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Biogeochemical cycling of sulphur and iron in Ithikkara lake; Kollam district, Kerala, India: a probable mechanism

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Abstract

The Ithikkara lake in Kollam District of Kerala State, India has been studied over a decade (2005-2015) in the context of biogeochemical cycling of iron and sulphur. The study includes the analyses of water and mud samples from the lake on a monthly basis. The parameters measured include pH and electrical conductivity of water and physicochemical and mineralogical characteristics (by XRD and thermal methods) of the mud. Simultaneously both the mud and water samples were tested for the presence of microorganisms. The water remained more or less pH neutral throughout the period of study. The mud is found to be rich in kaolinitic clays with the presence of minor minerals like pyrite, sulphur particles and calcareous materials. Different types of sulphur and iron bacteriae were present in both mud and water. A probable mechanism for the biogeochemical cycling of iron and sulphur aided by all types of sulphur and iron bacteriae, benthic organisms, fungi and water plants is described in this paper.

1. Introduction

Microbes present in the lithosphere and hydrosphere of earth influence the kinetics and course of reactions involving the dissolution of a number of minerals, and they can also influence the authigenic and diagenic formation of a number of minerals [1]. The minerals profoundly influence the survival, activity, gene expression and functions of microbes [2, 3, 4]. Therefore, the interactions between minerals and microorganisms are found to be essential to soil ecology, the environment and cycling of elements [5].

Recently, our research group started working on the biogeochemical cycling of various elements in a limnological perspective. Of late, our interest focused on Ithikkara lake (8°51′48″N 76°41′50″E), a fresh water shallow water body situated near the southern edge of Kollam town in Kerala State, India. The lake is part of a ternary system of water bodies consisting Ithikkara river, Paravur kayal and Arabian sea. However, the pristine nature of the lake is largely intact from salt water intrusion or chemical pollution due to human activities except by certain brick factories.

The lake can be categorized as one falling between oligotrophic and mesotrophic, thanks to the minimum level of biological or chemical pollution. Part of our studies mainly focused on the biogeochemical cycling of elements such as sulphur and iron, which mutually overlaps and are influenced to certain extent by climatic conditions as well. Phototrophic purple and green bacteria are found in nearly all aquatic environments and they are recognized when they form water blooms. The environments such as waste water stabilization ponds, polluted ponds, the hypolimnion of lakes etc. have the nutrients like H_2S, H_2 and simple organic compounds make the photosynthetic bacteria predominant [6]. In this paper, we describe a probable mechanism for the biogeochemical cycling of sulphur and iron in Ithikkara lake.

2. Material and Methods

2.1. Sample collection

Water and mud samples from the lake were collected all through the year for the past one decade on a monthly basis (2005-2015). The samples were collected from the middle of Ithikkara lake (20 meter away from the

shore) Kollam, Kerala State, India. These samples were analyzed physicochemically, mineralogically and microbiologically and the results of these are summarized.

2.2. Chemicals and culture media

Eosin methylene blue (EMB) agar, thiosulfate-citrate-bile salts-sucrose *(TCBS agar)*, pseudomonas agar base, serratia differential medium, potato dextrose agar, sodium benzoate, ammonium chloride, di-potassium hydrogen phosphate, magnesium chloride, resazurin, sodium carbonate, Sodium sulphide, KH_2PO_4, $CaCl_2$, $MgSO_4$, $FeSO_4$, $(NH_4)_2\ SO_4$, agar were procured from Hi-Media Laboratories Pvt. Ltd., Mumbai. All the chemicals and reagents used were of analytical grade and prepared in deionized water.

2.3. Physicochemical analysis

Analysis of water samples were conducted by the middle of every month of each year. The pH of water sample was determined by using a laboratory pH meter (Eutech); before taking the readings the pH meter was calibrated by using buffer solution of pH 4 and pH 9.2. All the readings were taken at 30 ^oC. Electrical conductivity (EC) was determined by electrometric method using a laboratory conductivity meter (Systronics digital 318).

