

# 4. Synergy in Sulfur Cycle: The Biogeochemical Significance of Sulfate Reducing Bacteria in Syntrophic Associations

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## Introduction

One of the most important processes governing the composition of the oceans and the redox balance on the earth's surface is the bacterial reduction of seawater sulfate to  $H_2S$ . Rates of sulfate reduction in marine sediments can vary over eight orders of magnitude (Jorgensen and Fenchel, 1974; Westrich and Berner, 1988). The sulfate reducing bacteria (SRB) that are responsible for the process are the most ubiquitous of organisms in the marine environment. Their growth rates may be low, but their high activity has wide implications. They dominate the anaerobic realms rich in sulfate. Of late, it is realized that their metabolic versatility accounts for their ecological competitiveness. SRB could bring about great many geochemical changes under different environmental conditions in sync with other microbes. The most important role however is their ability to link synergistically the various elemental cycles.

The evolution of the sulphur cycle synchronizes with the earth's history (Habicht and Canfield, 1996). The atmosphere over the planet was apparently reducing, containing little atmospheric oxygen, and with seawater sulfate concentrations estimated to be much lower than the present day concentrations. The accumulation of sulfate in the ocean to much higher concentrations was probably coincident with the initial accumulation of oxygen in the atmosphere and was the result of oxidative weathering of sulphide minerals on land. Logan et al. (1995), suggest that the biological production of sulfide by sulfate reducing bacteria would have taken place around 2,500-540 million years ago in the Proterozoic era. Biodegradation of algal products in sedimenting matter was complete, indicating that significant part of organic material was extensively reworked as it sank slowly through the water column by these bacteria.

In the present day the sulfate reducing bacteria (SRB) are diverse but they share the same physiology of using sulfate as the terminal electron acceptor for dissimilatory processes. The end product of sulfate respiration is invariably sulfide. As sulfate is a major ion in the sea next only to chloride and sodium, SRB are widespread in marine and brackish environments. Their hardy nature has made them ecologically competitive even in extreme conditions of temperature, pressure and salinity. This heterogenous assemblage of bacteria not only utilizes organic acids, fatty acids, alcohols and hydrogen as electron donors but also more complex compounds (hydrocarbons and sugars). Morphologically and ecologically diverse, the SRB include both gram positive and gram-negative strains. SRB are known to be members of the delta Proteobacteria, however, the novel bacteria are not restricted to this group. As they are now seen to have polyphyletic lineages, they are referred to as sulfate reducing prokaryotes or SRP. Several SRPs are closely related to microorganisms, which cannot perform anaerobic sulfate reduction for energy generation. They comprise a phylogenetically diverse assemblage of organisms consisting of members of at least 4 bacterial phyla and one-archael phyla. Archaea-Euryarchaeota; Bacteria- Firmicutes, Nitrospira, Thermodesulfobacteria and Proteobacteria.

SRB of different types are the most interesting microorganisms known and their means of energy production are physiologically unique and ecologically crucial. They are central to the global cycling of C, S, N, & P and are important ecologically in the re-cycling of the organic matter that enter anoxic aquatic systems. They are not only responsible for the turnover of as much as 50% of the total organic carbon (TOC) but also provide enough source of electrons for other chemolithotrophic and phototrophic bacteria (Gibson, 1990).

Though many microbes associate with others in nature the associations that SRB participate are biogeochemically significant in marine environment

especially since sulfate forms the third most important ion in sea water next only to sodium and chloride. SRB generally associate themselves syntrophically with other microbes and thus synergise many biogeochemical reactions. Syntrophy –literally meaning “eating together”, would lead to nutritional and metabolic interactions between microbes in proximity in a given environment. This phenomenon of cross feeding and interdependence would lead to synergy where the combined effect would be greater than the sum of the individual effect. The short review highlights some of these aspects of SRB in marine ecosystem.

