	1993	Oxic layer of hypersaline cyanobacterial mat Guerrero Negro, Mexico	10000
Caumette et al	1994	Microbial mats in HS ponds of mediterranean salterns Salins-de-Giraud - France	200-8200-17000
Parkes et al	1995	Deep marine sediment Japan Sea & Peru Margin	Without pressure: 54 With pressure: 4.9
Teske et al	1998	Cyanobacterial mats of Solar Lake (Sinai, Egypt)	100-2200
Brandt et al	1999	Great Salt Lake Utah	120 psu - 363±103 50-60 psu - 6131±835
Li et al	1999	Fresh water sediment Lake Kizaki - Japan	0.5
Ravenschlag et al	2000	Arctic Sediment	185-250
Schubert et al	2000	Chile Sediments	47-69 62 430

Table 3 B: Sulphate Reducing Activity: Comparative values (contd)

Reference	Year	Place	SRA
Jorgensen & Fenchel	1974	Limf Fjord surface sediment Denmark	55-135 $nM.cm^{-3}.d^{-1}$
Jorgensen	1977	Aarhus Bay surface littoral sediment Denmark	439-1013 $nM.cm^{-3}.d^{-1}$
Jorgensen & Cohen	1977	Solar Lake (Sinai) microbial mat	5400 $nM.cm^{-3}.d^{-1}$
Martens & Berner	1977	Long Island Sound salt marsh	0.77 $\mu M.cm^{-3}.d^{-1}$
Nedwell & Abraham	1978	Sediment surface of British salt marsh	0.1-0.5 $\mu M.cm^{-3}.d^{-1}$
Howarth & Teal	1979	Marsh peak England	1.4 $\mu M.cm^{-3}.d^{-1}$
Jorgensen et al	1979	Solar Lake Sinai - Egypt	5400 $nM.ml^{-1}.d^{-1}$
Skyring	1984	Shark Bay surface littoral sediment Australia	1800 $nM.cm^{-3}.d^{-1}$
Bottcher et al	2000	Intertidal mudflat Dangast site German, Wardden sea North Sea	14-225 $nm.cm^{-3}.d^{-1}$
Mudryk	2000	Sediments - Gulf of Gdnask - Baltic Sea	1.89-31-6 $nm.hr^{-1}.d^{-1}$
Present study:	2004	Ribander salt pan Goa - India	
85 psu 28 ^{0}C			$nM.cm^{-3}.d^{-1}$
0-2 cm			2050
2-5 cm			620
5-10 cm			408
330 psu 29 ^{0}C			$pM.cm^{-3}.d^{-1}$
0-2 cm			59.7
2-5 cm			21.5
5-10 cm			7.8

Table 4
Carbon Flux Table

Reference	**Year**	**Place**	**g.Cm^{-2}.d^{-1}**
Deuser	1971	anoxic water column Particulate organic matter in Black Sea	0.05
Howarth & Teal	1979	Salt marsh peat	2.76
Chapelle & Lovley	1990	Sandy Aquifer sediments	
Karl & Knauer	1991	Black Sea sediment	0.08
Muramoto	1991	Black Sea sediment	0.03
Kristensen et al	1991	Mangrove Forest (Thailand)	
Albert et al	1995	Black Sea water column sediment	0.67-1.2
Rysgaard et al	1998	Arctic Coastal Marine sediment	0.02
Alongi et al	1999	Shrimp pond of Mekong delta (Vietnam)	0.05
Alongi et al	2001	Mangrove Forest	0.01-0.41
Present study:	2004	Ribander saltpan Goa - India (Sampling 2002)	
^{35}S: 85 psu:			1.57
^{35}S 330 psu:			0.05

Fig 1: (A) location of the sampling site ‘Ribandar salt pan’.

(B) Shading represents the position of the slt pan.

