

ENVIRONMENTAL BIOCHEMICAL ANALYSIS OF SULFUR COMPOUND TRANSFORMATION OF NATURAL AND TECHNOGENIC GENESIS

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Abstract. Ecological and biochemical analysis of transformations of sulfur compounds of natural and technogenic genesis is carried out in the article. Biochemical analysis was based on metabolic models of bacteria *Thiobacillus* sp., *Acidithiobacillus* sp. etc. and the study of ecological trophic groups of microorganisms using the KEGG database to establish the regularities of sulfur transformations produced using secondary raw materials. The ways of attracting bacterially transformed sulfur by plant systems as an environmentally safe direction for improving S-nutrition in the ecosystem were determined.

Key words: ecological and biochemical analysis, sulfur compounds, metabolic models, agro-ecosystem, secondary raw materials

1. Introduction

Sulfur is a macro element essential for the development of plants. Its assimilation is an important factor that determines how fast the plants grow and adapt to different stress factors and therefore affects yields.

Inorganic sulfate penetrates through the roots of plants, and through the biosynthesis of cysteine it is introduced as organic sulfur. Fig. 1 shows a general scheme of sulfur metabolism pathways.

Basically two biochemical approaches are used for plant sulfur metabolism investigation:

- pathway-oriented approach aimed at understanding the pathway structure and coordination sulfur-including metabolites;
- impartial approach that include functional genomics using for determination of the sulfur metabolism pathway and its control within the entire plant system.

Theoretical substantiation of effective environmentally safe ways of sulfur absorption by “microorganisms-plants” symbiotic systems using secondary mineral resource is a very important task nowadays.



Fig. 1. Sulfur metabolism pathways [1]

2. Materials and methods

2.1. Review of the previous research

Sulfur compounds are essential for plant growth and development. Moreover, understanding the details of microbial mediated sulfur cycle with process of the release of soil sulfur based on molecular spectroscopic studies rather than chemical reactivity is more effective.

A number of scientific works of understanding how organic and inorganic sulfur compounds are converted into sulfur forms available to plants and how this process is regulated has made considerable advances in the last several years [2].

Sulfur (S) is a limiting element for increasing crop yield and harvest quality due to lower levels of available

S forms and varieties of crops that remove S from the soil at a faster pace.

Sulfur present in soil mostly in organic forms such as sulfonates and sulfate esters [3] (Fig. 2).

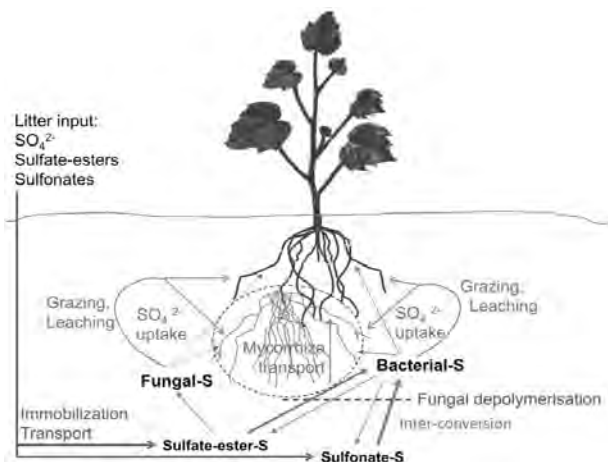


Fig. 2. Sulfur cycle in system “soil-plant” in rhizosphere [3]

These organo-S forms are not directly available to plants which rely upon microbes in soil and rhizosphere for organo-S mobilization. Plant root activity impacts physical and chemical properties of the soil through the release of organic compounds (rhizodeposition) which accounts for 15–30 % of photosynthetically produced carbon. This process provides soil organisms with the source of energy that enables them to fulfill their respective functional roles.

Thus, Fig. 2 shows that soil microbes transform S in two major ways: by organo-S pools or mineralized to inorganic S (green lines, thickness suggests the main pathway). Organo-S mineralised by fungi and bacteria becomes available for plant uptake in the form of sulfate [3].

The Sox system is the central pathway in the facultative bacteria from Alphaproteobacteria (e.g. *Paracoccus*) [4]. But the mechanisms of sulfur oxidation in chemolithotrophic bacteria, predominantly belonging to Beta – (e.g. *Thiobacillus*) (Fig. 3) and Gamma proteobacteria (e.g. *Thiomicrospira*) are not well determined.