## Synergy in Sulphur cycle

Many elemental cycles in an environment operate dynamically both in the oxidative and reductive mode fuelling each together. The syntrophic association in the sulphur cycle has been obvious since long and has been usually referred to as sulfureta. While fermentative bacteria break down organic matter anaerobically, the sulfide oxidizers fix carbon dioxide either chemoautotrophically or photoautotrophically. Between these two major groups, the SRBs act as important anaerobic terminal oxidizers of organic matter. They trophically link the heterotrophic degradative bacteria to the synthetic ones.

Laboratory experiments have succinctly elucidated how syntrophy promotes synergy in mixed cultures of *E. coli*, *D. vulgaris* and *Chromatium vinosum*, which are dependant metabolically (Loka Bharathi et al., 1980; 1982). The growth yield constant K (cell dry weight per solid substrate) of *E. coli* alone on glucose does not exceed 0.05. However, when cultured with two other physiologically different species, *D. vulgaris* and *Chromatium vinosum*, K increased to 0.46. (Loka Bharathi et al., 1980). On cellulose, the sulfate reducer improves the metabolic link between *Clostridium* and *Chromatium* by releasing the end products such as acetate and sulfide, which are easily metabolisable by the photosynthetic bacteria. Growth yield constant increased from 0.13 by cellulolytic bacteria to 0.31 for the association with *Desulfovibrio* and finally to 0.63 when both the sulfur bacteria ie *Desulfovibrio* and *Chromatium* are included.

The development of SRB is limited by the rate of fermentation of the organic substrate and growth of sulfide oxidizers. The fermentation of cellulose provides the sulfate reducer with the sources of carbon, electrons and the latter in turn releases catabolites, which serve as substrate for photo/chemosynthetic organisms. High organic matter of aquatic regions gives rise to high production of sulfide resulting from heterotrophic activities and sulfate reduction. At this stage photosynthetic bacteria grow

and break forth to re-equilibrate the system. The exacerbated activity manifests itself in the form of red water. Therefore, the occurrence of the bacterial red water phenomenon is rather an effect than a cause for precedent organic pollution. Without the intervention of the microorganisms the region would continue to remain toxic and the sulfur-cycle would remain open, the element being stocked in the form of sulfide. The catabolites and sulfide enclosed in the anaerobic system are converted by their activity to cellular matter, which constitutes the starting point of the food chains of the anaerobic realm through the zooplankton, which feed on them. Thus, a link between anaerobic bacterial production and secondary aerobic production is established (Loka Bharathi et al., 1980; 1982).

Most often in nature, SRB defy the generally accepted paradigm that environmentally available electron acceptors are depleted sequentially. This is because in nature such electron acceptors can be used simultaneously because of selective advantage through syntrophy. They have been shown to be present in the surface waters, beach sediments and coral ecosystems. While SRB were discovered to increase with depth in beach sediments (Loka Bharathi and Chandramohan, 1985), in mangrove swamps the trend was reversed (Loka Bharathi et al., 1991). The SRB of the mangrove swamps could mediate sulfate reduction through lactate, acetate, propionate, butyrate and also benzoate. The distributive abundance of the SRB in the estuarine and marine waters was dictated by the carbon regime and not deterred by elevated levels of oxygen encountered in surficial sediments or waters. Their abundance could vary from  $10^2$  g<sup>-1</sup> dry sediment in beach sediment to  $10^3$  to  $10^4$  in mangrove sediments, from  $10^2$  L<sup>-1</sup> in off shore waters to  $10^3$  L<sup>-1</sup> in near shore to  $10^4$  L<sup>-1</sup> in lagoon waters. The taxonomic affinities include *Desulfovibrio*, *Desulfococcus*, *Desulfobacter* and *Desulfobulbus*. The taxonomic distribution share the syntrophic benefits that these microbes derive from other fermentative bacteria (Loka Bharathi and Chandramohan, 1990; Loka Bharathi et al., 1991). Existence of such synergy and syntrophism with other groups has been demonstrated by strong relationship that SRB share with sulfur-oxidizing counterparts and general anaerobic bacteria. While 44% of the variation in anaerobic fermentors controlled the SRB variation, about 45% of the variations in these bacteria are responsible for the variation in *Thiobacillus denitrificans* like organisms (TDLO) (Loka Bharathi and Chandramohan, 1990).

