ECOLOGY

Встановлено раціональний якісний та кількісний склад гранул фосфогіпсу, що використовується у якості завантаження для систем біохімічного очищення газових викидів. Здійснено вивчення зон біотрансформації компонентів фосфогіпсу за допомогою растрової мікроскопії. Досліджено біоплівку, утворену сіркоокисними бактеріями на поверхні гранул, та елементарну сірку – метаболіт, відкладений у процесі окиснення сірководню. Визначено фізико-хімічні властивості біосірки, продукованої у результаті біохімічного газоочищення сульфурвмісних газових потоків у біофільтрах із завантаженням з фосфогіпсу. Проаналізовано моделі метаболічних шляхів сіркоокисних бактерій, що забезпечують окиснення сульфурвмісних сполук до легко доступних для рослин форм з використанням електронних баз даних KEGG database, MetaCyc та EzTaxon database. Визначено біохімічні механізми трансформацій біосірки при залученні її до процесу S-живлення рослин, що дозволить її утилізувати в агроекосистемах. Обґрунтовано комбіновану схему шляхів бактеріального окиснення сульфіду до сульфату. Оцінено видову структуру еколого-трофічних груп мікроорганізмів, що беруть участь в окисленні сірки, серед яких хемолітотрофні бактерії з роду Thiobacillus є домінантними. Розроблено загальну технологічну схему утилізації фосфогіпсу з продукуванням біосірки в системах біохімічного газоочищення. Отримано екологічні ефекти від впровадження запропонованої технологічної системи: видалено домішки (сірководню, вуглекислого газу) із газових викидів; утилізовано відхід хімічної промисловості – відвальний фосфогіпс; вироблено біосірку як продукт, що застосовується для покращення S-живлення в агроекосистемах

Ключові слова: біосірка, утилізація фосфогіпсу, біохімічне газоочищення, біотрансформація сульфурсполук, S-живлення рослин

UDC 502.174:66.074:631.461.7
DOI: 10.15587/1729-4061.2018.132240

RESEARCH INTO BIOTECHNOLOGICAL PROCESSES OF PLANT S-NUTRITION STIMULATION BY THE PRODUCTS OF PHOSPHOGYPSUM DISPOSAL IN GAS CLEANING SYSTEMS

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1. Introduction

Sulfur (S) is a physiologically essential element of nutrition for plants. However, deficit of S in soils is becoming more common in many areas of the world as a result of agronomic practices and a high increase in biomass. The forms of its getting into agro-ecosystems, including organic and inorganic, are of importance.

The study of processes of microbiological oxidation of elementary sulfur (S^0) as an alternative to increasing the S level in the soil is particularly relevant nowadays. In this case, ecological-trophic characteristics and diversity of S^0-oxidizing microorganisms in soils, specifically of the genus *Thiobacillus*, and biochemical mechanisms of S^0 oxidation in bacterial cells are the first to be explored [1].

Another important problem of environmental safety is cleaning gas flows of different origin (biogas, natural gas, industrial gas-air emissions, etc.) from sulfur compounds. It is necessary to remove hydrogen sulfide, contained in them, in order to avoid possible equipment corrosion and hazards for people and the environment. Biochemical technological solutions with the use of sulfur-oxidizing microorganisms that produce H_2S conversion into elemental sulfur are actively implemented in the world [2, 3]. These bacteria use CO_2 as a source of carbon to build up their own biomass. The ecological-trophic groups, which are resistant to

changes in environmental factors (for example, temperature fluctuations, humidity, pH, the ratio of O_2/H_2S), can be distinguished. It should be noted that implementation of integrated solutions to improve nutrition of plants in agro-ecosystems and bioconversion of sulfur compounds with their removal from gas flows is a relevant problem today.

2. Literature review and problem statement

Removal of H_2S and its conversion into elemental sulfur are carried out in one process within the framework of technological solutions THIOPAQ O&G System [2]. The useful resource, elemental sulfur, which is produced in this case, can be used as a fertilizer component and in liquid fungicides.