It should be noted that the combined sulfate / sulfide oxidation pulps derived from Chromatiaceae or Chlorobi can be ancestors of all sulfur-lithotrophic processes [5].

This, in turn, may have a significant potential for the development of processes of symbiotic transformation in the system of “rhizosphere microbiome – plant groupings” and the applied aspect of the development of environmentally friendly technological solutions to

improve plant nutrition with various nutrients, in particular sulfur.

Thus, microorganisms actively participate in transformation of sulfur compounds in the ecosystem, and this can be used to efficient sulfur transformation in agroecosystem using such secondary raw material as biosulfur to improve plant nutrition.

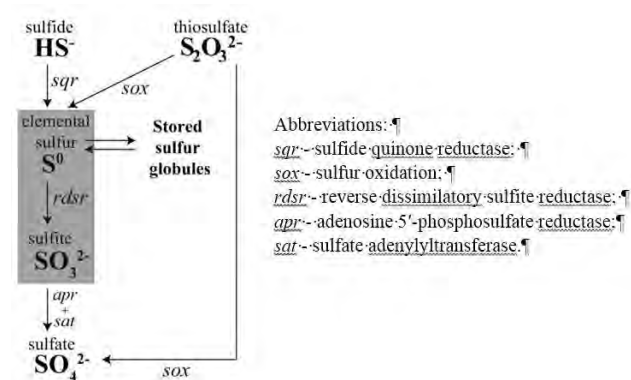


Fig. 3. Scheme of sulfur oxidation pathway in SUP05 bacteria [4]

This paper focuses on the environmental biochemical analysis of sulfur compound transformation of natural and technogenic genesis. To achieve the aim, the following tasks were set:

- analysis of metabolic models for sulfur conversion under rhizosphere;
- study of the biochemical reactions under sulfur metabolism in acidophilic groups of thiobacillus;
- study of the biochemical transformation of sulfur compound of technogenic origin.

2.2 Materials and methods

The methodology of investigation is based on the biochemical formalization using the system-synergetic approach for the description of the patterns of sulfur oxidation systems.

Special software was used in the research to identify the necessary ecologo-trophic groups of bacteria and implement the schemes of trophic interactions in associations of sulfur-oxidation microorganisms. The taxonomic classification was determined under the KEGG database use [6].

3. Results and discussion

Fig. 4 presents the existing sulfur transformation model for *A. ferrooxidans* under the bio-informatic analysis of the potential genes present in its genome.

In *A. ferrooxidans*, the expression of the genes involved in the oxidation of Fe(II) and sulfur

compounds in the respiratory pathways is dependent on the electron donor present in the medium. In addition, the genes involved in Fe(II) oxidation are dominated by those involved in sulfur oxidation, in the presence of both electron donors. In the regulator of the recombinant sensory two-component system RegBA was studied. The research [7] has shown that (i) the redox potential increases during Fe(II) oxidation but remains stable during sulfur oxidation and (ii) of the regulator RegA is able to bind to the regulatory area of a number of genes/operons involved in Fe(II) and sulfur oxidation.

Bacterial inorganic sulfur oxidation includes different chemical and fermentation reactions. The scheme of enzymatic oxidation of sulfur compounds presents on the table 1 [8].

Table 1

Bacterial oxidation of inorganic sulfur

Basic description	Conversion compounds
The main oxidation pathway	$S^{2-} \rightarrow S^0 \rightarrow SO_3^{2-} \rightarrow SO_4^{2-}$
Fermentation reactions that conducted with chemical reactions and the use of inhibitors	for $S^0 \rightarrow SO_3^{2-}$ $SO_3^{2-} \rightarrow SO_4^{2-}$
Oxidizing agents	$O_2, Fe^{3+},$ cytochromes

The bioinformatics analysis of the genome sequence of organism indicated various enzymes, enzyme complexes, and the electron transfer chain components that were located in different cellular compartments. In [9] based on metabolic models in

Acidithiobacillus species, hypothetical model for sulfur oxidation in A. thiooxidans A01 was developed with genome sequence analysis use and experimental results of study (Fig. 5).