In the clayey sediments of the mangrove swamps, SRB were restricted to or are maximum near the surface. Lactate oxidizers were high in numbers i.e. twice as abundant as acetate oxidizers at 5cm depth.

Butyrate and propionate also seemed to be good substrates for marine forms suggesting that fermentative bacteria producing these substrates were in sync with the SRB that use them. Propionate is a common end product of much fermentation. Besides, more propionate is produced from long chain fatty acids with odd numbers of carbon atoms by a syntrophic culture of hydrogen producing acetogenic bacterium and a hydrogen-consuming organism (Mc Inerny et al., 1979). Propionate-oxidizing SRB could be important mineralizers in anaerobic systems of mangrove swamps. The overall sequence in retrievability of numbers of SRB on the substrates was benzoate>lactate>butyrate>propionate>acetate. SRB of mangrove swamps were nutritionally versatile and ecologically competitive (Loka Bharathi et al., 1991).

As elsewhere, it has been shown that though SRB are anaerobes, their distribution is not inversely related to the oxygen content of the water column. The distribution and activity clearly point out they are not inhibited by the measured oxygen level of the surrounding water column. They are dictated more by the availability of utilizable organic carbon released by the aerobic heterotrophic or fermentative bacteria (Loka Bharathi et al., 1992). Thus, both SRB and sulfate reducing activity (SRA) were recorded in all depths of water tested with higher activities being recorded in shallow depths. Sulfate respiration and nitrate respiration were perhaps more temporally rather than spatially isolated. SRB are not only synergistically associated with heterotrophs and fermentative bacteria but also with those that utilize the metabolites that these microbes produce. The most prominent groups are either the anaerobic photosynthetic groups or aerobic chemolithotrophic ones.

Thus, the distribution of chemosynthetic bacteria involved in sulphide oxidation are linked to the distribution of SRB. TDLO (*Thiobacillus denitrificans* like organisms) are bacteria that oxidize sulfide at the expense of nitrate (Loka Bharathi, 1989; Loka Bharathi et al., 1988), such a link is demonstrated by the positive correlation that are statistically significant. Some of these bacteria have been identified as heterotrophic bacteria with affinities to either *Pseudomonas* spp or even *Alcaligenes* spp and they can participate in such processes (Loka Bharathi et al., 1994). At near shore and mud bank samples r-value was 0.265 (p<0.1). At mud bank region alone r was 0.816 (p<0.01). In river estuaries it was positive again at a value of 0.769 (p<0.001) suggesting their influence could vary from 7% to 66% (Loka Bharathi and Chandramohan, 1990).

SRB thriving at the hydrogen end may have a very limited capacity to degrade organic compounds but they can scavenge hydrogen with high affinity and use it as electron donor or carry out incomplete oxidations of ethanol or lactate with acetate as end product. Most of the species of the ubiquitous well-known genus of *Desulfovibrio* belong to this group. Under sulfate limiting conditions however, SRB may maintain metabolic activity by cleaving certain reduced fermentation products to acetate and hydrogen, thus providing substrates for methanogenic bacteria (Mc Inerny and Bryant, 1981). The first oxidation product of sulfide, elemental sulphur appears outside the cells of green sulfur bacteria and can therefore be oxidized further to sulfate or reduced by sulphur reducing bacteria. In defined syntrophic cocultures of acetate-oxidizing sulfur reducing bacterium *Desulfuromonas acetoxidans* with green sulfur bacteria, H<sub>2</sub> is produced from acetic acid via a light-driven S-cycle (Warthmann et al., 1992). The synergistic relationships can also evolve to symbiosis in higher organisms.