2.4. Heterophilic plate count

Heterophilic plate count (HPC) provides an indication of general population in the clay sample. HPC analysis was performed for two clay samples collected from two different locations. Using pour plate technique, the serially diluted clay samples were plated on Glucose Tryptone (GT) agar [7] (APHA 1998) and the total plate count was performed on colonies developed after incubation at 37^oC for 24 hours. This experiment was done in triplicates and the mean values were represented. Total number of colonies on each plate were counted with the help of colony counter and Colony Forming Unit (CFU) was counted using formula: CFU = No. of colonies x Dilution factor / sample volume (ml).

2.5. Isolation of bacteria and fungi from mud and water

Isolation of bacteria and fungi was performed by making serial dilutions of the clay samples. In this technique sample suspension was prepared by adding 1 g clay to 10 ml of sterile water and shaking vigorously for at least 1 minute. The dilute was then sedimented for a short period and 10^-2, 10^-3 and 10^-4 dilutions were made. The dilution was spread on nutrient agar and potato dextrose agar plates prepared according to the instructions and directions of the manufacturer. The bacterial plates were incubated at 37^oC for 24-48 h and the fungal plates were incubated at 25 ^oC for 72^oC. After successful growth of microorganisms the pure cultures of bacteria were sub-cultured in nutrient agar slants; incubated at 37 °C to achieve vigorous growth and then preserved in refrigerator. The cultural, morphological and biochemical characteristics of the respective isolates were compared with the criteria in Bergey's manual of Determinative Bacteriology [8]. Thiosulfate citrate bile salts sucrose *(TCBS) agar,* Pseudomonas agar base, Serratia Differential Medium, Eosin Methylene Blue agar (EMB agar) were used for the isolation of *Vibrio* sp., *Pseudomonas fluorescens, Serratia marcescens, E. coli* and *Proteus* sp. respectively. *Thiobacillus ferrooxidans* was isolated from the clay samples using nutrient agar medium and 9K solid medium [9]. Methanogenic bacterium was isolated through methanogenic enriched medium [10] 1974). Thiothrix, Beggiatoa were cultured as per the medium described by Williams et al. (1985) [11]. *Thiobacillus ferrooxidans* and green sulphur and purple sulphur bacteria were isolated using the medium described by Barron and Lueking (1990) [12] and Swoagar and Lindstrom (1971) [13] respectively.

2.6. Mineralogical analysis of the mud

The mud samples were analyzed using X-ray diffraction and thermogravimetry (TGA and DTA). The XRD pattern and TGA and DTA curves are shown in Fig. 1, 2 and 3 respectively. The XRD pattern was taken using a PAN analytical X' pERT PRO mpD (pw3040/60) diffractometer using the powder method, at $5^0 < 2\theta > 79^0$ intervals. Cu Kα and Co Kα (40 kv and 40 mA) radiations were used. The 2θ scanning speed was 0.02 degree per second. The thermogravimetric analyses were performed under N2 flow (40 cm^3/ min) over the temperature range from 25 to 900 ^oC at a heating rate of 10^oC/min using a Shimadzu thermobalance (TG/DTA) model DTG-60H.

3. Results and discussion

The pH did not vary considerably during different seasons over a decade, maintaining a nearly neutral value. The electrical conductivity of the water samples collected over different months showed consistent values (Table 1) for the entire decade long period. The heterophilic plate count of water ranged from 144 x 10^2 to 198 x

10^2 CFU/ml and the values for clay samples ranged from 149 x 10^3 to 170 x 10^3 CFU /ml respectively during different months. The chemical, physical and biological characteristics of water and the presence of aquatic plants influence the microbial population [14]. The microbiological integrity of water also depends upon its pH value [15]. *E. coli, Streptococcus* sp., *Proteus* sp., *Vibrio* sp., *Pseudomonas fluorescens, Thiobacillus ferrooxidans, Beggiatoa* sp., *Thiothrix* sp., green sulphur and purple sulphur bacteria and methanogens were found either in water or mud or both (Table 2). The fungal species observed was *A. niger and Penicillium sp.* (Table 3). Various factors like pH, bacterial quantity, mineral mass ratio and ionic strength can influence the complex process of adsorption [16, 17, 18].