As it is shown, that sulfur oxidation systems and electron transfer pathways occurred different cellular compartments.

The biochemical reactions under sulfur oxidation system are presented in table 2.

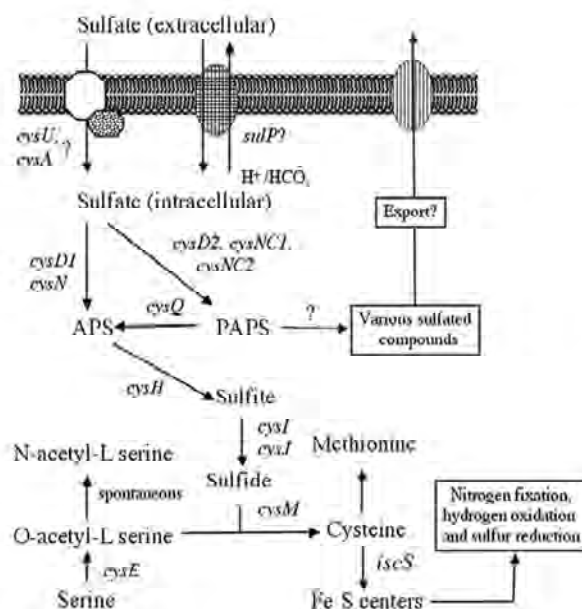


Fig. 4. Sulfur metabolic model for A. ferrooxidans [7]

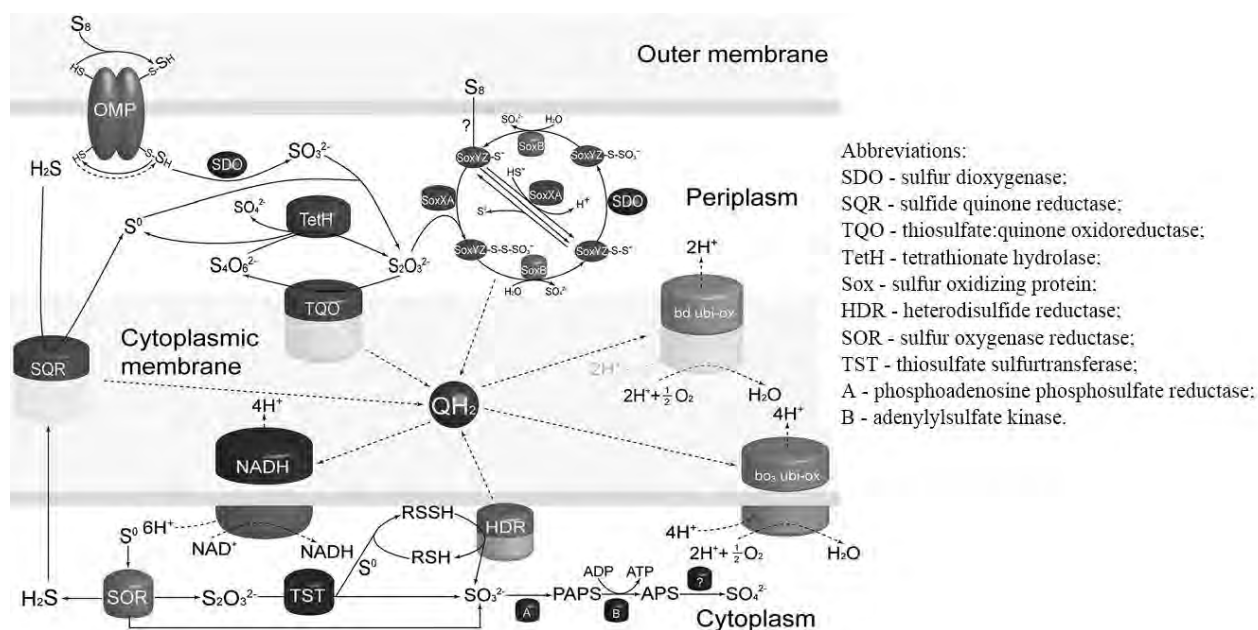


Fig. 5. Sulfur compound oxidation pathways in A. thiooxidans [9]