In the gutless marine oligochaete *Olavius algarvensis*, endosymbiotic sulfate-reducing bacteria produce sulphide that can serve as an energy source for sulphide-oxidizing symbionts of the host. Apparently these symbionts do not compete for resources but rather share a mutualistic relationship with each other in an endosymbiotic sulphur cycle, in addition to their symbiotic relationship with the oligochaete host (Dubiller et al., 2001).

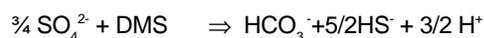
## DMS and SRB

From symbiotic associations in localized environments, their interactions could also have wider implications. They are known to participate not only in the degradation of dimethylsulfoniopropionate (DMSP) but also in the flux of the degradation product, DMS. These interactions are linked to either the heterotrophs that initiate the breakdown or chemoautotrophs or photosynthetic organisms that oxidize the sulfide that is produced. Thus, SRB are involved in demethylation of DMSP to yield MMPA (methylmercaptopropionate), carbonate and sulfide or oxidation of DMS to yield bicarbonate and sulfide (van der Berg et al., 1998).

### DMSP demethylation

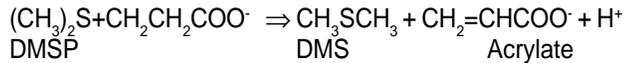


### DMS oxidation



Anoxic intertidal sediments abound in algal osmolyte DMSP. DMSP could release DMS by the intervention of SRB. This is known to have counter effective effect on global warming but also the potent green house gas methane (van der Maarel and Hansen, 1997).

Extracts of *Desulfovibrio acrylicus* from anoxic intertidal sediment, could cleave DMSP to DMS and acrylate and acrylate could be used as the terminal electron acceptor (van der Maarel et al., 1995).



Cells grown in the presence of DMSP contained high DMSP lyase activities (9.8 U/mg protein). DMSP can be metabolized by bacteria via cleavage to dimethylsulfide and acrylate or via an initial demethylation. An enzyme that specifically catalyses the demethylation of DMSP was isolated from the sulfate-reducing bacterium strain WN, (Jansen and Hansen, 2000). Unpublished work by the author and colleagues show that *Thiobacillus denitrificans* like organisms were better than heterotrophs, and sulfate reducing bacteria in the utilization of DMS. On a per cell basis SRB could oxidize DMS at 0.32 fM.cell<sup>-1</sup>.d<sup>-1</sup> as compared to TDLO strains that could utilize at the rate of 0.6 fM.cell<sup>-1</sup>.d<sup>-1</sup> or heterotrophs that could do so at the rate of 0.23 fM.cell<sup>-1</sup>.d<sup>-1</sup>. On culture basis however, these values were 0.7, 2 and 1.3 nm.d<sup>-1</sup> respectively thus indicating that TDLO were more active utilizers because of their higher abundance and perhaps because of their higher affinity to DMS.

## Climate

Thus the activities of SRB alone or with others could have profound influence on the climate. Either through the interaction with DMS an anti green house gas or with methane a green house gas. The bacterial oxidation of methane by SRB could have several significant effects. It could prevent upward diffusion of hydrate-derived methane in the overlying sediments (Hoehler et al., 2000), which could ameliorate or buffer climate impacts resulting from slow hydrate dissociation (Orcutt et al., 2004). The strong coupling between SRB population and methane concentrations could affect the alkalinity, which indirectly favors carbonate precipitation (Zhang et al., 2002). Thus, it may lead to sequestration of large amounts of carbon that otherwise would enter the water column and atmosphere. It may also lead to a significant transfer of carbon to higher trophic level consumers as well as to mobile predators (Carney, 1994; MacAvoy et al., 2002). Michaelis et al. (2002) have found that massive microbial mats cover about 4-meter-high carbonate buildups and prosper at methane seeps in anoxic waters of the

northwestern Black Sea shelf. These mats were basically composed of aggregates of archaea and sulfate reducing bacteria.