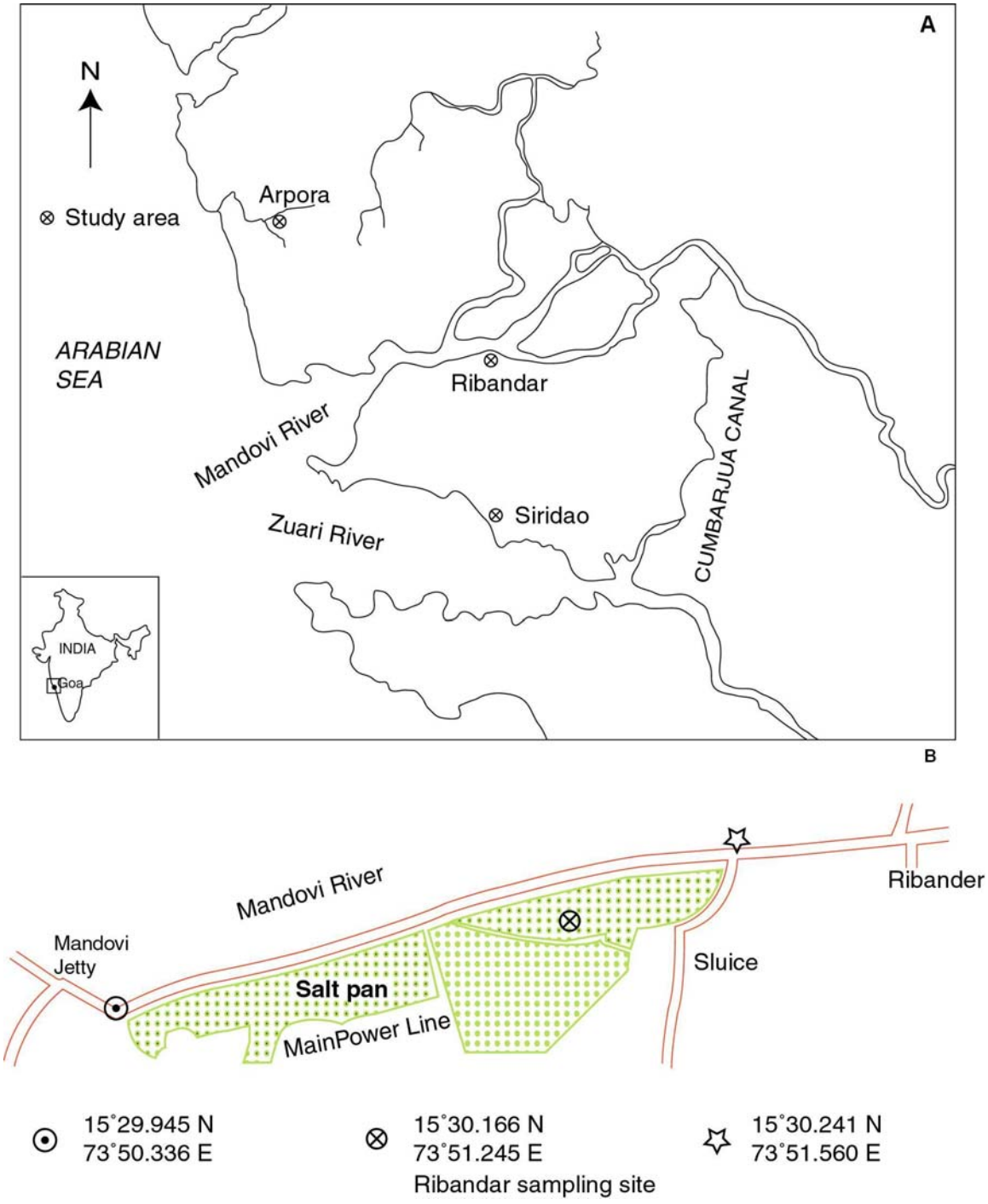


Fig. 2: Effect of salinity on sulphate reducting activity in overlying saltpan water.