Table 1: pH, EC and HPC values

Months	pH	Conductivity (mho)	HPC of water sample (CFU /ml)	HPC of clay sample (CFU /ml)
January	6.9	2.21	162 x 10^2	149 x 10^3
February	6.9	2.2	160 x 10^2	153 x 10^3
March	6.9	2.2	153 x 10^2	160 x 10^3
April	6.9	2.1	148 x 10^2	165 x 10^3
May	6.9	2.2	144 x 10^2	163 x 10^3
June	7.0	2	190 x 10^2	170 x 10^3
July	7.0	2	198 x 10^2	168 x 10^3
August	7.0	2	179 x 10^2	170 x 10^3
September	7.0	2	180 x 10^2	165 x 10^3
October	7.0	2	185 x 10^2	160 x 10^3
November	6.9	2.1	165 x 10^2	167 x 10^3
December	6.9	2.1	170 x 10^2	158 x 10^3

A large number of bacterial species were isolated which include sulphur bacteria of several types and iron bacteria of different nature as shown in table 2. Fungal isolates are given in table 3. These organisms derive energy from redox reactions involving S and Fe in the presence and or absence of sunlight, oxygen and carbon sources. Chemical analysis of the mud and water samples have shown the presence of several sulphur compounds such as sulphides, sulphates of Fe and Ca and elemental sulphur as well Chandran et al. (2010) [19]. The mud samples are always enriched by the presence of black pyritic sediments, embedded in a clay matrix consisting kaolinites and bentonites. Sulphur can exist in any oxidation state from +6 to -2 including zero (elemental) and iron can exist either as +2 or +3, very often shifting the equilibrium maintaining pH neutrality. In other words, a combined mechanism for the biogeochemical cycling of sulphur and iron is the characteristic of the lake which can be envisaged as given in figure 1.

Sulphate reducers, sulphur oxidizers, iron bacteria, acetogens, methanogens and fungal mass take part in the complex process of the biogeochemical cycling of S and Fe. The adhesion of bacteria onto solid surfaces is a necessary event in nature for the utilization of inorganic and organic components and for the enhanced growth of bacteria [20]. It is suggested that the adsorption of the *Bacillus subtilis* on to corondum increases with decreasing pH, increases with bacterial quantity, increases with mineral mass ratio and decreases with ionic strength [21]. Certain benthic creatures such as molluscs and crabs also contribute. Redox reactions involving ferrous and ferric ions sometimes can be purely chemical in nature, which maintains the ecological balance and biodiversity of the lake, allowing the safe growth of benthic organisms [22].

The lake has the following features, which are maintained in all seasons,

1) pH neutrality of soil and water.
2) Absence of eutrophication.
3) Availability of benthic organisms.
4) Adequate light penetration and aeration (oxygenation).
5) Temperature stability between 23-33°C.
6) Absence of salinity.
7) Presence of $CaCO_3$ and FeS_2 in the mud.
8) Presence of limited number of algal species and floating plants.

Table 2: Bacterial population found in the lake, water and mud

Sl. No.	Bacterial species found		
	Both in mud and water	Mud only	Water only
1	*Serratia marcescens*	Nil	Nil
2	Nil	Nil	*Bacillus* sp.
3	*E. coli*	Nil	Nil
4	*Streptococcus* sp.	Nil	Nil
5	*Proteus* sp.	Nil	Nil
6	*Vibrio* sp.	Nil	Nil
7	*Pseudomonas fluorescens*	Nil	Nil
8	Nil	Nil	*Staphylococcus* sp.
9	Nil	*Thiobacillus ferrooxidans*	Nil
10	Nil	Nil	*Beggiatoa* sp.
11	Nil	Nil	*Thiothrix* sp.sulphur bacteria
12	Green sulphur and purple sulphur bacteria	Nil	Nil
13	Methanogens	Nil	Nil

Table 3: Fungal population found in the lake, water and mud

Sl. No.	Fungal species found		
	Both in mud and water	Mud only	Water only
1	*A. niger*	Nil	Nil
2	*Penicillium sp.*	Nil	Nil

Two points to be noted are the maintenance of pH neutrality and temperature stability throughout the year. The level of oxygenation and the extent of light penetration are maintained constant all through the seasons, because of the tropical climate.