## Synergy in carbon cycle

Actually all the elemental cycles are intimately connected to the carbon cycle. Quantitative experiments carried out by Jorgensen and Fenchel (1974) showed that more than half of the added organic matter was completely degraded to CO<sub>2</sub> in the course of sulfate reduction thus showing that they function as terminal oxidizers. Interspecies transfer of fermentation products stimulates the growth of fermentative and sulfate-reducing bacteria. Sulfate reducing bacteria react with the fermentors at one end and with the methanogens at the other end of anaerobic trophic chain.

Like in sulfureta, syntrophic relationships are important in anaerobic food chains as well. Some fermentations of substance require the activities of two different organisms working in concert with one another syntrophically. Fermentation of ethanol to acetate and methane is mediated by ethanol fermentor and a methanogen. When conditions become anoxic and alternate acceptors are scarce, fermentative catabolism predominates but in many cases requires syntrophic interactions.

## Interspecies hydrogen transfer

The preferred advantage of synergy through syntrophy is particularly significant in hypoxic or anoxic environments ie the environments with very little or no free oxygen. This is because anaerobic metabolism yields little energy and calls for high metabolic efficiency. While the fermentation of short chain fatty acids is energy consuming and therefore endergonic under standard partial pressure of hydrogen, at very low pH 2 it is exergonic. Hence, syntrophic hydrogen consumption by SRB, homoacetogens and methanogens could be the metabolically advantageous (Douglas, 2004). Fermentative organisms can convert large organic molecules to low molecular weight acids, carbon dioxide and molecular hydrogen which act as feed back inhibitor of the process. Methanogen can increase the activity of SRB and fermentors by removing hydrogen and reducing the feed back inhibition (Rogers and Whitman, 1991). Interspecies hydrogen transfer is a typical example for syntrophic associations. Interspecies hydrogen transfer is the interdependent sequence of reactions involved in the anaerobic conversion of complex polymers to methane. Interdependent transfer of hydrogen generally culminates in methane sink. Thus, in most

anoxic systems the rate limitations of methanogenesis are the steps involved in the production of acetate and hydrogen by syntrophs. As soon as hydrogen is released, it is taken up by a methanogen, homoacetogen or SRB. Thus, syntrophs tend to form flocs or aggregates continuing as hydrogen producer or consumer- in close proximity for effective transfer.

The reduction of sulfite to H<sub>2</sub>S and the reduction of CO<sub>2</sub> to CH<sub>4</sub> rely on the transfer of electrons from fermentative products (fatty acids and alcohols) up a potential gradient to molecular hydrogen. For example the production of hydrogen from lactic acid by *Desulfovibrio* sp. requires the transfer of electrons up a potential gradient from -185 mv to -450 mv. Though certain reactions seem thermodynamically impossible, kinetics show that molecular hydrogen is produced and utilized as the electron source for methanogenic bacteria. An interesting symbiotic relationship occurs between SRB and the methanogen. Sulfate is used as the electron acceptor, until the concentration is low. At this point electrons flow to produce molecular hydrogen, which is kept at a low partial pressure by the methanogenic bacteria in their reduction of carbon dioxide to methane. The regulation of electron flow is mediated by the low molecular weight multihaeme cytochrome C<sub>3</sub>. Cytochrome C<sub>3</sub> contains 4 haemes, which operate at 4 different redox potentials. The unique cytochrome regulates electron flow in a multifunctional mode with pathways to sulfite reduction and hydrogen formation thus demonstrating that certain critical processes in the anaerobic environment are regulated by kinetic rather than by thermodynamic considerations (Wood, 1983).

Anaerobic biological processes take place within reduced micro-environments in the sediment. Interspecies transfer of fermentation products stimulates the growth of fermentative and sulfate-reducing bacteria. Such micro-environments influence the syntrophic growth of fermentative and sulfate-reducing bacteria (Shiba, 1985).