THIOPAQ process [3] was designed to remove H_2S from the biogas streams of low pressure. In this process, the gas flow containing H_2S, is in contact with aqueous solution of caustic soda with formation of *NaHS*. Dissolved hydrogen sulfide (*HS*) from the scrubber is fed to the biofilter. The process occurs at atmospheric pressure and under aerobic conditions. Metabolism of bacteria is accompanied by reactions of biodesulfurization with formation of elemental sulfur at pH from 8.2 to 9.0 [4]. But this range of pH in the biofilter prevents the growth of many bacterial species of thiobacilli. Oxisulfide bacteria Thiobacillus sp. are gram-negative, aerobic, motile cells that grow well at 20–35 °C and pH of about 2–6 units.

Purified gas meets specification of up to 5 ppm H_2S (typical requirements for biogas of EU countries). The range of sulfur production is from 45 kg per day to 20 tons a day [3, 5].

Using the carrier for immobilization of microorganisms provides for expansion of the surface for the growth of microorganisms and protects against inhibiting action of toxic impurities [6]. Granular activated carbon (GAC) is often used for immobilization of microorganisms. To remove hydrogen sulfide, loading of biological filters, consisting of peat and compost, alginate, polypropylene rings and polyurethane foam, etc., is used [7, 8].

According to the results of conducted research with the use of alginate, activated carbon, cellulose beads Mavicell and plastic rings Kaldnes K1 as carriers to sulfide oxide bacterium *Thiobacillus thioparus*, it was found that using two latter kinds of loading, it is possible to achieve high indicators of effectiveness of hydrogen sulfide removal at the level of 95–100 % [9]. The strain of *Accinetobacter ST-550*, capable of producing indigo, immobilized on the polyurethane carrier was used, applying a new method of immobilization with the use of adhesive bacteriofibrous protein AtaA from the family of three-dimensional adhesim autotransporters (TAA) [10].

The mentioned above processes of immobilization need supplying additional nutrients into the space of a bioreactor to stimulate the development of oxisulfide bacteria. And neutrophilic and alkophilic modes require correction of pH values using chemical reagents. It affects economic performance of the gas cleaning process and degree of cleaning.

It should be noted that acidophilic groups of microorganisms, the use of which is limited in the following technological solutions, are involved into the processes of oxidation of sulfur compounds to elemental sulfur.

A new type of mineral carrier based of industrial waste, specifically phosphogypsum, was developed at the laboratory base of Sumy State University (Ukraine) [11]. Biosulfur is formed when implementing the process of gas cleaning in a sewage filter with a granulated carrier from phosphogypsum [12]. The resulting product has different physical, chemical and biochemical characteristics in comparison with elemental sulfur/biosulfur. In this connection, there is a need to conduct qualitative and quantitative research into biosulfur.

Sulfur is the basic nutritional substance among microelements that are absorbed by plants and its consumption volumes can be compared to the physiological need in phosphorus. But the major part of sulfur is absorbed by roots of plants, especially in the area of root hairs in the form of water soluble sulfate and comes into plant cells using protein-agents and sulfate ions. Inside a plant, sulfate-ions move with the transpiration flow and then are accumulated in vacuoles of plant cells or participate in a number of biochemical reactions. In addition, leaves of plants absorb sulfur dioxide (SO_2) from the atmosphere, but usually in the amount that does not exceed 1 kg S/ha·year. Most part of sulfate sulfur, absorbed by roots, is restored and form the part of cysteine in chloroplasts of leaves, which is primary connection, from which other sulfur-containing organic compounds are subsequently formed in plants. Other important sulfur-containing amino acids are cysteine (two interconnected molecules of cysteine) and methionine. In smaller quantities, sulfur is included in such important organic compounds as coenzyme A, biotin, thiamine, glutathione, as well as sulfolipids [13].