3.1. Sulphur Cycling

It involves four major steps such as:

i) Mineralization of organic sulphur to inorganic sulphates and H_2S. In the formation of H_2S, sulphate reducers (SRB), acetogens and methanogens play major role and the rate of mineralization is slow in summer.

ii) Immobilization of the sulphates to sulphate esters and carbon bound sulphur by plants, fungi and prokaryotes. Mostly sulphur is reduced to -2 oxidation state in this process. The rate of immobilization could be high in monsoon.

iii) Oxidation of H_2S and FeS_2 to elemental sulphur, thisoulphates and other inorganic sulphates, occurs both in the aerobic and anaerobic pathways in the presence of light. Green and purple sulphur bacteria (phototrophic) in presence of light convert H_2S to elemental sulphur as per the equation $H_2S \longrightarrow S^o + 2H^+ + 2e^-$. Oxidation of H_2S generates energy required to fix carbon.

iv) Reduction of sulphates through an assimilatory route by green plants or dissimilatory path way by sulphate reducers in the absence of oxygen results in the formation of H_2S or FeS_2. H_2S can partly escape the system while FeS_2 gets embedded in the clay matrix at the benthic zone and will undergo further oxidation as shown in the cycle.

3.2. Iron Cycling

This is a rapid redox cycling. Since iron is a metallic element, it cannot exist in the zero oxidation state unlike sulphur. Nevertheless it undergoes rapid cycling between +2 and +3 oxidation states, biogeochemically. Iron bacteria (e.g. *Thiobacillus ferroxidans*) and a variety of sulphur bacteria (green sulfur and purple sulfur) contribute synergistically in this process. In addition to this, ferric ion reacts chemically with pyrite forming Fe^{2+} as shown in figure 1, generating H^+ ions which leaches clay minerals as well as calcarious shells and thus get neutralized. Ferrous ions are rapidly converted to ferric ions by microbial oxidation and then the cycle continues.