Thermophilic sulfate reducers may serve as potential acetogens for the degradation of organic acids and of ethanol in syntrophy with thermophilic methanogens. Pure cultures of thermophilic 'fermentative' bacteria can degrade a variety of different sugars and polysaccharides, irrespective of their origin (Winter and Zellner, 1990).

Many of the products of fermentative metabolism are themselves energy source for other fermentative organisms. For example, succinate, lactate and ethanol produced from the fermentation of sugars can be further fermented by other microbes. The bacterium *Syntrophobacter pfennigii* oxidizes propionic acid and lactic acid in cooperation with the

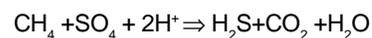
hydrogen- and formic acid-utilizing *Methanospirillum hungatei* (Wallrabenstein et al., 1995). Secondary fermentation of these primary fermentation products lead to the production of acetate H<sub>2</sub> and carbon dioxide- ideal substrates for methanogenic archaea. Finally, the fermentation end products i.e., the ultimate products of anoxic decomposition are CO<sub>2</sub> and CH<sub>4</sub>- the most oxidised and the most reduced form of carbon.

Thus, interspecies hydrogen transfer is responsible for increased carbon turnover, production of more oxidized end products, high energy conservation, higher growth of all organisms and the displacement of unfavorable reaction equilibrium towards homeostasis (Roger and Whitman, 1991) and the role of SRB is highly significant since they have can scavenge hydrogen very efficiently.

## SRB and methanogenesis and reverse methanogenesis

Syntrophic associations of sulfate reducers along with other fermentors fuel methanogenesis. It is dependant on the production of few carbon compounds by other organisms from complex organic matter-methanol, formate methyl mercaptan and acetate and methylamines. High organic loading (4-5 wt%) and sedimentation rates (1.4 mm yr<sup>-1</sup>) lead to anaerobic conditions within the uppermost 15 cm. Intense bacterial sulfate reduction (0.011-0.15 mM SO<sub>4</sub><sup>2-</sup>yr<sup>-1</sup>) exhausts dissolved sulfate around 150 cm sediment depth, resulting in methanogenesis at greater sediment depth by carbonate reduction (1.8-8.5 mM CH<sub>4</sub>yr<sup>-1</sup>). After exhausting the sulfate, SRB can produce conducive environmental conditions for methanogens to proliferate and produce methane.

On the contrary, SRB can also participate in the oxidation of methane in syntrophic association with methanogenic bacteria. Methane can be oxidized to produce some bioclastic limestones by these consortia. Hansen et al. 1998 proposed that a consortium of methanogenic bacteria and sulfate reducers is responsible for net oxidation of methane under anoxic conditions, a process called 'reverse methanogenesis'.



Such moderation of methane concentration by sub-surface dwelling SRB at higher abundance have been encountered in sediments off east coast of India. (Unpublished data)

Methane-derived carbonate cementation of Holocene marine sediment occurs at several locations in the Kattegat, Denmark. Generally, the carbonate-

cemented sandstones occur at the seafloor as individual slabs or widely distributed, thinly lithified pavements (Jorgensen, 1992).

A structured consortium of archaea and sulfate-reducing bacteria is symbiotic, with the archaea growing in dense aggregates surrounded by sulfate-reducing bacteria. These aggregates were found abundantly in gas-hydrate-rich sediments with extremely high rates of methane-based sulfate reduction (Boetius et al., 2000). Such reversals by a consortium of methanogenic archaea and bacteria seem to be widespread. Biomarker evidence for widespread anaerobic methane oxidation in Mediterranean and in some localized settings have been reported by Pancost et al. (2000).

The isotope data of Thiele et al. (2001) imply that the biosynthesis of the archaeal isoprenoids occurred *in situ* and involved in the utilization of isotopically depleted, i.e. methane-derived, carbon. Apart from archaeal markers, the carbonate and the mat contain authigenic, framboidal pyrite and isotopically depleted fatty acids, namely iso-, and anteiso-branched compounds most likely derived from sulfate-reducing bacteria (SRB). The indications for a tight association of these normally competitive organisms support a model invoking a syntrophic relationship of SRB with Archaea responsible for the anaerobic oxidation of methane (Thiele et al., 2001).