Biological oxidation of sulfur granules is a limitation process to enhance effectiveness of using elemental sulfate fertilizers, because it turns sulfur into accessible sulfate [14]. Models of metabolic pathways of oxisulfide bacteria were studied in [15–20], some of them are shown in Fig. 1–3.

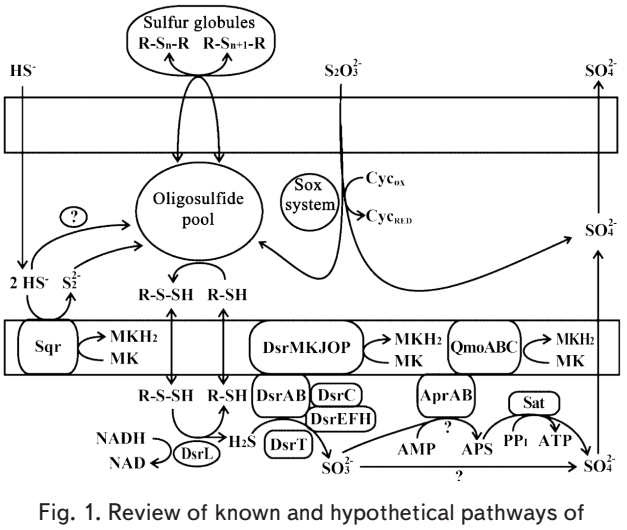

Fig. 1. Review of known and hypothetical pathways of electronic and inorganic sulfur compounds to *Chlorobaculum tepidum* [18]

The thin arrows determine the pathways of propagation of electrons, and the thick arrows determine metabolic or transport pathways of substrates or products. ? is hypothetically; APS is adenosine 5' – phosphosulfate; Dsb – homologs, encoded by genes in a cluster of genes thedsb, which encode thiol: disulfide interchangeable proteins; Dsr are proteins that are encoded by genes in the cluster of gene dsr, which

encodes proteins of dissimilation sulfitereductase; [S_0] is the zero valence or equivalent (atom of sulfur in organic and inorganic polysulfide, elemental sulfur); Sox are the proteins that are encoded by genes in the cluster of genes of sox.

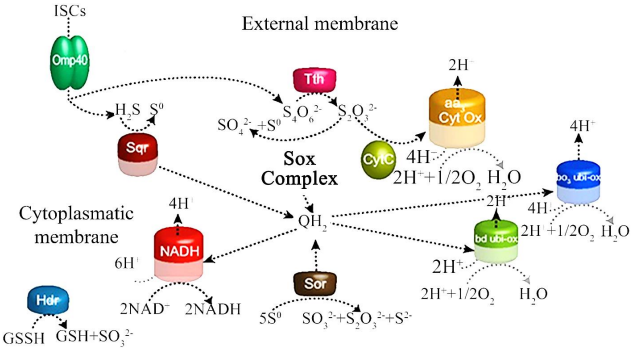

Fig. 2. Model for metabolism of inorganic sulfur compounds in *Acidithiobacillus caldus* [19]

ISCs are inorganic sulfur compounds; *Sox* are the systems of sulfur oxidation; T*th* is hydrolase tetrathionate; *Hdr* is the heterodisulfidereductase; *Sqr* is sulfide quinone reductase; *Sor*, sulfur oxygenase reductase; *hyp*, hypothetic protein.