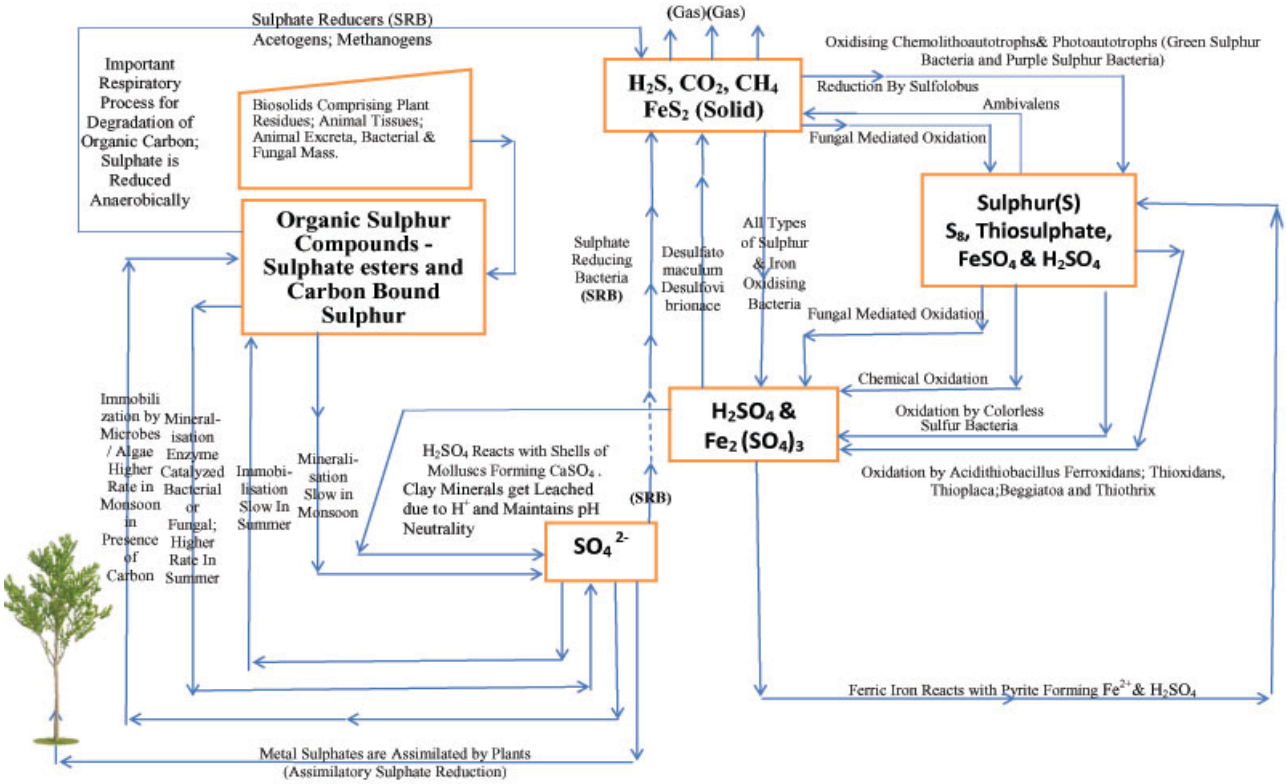

Figure 1: The proposed mechanism of biogeochemical cycling of sulphur and iron

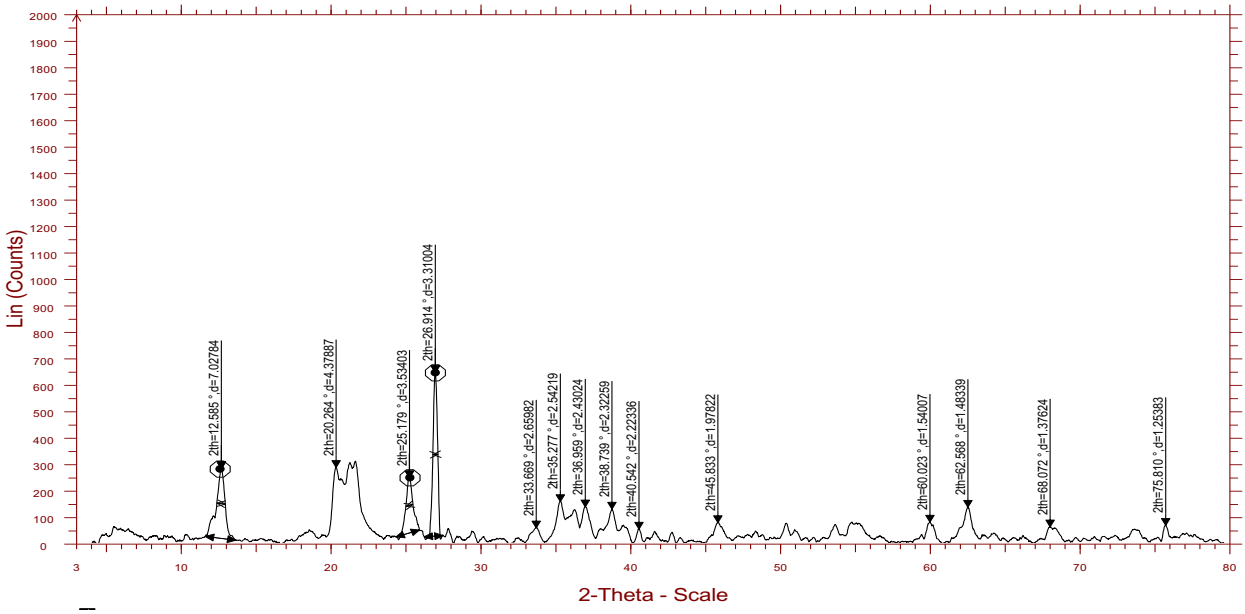

Figure 2: A typical XRD pattern of the lake mud sample

The XRD pattern (Fig. 2) shows that the mud is rich in disordered kaolinite (d values corresponding to 7.027 A^0, 4.378 A^0 and 3.534 A^0). Quartz is also present (d value corresponding to 3.33 A^0). Elemental sulphur (d value 2.659 A^0) [23]. Hematite (d value 2.542 A^0), $CaCO_3$ (d value 2.430 A^0) and pyritic mineral (d value 3.22 A^0) were also present in the mud. The TG/DTA curve (Fig. 3) as well as XRD pattern show that the mud is mainly kaolinitic in nature [24]. The endothermic peak around 500 ^oC in DTA is typical of kaolintic clays. Other subsidiary minerals present in the mud did not show very specific peaks in the thermal analysis. This could be due to the low percentage present.

