

Review Article

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Different Applications of Sulphur Oxidizing Bacteria: A Review

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ABSTRACT

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Sulphur is an essential nutritional element for plants, animals, microorganisms and humans; as it is integral component of many compounds including protein. Sulphur is metabolized into different forms with the help of sulphur oxidizing bacteria, which use sulphur as energy producing ingredient. Sulphur oxidizing bacteria have an interesting property of adaptability in wide range of habitats. Due to this property they have many applications in different fields like for improving quality of crops, bioleaching and extraction of metals from ores, in waste water treatment plants, for removal of hydrogen sulphide gas, concrete bridge structure and for bioremediation etc.

Introduction

Sulphur (S) is an important component of organic matter. It is an essential macronutrient for plants, animals, microorganisms and humans. In both plants and humans, S comes after K, Ca and P in abundance (Scherer, 2009). It is found in amino acids, which are building blocks of protein. Sulphur is also part of many other biologically active molecules found in reduced forms (Tandon and Messick, 2002; Kertesz and Mirleau, 2004; Jamal *et al.*, 2010). All living organisms have requirement of S as an elemental component. Sulphur is taken up mostly in the form of sulphate, with

further reduced to sulphide to form further necessary compounds. The average amount of sulphur content found in organisms is up to 0.2% of dry weight though some organisms use S compounds higher amounts in many ways like for anabolic processes, reducing power (electron donor), source of energy or as electron acceptor (Camacho, 2009).

The source of sulphur in soils is mainly derived from the sulphur containing minerals found in parent materials and the plants and animals remains or from the addition of elemental S from outer sources. Sulphur is mostly found in the form of sulphates

sulphides and organic fractions associated with nitrogen and carbon compounds. The important sulphur bearing minerals in rocks and soils are gypsum, anhydrite, epsomite, iron pyrite, sphalerite, chalcopyrite, galena and arsenopyrite. Sulphur mainly occurs in organic and inorganic forms and is cycled in between these forms via mineralization, mobilization, immobilization, oxidation and reduction processes (Jamal *et al.*, 2010). S exists in all three phases i.e. solid, liquid or gaseous. Plants mainly take up sulphur in the form of SO_4^{2-} and reduce it to form S containing amino acids and other compounds. In plants cysteine is the main source of sulphur for most of the other S-compounds. The recommended dose of dietary allowance for sulphur amino acids for humans is 14 mg kg^{-1} of total bodyweight (Prasad, 2016).

Extent of sulphur deficiency

Sulphur is involved in the synthesis of amino acids and proteins, enzymatic and metabolic activities taking place in plants. S deficiency in soils and plants leads to an inhibition of protein synthesis and also results in accumulation of non-S containing amino acids particularly asparagine, glutamine and arginine in plant tissues. The deficiency of sulphur in areas is seen more where high S utilizing crops like oilseeds and pulses are grown regularly. Approximately 41% of our Indian soils are deficient in sulphur and deficiency of S is developing fast in areas where, intensive cropping system, taking high yielding varieties with use of high analysis chemical fertilizers particularly sulphur free S-free fertilizers like diammonium phosphate and urea etc. are being used (Singh, 2001).

Effects of sulphur deficiency

Plants suffering from S deficiency resemble in symptoms in nitrogen deficiency as the leaves turned to light green or yellow in colour. The

most common visible symptoms of sulphur deficiency in oilseed and leguminous crops generally seen on young foliage as pale chlorotic leaves, high red tints at leaf margins reduced nodulation, stunted growth, poor branching, thin slender stem etc. In cereal grains, stunted growth, delayed maturity, small and spindly with slender stalks are common symptoms. Hence, S is essential for plant nutrition and its shortage can really affect the yield and quality. In sulphur deficient plants greater decrease in chlorophyll content in leaves, inhibition of protein synthesis and carbohydrate metabolism, composition of proteins, supply of mineral nutrients, nitrogenase activity in root nodules etc. take place which ultimately affect the growth, yield and quality of crops and produces. For example, a decline in cysteine content of cereal grains like wheat further lessens the baking quality of flour because disulphide bridging of the gluten fraction is responsible for the polymerisation during doughing. In mustard family the content of glucosinolates is directly related with the supply of SO_4^{2-} . When plants are grown at sites with low S supply, the breeding hybrid cultivars are more sensitive to S deficiency than the traditional crops. The deficiency may be due to the role of glucosinolates as transient storage of S-compounds (Shreeja, 2016).