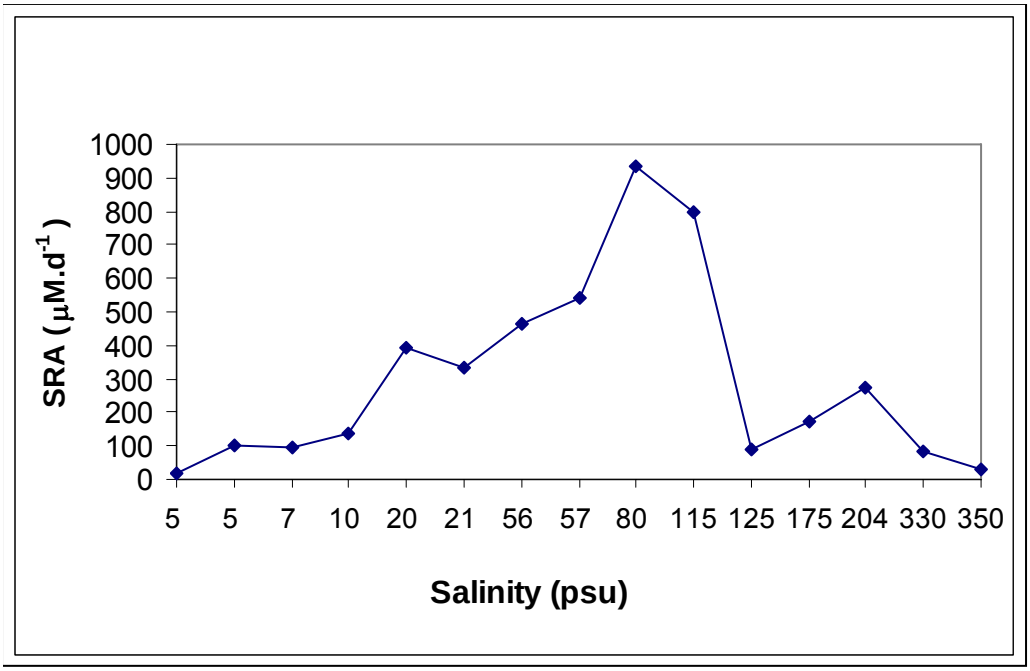
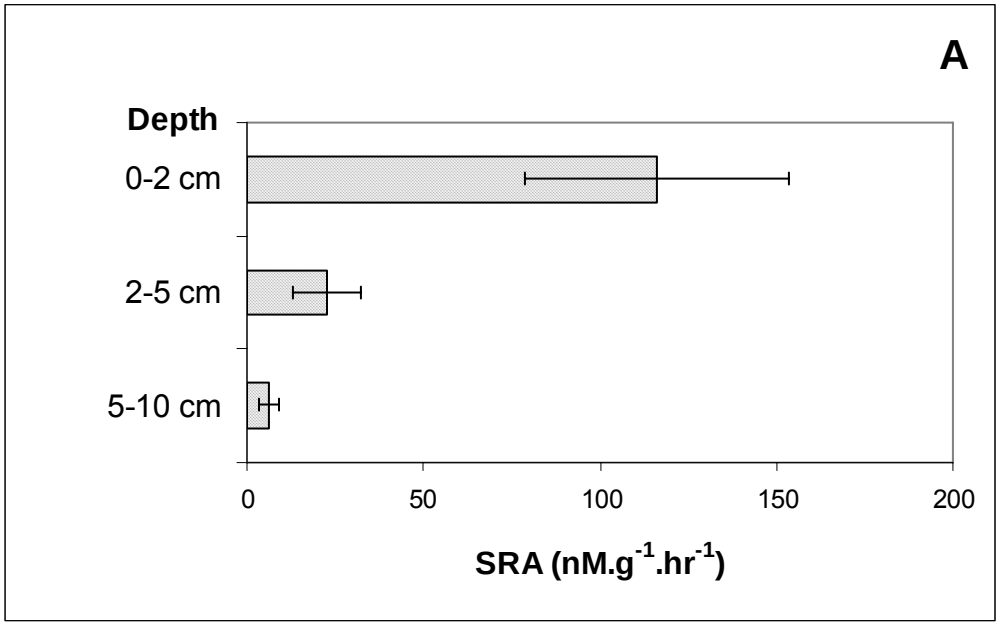


Fig 3: SRA in unammended sediments: at 85 psu (A) and 330 psu (B)