In gas-saturated areas as in CH<sub>4</sub> vents at an arctic mud volcano) the development of bacterial mats with a predominance of filamentous colorless sulfur bacteria, along with benthic symbiotrophic community with prevailing pogonoforas was observed. The participation of association of methane-forming and sulfate-reducing bacteria is evident. About 0.15 l of methane are oxidized each day in one square meter of sediment cover, 0-20 cm thick. The rate of methane-derived carbon incorporation into the bacterial biomass is 11 mg/m<sup>2</sup> per day. The carbon dioxide that is formed from methane oxidation is used for the formation of carbonate illite-calcite barite chimneys, crusts, and nodules (Lein et al., 2000). Sometimes methane-driven sulfate reduction shapes the entire sulfate gradient. Methane oxidation could be complete, but the process could be very sluggish with turnover times of methane within the sulfate-methane transition zone of 20yr or more (Jorgensen et al, 2001).

## SRB and Oxygen

SRB are generally considered as strict anaerobes though they can tolerate limited exposure to oxygen. Enzymes like catalase confer protection against oxygen (Sass et al, 1996). However, it has now been

shown that SRB are capable of aerobic growth in the presence of other microbes. Oxygen-dependent growth of the sulfate-reducing bacterium *Desulfovibrio oxycliniae* in coculture with *Marinobacter* spp has been demonstrated in an aerated sulfate-depleted chemostat (Sigalevich et al., 2000). The patterns of consumption of electron donors and acceptors suggest that aerobic incomplete oxidation of lactate to acetate is performed by *D. oxycliniae* under high oxygen input. Both organisms were isolated from the same oxic zone of a cyanobacterial mat where they have to adapt to daily shifts from oxic to anoxic conditions. This type of syntrophic association may occur in natural habitats, enabling sulfate-reducing bacteria to cope with periodic exposure to oxygen.

## SRB in extreme environment

Likewise in the salt pans of Goa both abundance and diversity of SRB was higher in surficial sediments than in deeper layers or in overlying water. They share a strong relationship with the abundance of Thiobacilli like organisms (Kerker, 2004). Traditional methods of identification showed the abundance of *Desulfobacter postgatei* > *Desulfovibrio desulfuricans* > *Desulfococcus multivorans* under mesohaline conditions and *Desulfovibrio halophilus* > *D. desulfuricans* > *Desulfococcus multivorans* under hypersaline conditions. Very interestingly the identity of *D. halophilus* by 16S rDNA technique conforms to the taxonomic affinity established by the conventional method. Surprisingly sulfate-reducing activity has been noted in hitherto unreported microbes. The sulfate reducing ability in hypersaline *Halomonas elongata* and *Chromohalobacter israelensis* has been demonstrated for the first time (Kerker, 2004).

## Culture to nature

While it is becoming more and more evident that < 0.01% of the bacteria can be cultured, attempts are being made to improve culturability to reduce the anomaly. Besides, very often it is difficult to extend what is observed in the laboratory to the field conditions and draw similarities. However, under certain circumstances, it is becoming possible to make such parallels. Purdy et al. (2003) showed that their study of the use of 16S rRNA-targeted oligonucleotide probes to investigate function and phylogeny of sulfate-reducing bacteria and methanogenic archaea in a UK estuary complement the understanding of the ecophysiology of the organisms detected. Further they have been able to make a firm connection between the capabilities of species, as observed in the laboratory, to their roles in the environment.