Toxic effects of sulphur

S may be toxic to the plants due to high SO_2 amount in the air. The critical level of SO_2 for plants is 120 $\mu\text{g}/\text{m}^3$. SO_2 may be removed from the environment mainly by precipitation or direct contact of the gas particles with soil and vegetation in a process called dry deposition.

The amount of sulphur brought to the earth surface in precipitation form generally varies broadly at different places to other and is affected by many factors like by volume of

precipitation (rainfall); industrial areas set up, proximity to sea and the usual winds out there. Although the amount of SO₂ are numerous times higher than that of normal levels near industrial areas. The cause of Sulphur dioxide (SO₂) toxicity also due to its liberation from different sources. SO₂ captivated by the vegetation mixes in the moist surfaces of mesophyll cells present in the stomatalvoids. The sulphurous acid (H₂SO₃) forms from the reactions of SO₂ dissociates giving rise to H⁺, HSO₃⁻ and SO₃²⁻ and SO₄²⁻ ions. Further the disruption of chloroplast membranes can also takes place due to sulphur dioxide toxicity. The availability of SO_x along and NO_x in the environments causes acid rains, which also damage the crop to a huge extent (Shreeja, 2016).

What are sulphur oxidizing bacteria?

Sulphur bacteria forms many mutualistic interactions depending on the types of sulphur compounds, whether they interact with oxidized or reduced form. Though, direct interactions (physical contact/close trophic relationships) may also takes place. An example of stable structural association (most evolved symbiosis in prokaryotes) of green sulphur bacteria is with motile chemotrophic bacteria, which are responsible for syntrophic growth based on the exchange of inorganic and organic S-compounds. (Camacho, 2009)

The sulphur bacteria, which are having capability to oxidize the reduced forms of sulphur compounds with sulphate as a final product, are known as sulphur oxidizing bacteria (SOB). In nature Scycled (sulphur cycle) between many different biological forms, which takes place absolutely by the action of microorganisms. Sulphur is absorbed by plants in the form of SO₄²⁻, which further undergoes a continuous conversion in series before its assimilation into the different S-compounds (Katyal *et al.*, 1997). Sulphur

oxidizing bacteria generally belongs to genus like *Thiobacillus*, *Beggiatoa*, *Thiothrix*, *Thiomicrospira*, *Desulphuromonas* and *Achromatium* (Das *et al.*, 1996) but the oxidation process is not limited to the true sulphur bacteria; it takes place as well in the bacteria having heterotrophic mode. These sulphur oxidizing heterotrophic bacteria, belongs to the genera *Pseudomonas*, *Escherichia coli*, *Alcaligenes* and *Xanthobacter* and isolated from different environment (Starkey, 1935; Kuenen and Beudeker, 1982).

Two different kinds of metabolically active groups exist: one is obligate chemolithotrophic bacteria, which can utilize the oxidizable S compounds only (and CO₂ as the source of carbon) and the other is heterotrophic, which may also follow the chemolithotrophic mode of nutrition (Chaudhary, 2018).

The typical examples of obligate chemolithotrophs, are *Thiobacillus neapolitanus*, *Thiobacillus thioparus*, *Thiobacillus denitrificans* (denitrifiers) and *Thiobacillus thiooxidans* (extreme acidophile). Some other examples of this group are *Thiobacillus ferrooxidans* (acidophilic iron-oxidizer), *Thiomicrospira* and some species of *Thiobacillus halophilus*. The most common example of heterotrophs in this category include *Thiobacillus acidophilus*, *Thiobacillus novellus*, *Paracoccus denitrificans*, *Thiobacillus aquaesulis* (moderate thermophile), *Thiobacillus intermedius*, *Xanthobacter tagetidis*, *P. versutus*, *Thiomicrospira thyasirae* and *Thiosphaera pantotroph* (Kuenen and Beudeker, 1982). A number of chemolithotrophic and non-filamentous sulphur oxidizing bacteria like *Thiospira* or *Thiomicrospira*, *Sulfolobus*, have also been isolated from some special environments (Starkey, 1934; Vidyalakshmi *et al.*, 2009).