Table 2

Enzyme properties and biochemical reactions involved in sulfur oxidation

Enzymes	Bio-chemical reaction
1	2
Hydrogen sulfide: Flavocytochrome c and oxidoreductase	
Sulfur: oxygen oxidoreductase (hydrogen-sulfide- and sulfite-forming)	
Parathion, NADPH--hemoprotein reductein: oxygen oxidoreductase (paraoxon-forming)	

The previous study in the laboratories of Sumy State University [10–11] proved the expediency of using phosphogypsum (PG) as a mineral substrate for the development of microorganism association that oxidize sulfur compounds under aerobic conditions during desulfurization of biogas and the use of the processed product – bio-sulfur as environmentally safe material in environmental protection technologies.

The basic composition of phosphogypsum granules in terms of oxides is as follows: CaO (36.73 %–48.0 %). SO₃ (38.22 %–50.1 %). SiO₂ (4.3 %–10.0 %). P₂O₅ (1.10 %–2.75 %). Fe₂O₃ (3.80 %–4.50 %). Al₂O₃ (0.87 %–0.93 %). MgO (0.10 %–0.12 %). Na₂O (0.32 %–0.41 %). K₂O (0.08 %–0.10 %). CuO (0.01 %–0.02 %). ZnO (0.02 %–0.03 %). H₂O_{crystal} (20.73 %–24.43 %)

Fig. 6 shows the basic scheme of interactions that was formed according to the analysis of the

pathways of biochemical interactions of the phosphogypsum mineral carrier and biofilms of sulfur-oxidizing bacteria.



Fig. 6. Granulated PG as a carrier for the growth of sulfur-oxidizing microorganisms

The acidophilic groups of thiobacteria were used for phosphogypsum conversion in the process of biogas bio-desulfurization. Thus, acidophil

association of microorganisms was formed during the desulfurization process [11]. Representatives of species of the genus *Acidithiobacillus*, in particular *A. ferrooxidans* play a major role in biomineralization processes by recycling ferric and sulfur compounds with the release of sulfuric acid that chemically interact with minerals, which leads to the release of metal ions into the solution.

This association is able to oxidize hydrogen sulfide forming bio-sulfur in acid medium and transform PG with metals release into a liquid phase and their removal from the system by reagent precipitation.

The granulated PG as a carrier for microorganisms and as nutrient supplier has such aspects of using: it has low cost; it stimulates the growth of necessary ecological trophic groups of microorganisms; it creates suitable conditions for the development of bio-film on their surface; it extends the contact surface with the gas stream; it is resistant to hyperacidity; it increases the yield of bio-sulfur in gas purification systems.

The natural sorption mechanism is characteristic of bacteria living cells. This mechanism provides important minerals (microelements and macronutrients) for microorganisms in appropriate concentrations, which come from mineral substrate – PG granules. Cells with specialized transfer systems that use energy of ATP hydrolysis provide transfer of ions into the cell or their release in the extracellular space. Inside the cell metals are released as ions or in the form associated with various components of the cytoplasm.

The advantage of immobilizing bacteria adsorption method on the PG surface is that it allows binding of bacteria in the matrices of the supportive medium. This creates a stable bio-mineral structure with effective sulfide conversion to bio-sulfur that can be used as a source of sulfur forms available for plants.

Conclusions

The biochemical and fermentation reactions of sulfur compounds transformations was analyzed according to the concept of environmental safety. The biochemical analysis is based on the metabolic models of *Thiobacillus* sp., *Acidithiobacillus* sp. etc, as well as the analysis of the sequence of biochemical reactions with the use of KEGG database and experimental results from the previous research. The technogenic resource of sulfur, namely, PG utilization as a mineral substrate for the development of the association of sulfur-oxidizing microorganisms under aerobic conditions during biogas desulfurization was

considered. Acidophilic groups of thiobacilli were used for bioconversion and leaching of PG in the process of biogas bio-desulfurization.

Thus, sulfur-oxidizing and sulfide-oxidizing microorganisms actively participate in the transformation of sulfur compounds in the ecosystem and this can be used in efficient sulfur transformation in the agroecosystem in the environmentally safe way of recycling secondary raw materials such as bio-sulfur from the system of bio-desulfurization of gas streams with PG processed as a mineral supply.

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