The cycling of both sulphur and iron are dynamic and well-balanced and the lake provides a safe ecosystem for the benthic organisms. Also it could be depicted that there is a perfect parity in the population of sulphate reducers (SRB) and sulphur oxidising bacteriae (SOB) at any given time in the lake (both mud and water) allowing the active presence of methanogens and fungi.

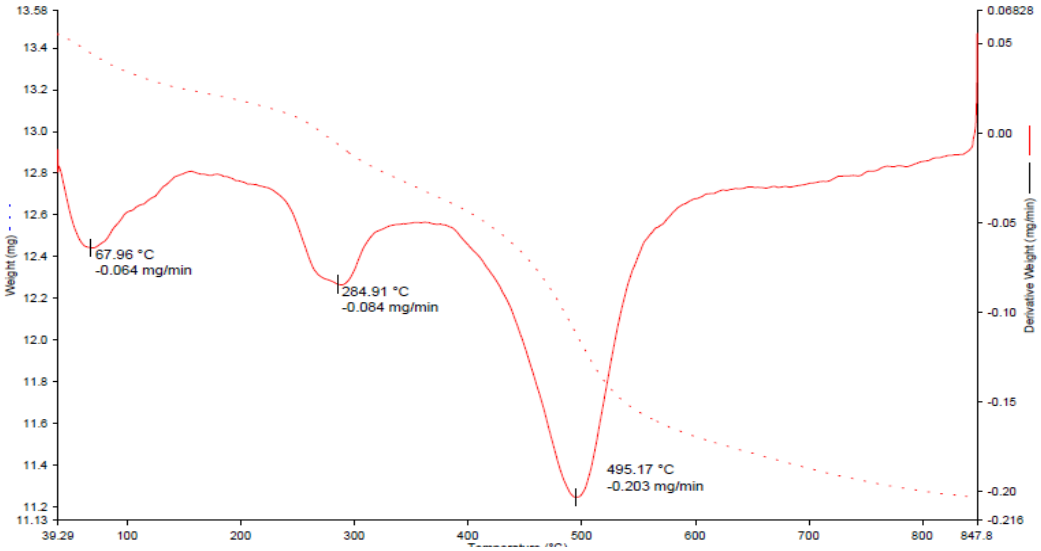

Figure 3: TGA and DTA curves of the lake mud sample

Conclusion

Ithikkara lake can be considered as a less polluted shallow water body with a perfectly balanced biogeochemical cycling of Fe and S, which remains largely intact. A large number of microbial and fungal populations including all kinds of sulphur and iron bacteria help to maintain the biogeochemical cycling perfectly balanced, without altering the pH neutrality in all the seasons throughout the year. Water plants, some algaes and benthic organisms like molluscs also contribute to the cycling. Also, the addition of acidic components to the lake via precipitation or acid-mine drainage is only nominal in the decade studied.

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