Sulphur oxidation in rhizosphere

The soil environment surrounding plant roots is the zone of intense microbial activity. A large number of microorganisms capable of sulphur oxidation have been isolated from the root region of crop plants.

Shi *et al.*, (2011) demonstrated a sulphur oxidizing strain HT1, isolated from rhizosphere of rice soil having Pb pollution, using thiosulphate as electron donor at pH 7.0 and on the basis of 16S rRNA gene sequencing, the isolated strain found belonging to γ -proteobacteria, *HaloThiobacillus*.

Chaudhary *et al.*, (2017) isolated three potential strains of sulphur oxidizing bacteria namely SSF7, SSA21, and SSS6 from rhizosphere of mustard, showing sulphate ion production concentration of 2.268, 3.102, and 2.785 mM respectively. They were characterized as *Xanthobacter*, *Pseudomonas* and *Pseudomonas* sp. for SSF7, SSA21 and SSS6 respectively.

Mechanism of sulphur oxidation

The oxidation of reduced sulphur in soil is usually regarded as a microbial process (Katyal *et al.*, 1997). Wide spectrums of microorganisms are capable of oxidizing sulphur. Microbial sulphur oxidation is, on the whole, beneficial to soil fertility, resulting in the formation of SO_4^{2-} , which can be used by the plants, while the acidity produced by process of oxidation is used to solubilize the other plant nutrients specially that of phosphorous and improve the alkaline soils (Wainwright, 1984). Most of the soil contains many sulphur oxidizing microbes and this number increases rapidly on sulphur addition (Swaby and Fedel, 1973). Skiba and Wainwright (1984) observed that following addition of elemental sulphur to a fertile loam

soil, both thiosulphate and tetrathionate had transitory appearance whereas sulphate accumulated throughout the incubation period. These studies suggest that elemental sulphur is oxidized to sulphate in soils through a polythionate pathway.

The sulphur cycle includes an interconnected sets of oxidation-reduction potential of organic and inorganic S complexes with an alteration in the reduction form of the sulphur from -2 reduced (sulphide) to +6 (sulphate) oxidation state through several common intermediate compounds for example, elemental sulphur (S^0), polysulphide, thiosulphate, polythionates and sulfite as shown in figure 1.

Applications of SOB in agriculture

The microorganisms present in soil improve the plant growth by providing nutrients and defending them against stress and pathogens.

Anandham *et al.*, (2007) demonstrated the use of *Rhizobium* co-inoculation with the sulfur (S)-oxidizing bacterial strains. Clay-based pellets of *Thiobacillus* were formulated (2.5107 cfu/g pellet) and their efficacy to enhance plant growth was tested in groundnut under pot house and field conditions with sulphur-deficit soil. Experimentation in pot house yielded promising results on groundnut by increasing the plant biomass, nodule number and pod yield and co-inoculation of *Thiobacillus* sp. (60 kg/ha) with *Rhizobium* under field condition also recorded significantly improvement in all characteristics. Also inoculation of SOB increased the available S of soil from 7.4 to 8.43 kg/ha and oil content of groundnut.

Anandham *et al.*, (2008) tested the thiosulfate oxidizing bacteria for their traits related to plant growth promotion. In gnotobiotic experiments, *Pandoraesputorum* ATSB28,

enhanced the primary root length of canola by 166% and inoculation of *Pandoraea* sp. strain ATSB30 with RP and thiosulfate significantly increased the water extractable-P (1147 µg P g/RP) and bicarbonate extractable-P (1144 µg P g/RP) on 45th day.

Abhijit *et al.*, (2014) carried out a field experiment to study the effect of sulphur oxidizing strains of *Thiobacillus thiooxidans* along with sulphur to observe the effect of amendments on the yield parameters of mustard (Mustard, var. B-85) At maturity, the average seed yield was 14.5% higher in S⁰ over control which further increased upto 30.6% along with inoculated sulphur oxidizing strains, while the oil yield was found to be 25.7% higher in S⁰ or gypsum over the control, which increased up to 42% after 2nd year with sulphur oxidizing inoculants RS 004.

On an average, the S increment was recorded 19.1% with gypsum over the control which further increased up to 22.3% when amended with sulphur oxidizers.

Chaudhary *et al.*, 2018 also applied SOB with the seeds of mustard for studying their effect on mustard growth.