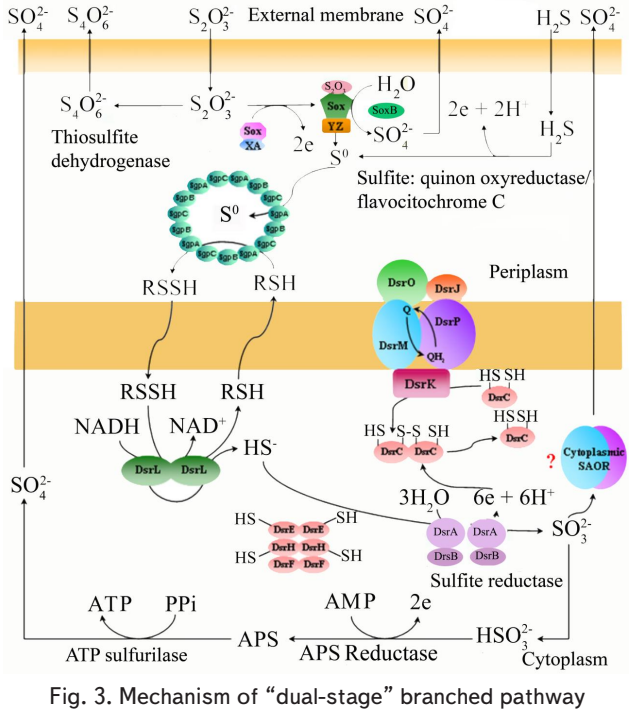

Fig. 3. Mechanism of "dual-stage" branched pathway of thiosulfate oxidation in sulfur oxide aniogenic photolithotrophic, as well as in optionally aerobic chemolithotrophic bacteria [20]

Initial oxidation of covalent binding of thiosulfate with SoxYZ is implied as SoxXA and SoxB then hydrolytically releases sulfate as in the Sox- indirect way. Since these organisms are short of SoxCD, sulfur atom of sulfur, still bonded with SoxY, can not be directly oxidized further. Instead, it is transferred to increasing sulfur globules in bacterial periplasma. Extracytoplasmatic sulfur globules, which are wrapped by proteins SgpA, SgpB and SgpC, are reduced and transferred to the cytoplasm. Subsequently, they are oxidized to sulfite by reverse activity of DsrAB. The latest model of interaction of proteins Dsr to achieve oxidation of globular sulfur to sulfite states, with which DsrAB specifically interacts with DsrL, on the one hand, and DsrMKJOP and DsrEFHC, on the other hand. The electrons that are released during oxidation of sulfide DsrAB, are supplied to the photosynthetic electron transfer via DsrC and DsrKMJOP with DsrJ as a terminal acceptor. It is believed that sulfite is oxidized to sulfate by sequential retroactive action of cytoplasmatic AMP-dependent APS-reductase and ATP-sulfilase. However, in a number of phototrophic organisms, a unique cytoplasmatically located AMP-independent SAOR can implement this final oxidation stage.

But as we can see, these models represent the set of possible pathways of transformations of sulfur compounds for certain types of microorganisms, while not all enzymatic transformations have been proven so far (designated by the "?" sign).

Hemotrophic bacteria, particularly from genus *Thiobacillus* [21] (*thioparus, thiobacilli, denitricans, thiooxidans, ferrooxidans*) and other microorganisms (*chlorobiaciae*, *xanthomonas*), which convert H_2S into reduced sulfur compounds, are used in the technologies of biodesulfurization of gas flows [22].

Complete oxidation of this pool to sulfate depends on direction of dissimilation sulforeductase, which determined the concentration of oxygen and sulfide. Under conditions of oxygen limitation, that is, at concentration of O_2 below 0.1 mg/dm^3, biosulfate is the final product of sulfide oxidation, whereas sulfate is formed under conditions of sulfide limitation, which was considered in [23].

Thus, determining qualitative differences of the resulting product of disposal of a carrier from phosphogypsum in the systems of desulfurization – biosulfur, will make it possible to establish the possible scope of its use for improvement of S-nutrition of plants.

3. The aim and objectives of the study

The aim of the present research is to study the possibilities of plant nutrition with the use of biosulfur – the product of phosphogypsum disposal in biochemical gas cleaning systems.

To accomplish the aim, the following tasks have been set:

– to explore biotransformation of phosphogypsum components in the process of hydrogen sulfide oxidation by thiobacteria with biosulfur production;

– to substantiate theoretically the expediency of the use of biosulfur for improvement of S-nutrition of plants;

– to develop a general technological scheme of phosphogypsum disposal with biosulfur production in biochemical gas cleaning systems.