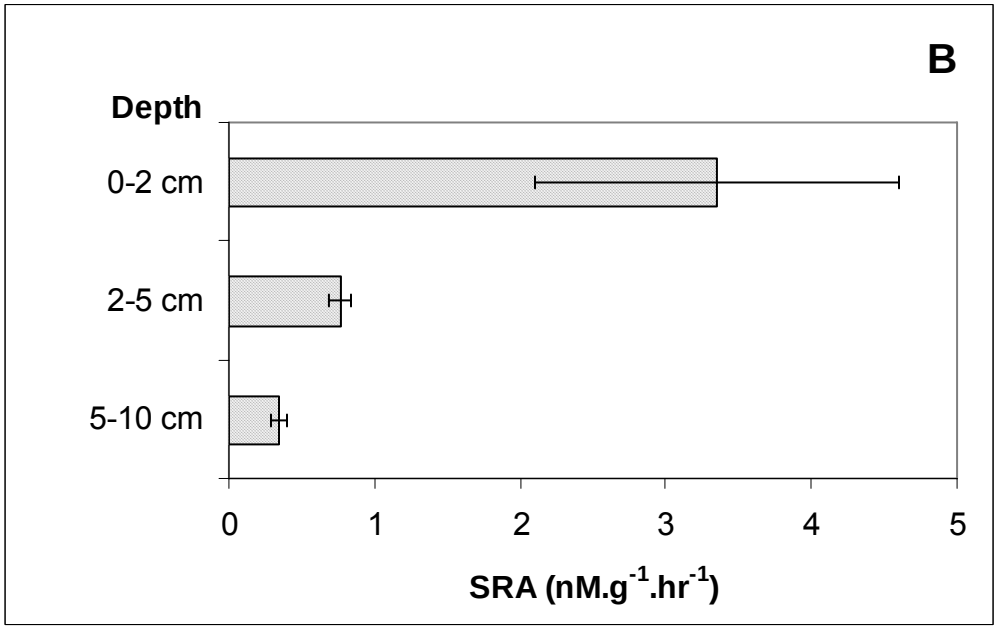
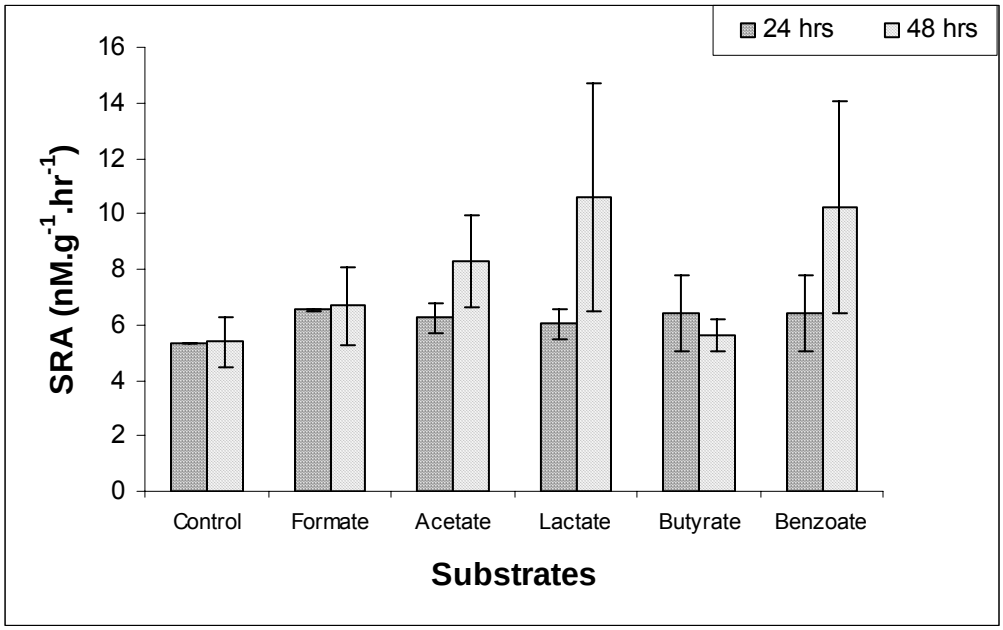



Fig 4: SRA in 0-2 cm sediment at 330 psu amended with different substrates at 24 and 48 hours.