A total of 15 treatments comprising three controls and six treatments for each bacterium were formulated and different growth parameters were observed. A positive improvement in mustard crop growth with respect to different parameters was recorded after inoculation of SOB.

Inoculations of sulphur oxidizing bacteria with mustard seeds oxidized the reduced sulphur compounds and make them available to plants in sulphate form, which further result in improvement in plant growth parameters such as length, weight, no. of siliquae, seed weight, oil content and chlorophyll content.

Other applications of SOB

Besides having application in agriculture or for improving plant growth there are reports of many other applications of SOB. SOB has noticeable contribution in maintaining healthy environment. These are applied for bioleaching and extraction of metal from ores, waste water treatment plants, degradation of concrete bridge structures and for bioremediation purposes, etc. An interesting fact of SOB is their adaptability in all kinds of habitats ranging from normal soil, water environments to extreme natural sources like geothermal areas, black shale, volcanic eruptions and acid rock drainage.

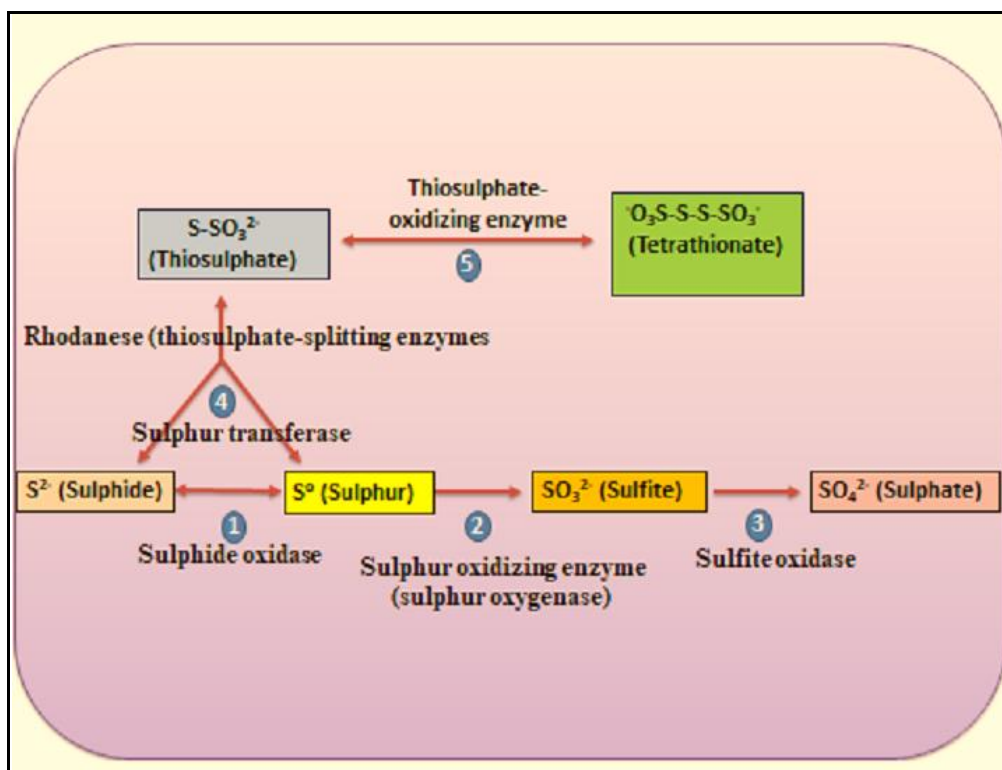
Applications of SOB in heavy metal Removal

The contamination of aquatic sediments with metals is a widespread environmental problem. The conventional technologies used for metal removal has many drawbacks like time consuming, more use of chemicals etc., so environmentally friendly biotechnological approaches (bioleaching) are gaining interest in this field as a promising strategy for the eventual treatment of contaminated sediments and solubilization of heavy metals. Iron and sulphur oxidizing bacteria are exploited for large-scale operations of metal recovery from ores (Seidel *et al.*, 2006; Ilyas *et al.*, 2014). Sulphur oxidizing microorganisms are being used for the extraction of toxic metal ions from contaminated environment by decomposition and erosion methods. Therefore, they can have potential effect on metal retrieval and decontamination of waste products of sewage sludge, industry, coal mine and heavy metal contaminated soils. Acidophilic SOB (*Acidithiobacillus ferrooxidans* and *A. thiooxidans*) converts the toxic metal sulphides forms to less toxic sulphates (Roy and Roy, 2015).