4. Materials and methods to study biosulfur as a product of phosphogypsum disposal in the biochemical gas cleaning systems

4. 1. Characteristics of the carrier made from phosphogypsum for the biochemical technological systems

As a result of carrying out a series of experiments [11, 12, 24], we determined the rational content (in %) of components in modified phosphogypsum granules for immobilization of sulfate oxide microorganisms (in terms of oxides of metals and nonmetals) (Table 1).

Table 1

Basic chemical components of modified phosphogypsum granules (samples, dried at 333 K)

Components of granules	CaO	SO_3	P_2O_5	MnO	SiO_2	Fe_2O_3	Na_2O	CuO+ZO
Mass fraction, % by weight	36.0–46.0	38.0–50.0	1.1–2.5	3.0–3.5	5.5–10.0	3.8–4.5	0.5–1.5	0.04–0.05

In the modified phosphohypsum granules, mass fraction of the basic substance of calcium sulfate dehydrate ($CaSO_4 \cdot 2H_2O$) in terms of dry two-water gypsum is not less than 80 %, and mass fraction of fluoride compounds in terms of fluoride F_2 – not more than 0.003 %.

4. 2. Methods of research into the product of disposal of mineral carrier from phosphogypsum in the biochemical gas cleaning systems

Physical-chemical research. X-ray microanalysis by area was used to conduct high-quality (element composition) and quantitative analysis of chemical composition of the explored samples. Research was performed by using scanning electronic microscope REMMA102 (JSC "Selmi"), which is equipped with a multi-channel X-ray spectrometer with wave dispersion and dispersion by production energies.

To understand the forms of binding metals and organic substances, the mineral composition of active sludge was studied. X-ray diffraction research into the structure of material was performed on the automated diffractometer DRON-4-07. Automation system DRONE-4-07 is based on a microprocessor controller that provides management of goniometer GUR-9 and the transfer of data in a digital form on a PC. The experimental results were transferred directly to software package of support of the experiment DifWin-1 (TOO "Etalon PTC") for preliminary processing. Identification of crystalline phases was carried out using a card index JCPDS (Joint Committee on Powder Diffraction Standards).

Microbiological and biochemical research. Bioinformation databases were used in the study to identify the necessary ecological-trophic groups of microorganisms and implementation of schemes of trophic interactions in associations of different ecological and trophic groups of microorganisms. Specifically, the taxonomic classification and review of metabolic pathways of transformation of sulfur compounds was carried out using electronic KEGG databases (the Kyoto encyclopedia of genes and genomes), MetaCyc and the Ez-Taxon database.

MetaCyc is a curator of database of experimentally identified pathways of metabolism of all areas of life. MetaCyc contains the pathways associated with both primary and secondary metabolism, as well as metabolites, reactions, enzymes and genes.

In assessing the dynamics of development of sulfur oxidizing bacteria in the process of gas cleaning, modified phosphogypsum granules were selected directly from the space of the biofilm along with the bacterial matrix that develop on it. In this case, removal of aerobic bacteria occurred on the surface in a laboratory thermostat TSU-01-200, when microorganisms got oxygen directly from the air.

The mechanical cultivation method was used: the studied aerobic culture (eyedropper + spatula) was introduced in the agarized nutritious environment, which was molten and cooled to 303 K, stirred and poured from top by the medium. Favorable conditions for cultivation of microorganisms were created.

Introduction of modified phosphogypsum granules was caused by the fact that it creates an environment for flowing of electrophilic reactions (SO_4^{2-}) while getting energy and acts as a source of many mineral elements required for metabolism of bacteria.

Petri dishes with samples were kept in the thermostat TSU-01-200 during a week to maintain the mesophilic temperature mode (309 K).