## Application

The synergy existing between SRB and other microbes could be effectively used in bioremediation and ecosystem management and modelling carrying capacity of an environment in question. Syntrophic associations between sulphate reducing and sulfur oxidizing bacteria could be gainfully used in the bioremediation of oil wells polluted by sulfide production (Loka Bharathi et al., 1997). While heterotrophic bacteria in a metal contaminated system could provide the necessary fatty acids that could put particulate metal in soluble phase, SRB could reduce the effect by precipitating the metals as metal sulfides. It is perhaps for these reasons that anaerobes especially SRB have higher tolerance to metal contamination as compared to the aerobic counterparts (Loka Bharathi et al., 1990) and therefore they could be effectively used to precipitate heavy metals. Hypersaline SRB were found to be more effective (Haristha et al., 2002 ; Kerkar et al., 2003). Kerkar et al. (2003), hypothesized that the growth substrate influences the extent of tolerance. While some isolates showed maximum tolerance to mercury on lactate and acetate, the response was stimulatory on benzoate and inhibitory on butyrate at 50ppm of mercury. The most tolerant isolates could do so with the help of extra- chromosomal DNA. Some of them harboured plasmid of 5kb size.

SRB can also be harnessed for hydrogenases. *D. vulgaris* possesses one of the most active hydrogenases known and it could be the enzyme of choice for any biotechnological processes. It can be extracted in an oxygen tolerant form (Van Berkel Arts et al., 1986).

Sulfur bacteria have other biotechnological applications as well. In biochemical fuel cells organisms that generate a redox or pH gradient are particularly suitable for such uses. While SRB lower the redox potential, Thiobacilli reduce the pH. Sometimes both types of organisms are combined in a process for the electrochemical neutralization of acid mine drainage.

## Future scope

It is becoming more and more evident that SRB are more metabolically versatile. They have been shown to reduce nitrate, fix their own nitrogen and even participate in chemolithotrophic production. The syntrophic association with *Marinobacter* spp and *D. oxyclinae* that has been suggested to occur in natural habitats, enable the latter, ie to cope with periodic exposure to oxygen (Sigalevich et al., 2000).

Under these circumstances it would be more beneficial to extend the use of anaerobes to aerobic realms than the other way about.

As it is with other microbes, it is probable that only a minor fraction render themselves cultivable. Most probable number technique (MPN) give higher yield but more strategies could be evolved to reduce the difference between the cultivable and the non-cultivable. The unculturable majority could be identified by molecular techniques to understand the diversity of their lineages and functional genes. Process based and culture based approach could complement molecular approach. As each method has its positive and negative aspects combining all the techniques would give an integrated and holistic study of the ecology of these organisms. This approach would not only yield new microbes yet to be discovered but also new functions in established forms.

## Summary and Conclusion

The bacterial associations with SRB lead from syntrophy to synergy and the sum of the total is more than its parts. Alone they could contribute heterotrophically or chemolithotrophically and in combination SRB could bring about great many geochemical changes under different environmental conditions in association with other microbes. The reduction of sulfate to sulfide is important since it influences many other metabolic processes. The production of sulfide ions has a profound effect on the availability of trace metals and directly influences their uptake by marine biota. Their associations with methanogens from mutual exclusivity to reciprocal mutuality is highly dictated by environmental conditions like substrate depleting or repleting condition. In the sulphur cycle the sulfide that is produced in the process is again synergetically used by photosynthetic bacteria under phototrophic conditions and by chemosynthetic sulfur bacteria under autotrophic. Their interactions with DMSP, an osmolyte of planktonic origin can range from having local to global implication on climate. The bacterial associations with SRB can lead from syntrophy to synergy and the sum of the total is more than its parts. Alone they could contribute heterotrophically or chemolithotrophically and in combination they can set a cascade of reactions in series. The growing understanding of these microbes can help us to tame and harness their potential better- from the coastal realms where they compete successfully with aerobes

in the biodegradative processes to the dark abysses of deep ocean beds where they biogeochemically control the rich mineral resources.

## Acknowledgements

Dr. R. Matheron University of Aix Marseilles III introduced me to the anaerobic world and to the vagaries of SRB. He also helped improve the contents of this article. I record my gratefulness to him.

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