The main products of these mechanisms are sulfuric acid and ferric ions. The most commonly used strains for metal removal are *Leptospirillum ferrooxidans*, *Acidithiobacillus ferrooxidans* and *At.thiooxidans* but other species like *Aspergillus* also known (Dopson and Johnson, 2012; Johnson, 2012) In addition, iron-oxidizing and sulfur oxidizing strains among Archaea have also been identified (Brierley and Brierley, 2013; Vera *et al.*, 2013). For example, thermophilic bacteria, *Acidianus brierleyi* is a facultatively chemolithoautotrophic, extremely acidophilic Archaeon and aerobic bacteria growing in the presence of S^0 , metal sulphur and ferrous ion.

The solubilization process metal sulphides by these bacteria take place by two independent mechanisms: a ‘direct mechanism’ (direct enzymatic oxidation of sulphur moiety of metal sulphide) and an ‘indirect mechanism’ (non-enzymatic metal sulphide oxidation by Fe(III) ions along with enzymatic (re)-oxidation of the resulting Fe(II) ions; Sand *et al.*, 2001). SOB also form biofilm with the mineral surface in a contact sub-mechanism, while some planktonic also perform by remain floating in the bulk solution (non-contact sub-mechanism) (Ackil, 2014).

Fig.1 Oxidation-reduction reaction of sulphur compounds



Applications of SOB in H₂S removal

The sulphur oxidizing bacteria also play an important role in removal of poisonous hydrogen sulphide (H₂S) from the atmosphere. The strong unpleasant smell due to hydrogen

sulphide emission is one of the major problems of wastewater treatment plants (WWTP). Also the gas is responsible for atmospheric pollution and corrosion thus affecting human and environmental health (Tang *et al.*, 2009). Three dominant bacterial

families: Hydrogenophilaceae, Xanthomonadaceae and Spirillaceae found in biofilter packed with marble having acidic (pH < 3) can remove the odour of H₂S (Chouari, 2015; Heydarzadeh, 2014).

In other experiment conducted by Lestari, 2016, a packed bed reactor was evaluated for hydrogen sulphide removal by sulfur-oxidizing bacteria attached as a biofilm on salak fruit seeds (SFS).

The bacteria were isolated from wastewater sludge biogas plant decreased H₂S in biogas from 142.48 ppm to 4.06 ppm (97.15% removal efficiency) along with biogas flow rate of 8550 g/m³/h in residence time of 4 h.

Giordano *et al.*, (2015) studied the potential of biological sulphur-oxidizing potential of indigenous sludge of a plant treating tannery wastewater, Italy. It was demonstrated that the use of different fundamental techniques was a basic step in order to detect the large number of SOB components. In addition, the significance of using the primary sludge as inoculum for sulphur oxidizing reactors was ascertained.

Other mixed applications

Purple sulphur bacteria and other colourless sulphur-oxidizers also used as a part of mixed microbial communities for sewage treatment processes and might also be used in H₂S removal.

Sulphur bacteria can also be used for production of biopolymers such as poly β-hydroxybutyrate and molecular hydrogen. Some sulphate-reducers for example *Desulfovibrio desulfuricans* are having the capacity of reducing uranium, for concentrating and removal of radioactive uranium (Shreeja, 2016).

Future perspective

The efficiency of sulphur oxidizing bacteria can be improved to get more and efficient mechanisms for different uses. The limitations like *Thiothrix* cause problems in the industrial process, needs to be studied and find a solution. Also there is scope of enhancing efficiency of SOB especially in mining activities, where acidophilic sulphur bacteria such as *Thiobacillus ferrooxidans*, *T. thiooxidans* and *T. acidophilus*, which are being used in the recovery of metals, are too poor for conventional metallurgical extraction. Recoveries of up to 70% of copper from low-grade ores can be increase up to maximum potential. It can be concluded that sulphur oxidizing bacteria have enormous potential to metabolize the reduced S-compounds. Due to this they have wide applications in agriculture (crop improvement), maintaining environment, metal removal, tannery waste water treatment by removing H₂S etc.

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