Microphotos of microbial preparations were obtained and processed by using the digital image output system "SEO Scan ICX 285 AK-F IEE-1394" and morphometric program "SEO Image Lab 2.0" (Sumy, Ukraine). Images of microbial colonies were acquired using the digital camera Canon Powershot SX30 IS with a matrix resolution of of 10 Megapixels.

Research into the form and cellular structures was performed on the transmission electronic microscope EMR 100 AK (NPO "Eelectron", Sumy, Ukraine).

5. Results of research into biosulfur – the product of phosphogypsum disposal in the biochemical gas flow cleaning systems

The study of areas of nutritious medium revealed the formation of metabolite in the development of sulfur oxidizing bacteria on the load from phosphogypsum. In Fig. 4, raster microphotos with different magnification of granules from phosphogypsum were overlaid to highlight the areas of biotransformation of phosphogypsum components. The clusters of bacteria of the genus thiobacilli with deposits of metabolite – elemental sulfur in the process of oxidation of hydrogen sulfide – were displayed separately.

Developing on the surface of modified granules of phosphogypsum, a biofilm begins to grow in the form of individual cellular components, sorbing dissolved compounds from the gas phase and transforming them into their own metabolites.

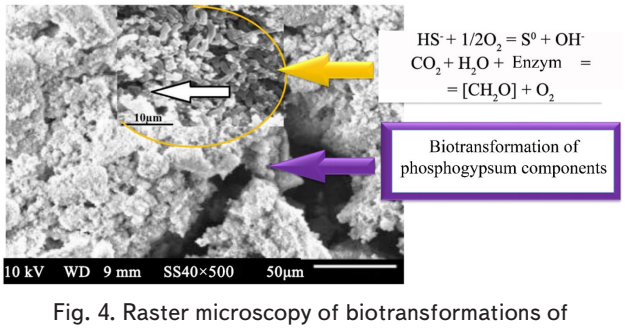

Fig. 4. Raster microscopy of biotransformations of phosphogypsum granules in the process of oxidation of hydrogen sulfide with thiobacteria. The arrows of different colors show: white – clusters of the formed biosulfur (GS. 10 μm); yellow – bioactive layer in a crack of modified phosphogypsum granules (GS 10 μm); purple – the zone of a crack of a biotransformed granules (GS 50 μm)

The influence on bacterial cells of normal and destructive tensions of the filtration gas flow is observed before the

formation of a single bacterial biofilm matrix. In this case, sulfur is found on the surface of the granules and is subjected to removal.

Fig. 5 shows general view of biosulfur after drying and rounding

Fig. 5. General view of biosulfur

Based of the diffractometer research and rastrum microanalysis of the structure of biosulfur, it was found that the content of orthomolecules S_8^0 in its composition reaches 60 %. Existence of organic sulfur inclusions (S_{org}) and particles of components of the transformed granules ($CaSO_4{\cdot}2H_2O$, $CaCO_3$, $Ca_3(PO_4)_2$, CaF_2) was established. The composition of the formed product is shown in Table 2. The "trace" content of fluoride in the final product was revealed, which according to the diffractometer research is in the form of CaF_2 that is insoluble in water and organic solvents, which corresponds by composition to natural mineral fluoride – fluorite. It should be noted that the content of biosulfur compounds of fluorine depends on its quantity in photogypsum and, accordingly, it was not identified in all samples.

Table 2

Content of basic elements in biosulfur

Content of basic elements in mineral spectrum of biosulfur, %							
S		Ca			P		
55.65–68.21		16.45–34.7			1.8–4.1		
Organic inclusions of biosulfur							
Bacterial cells	$C_{4,40}H_{6,8}O_{1,31}N_{0,86}P_{0,11}S_{0,14}$						
	Elemental composition of biomass , %						
	C	H	O	N	P	S	Ash
	52,9	6,8	21,0	12,0	3,5	4,5	4,0

Thus, the population of thiobacteria is useful for the environment. In the course of their own metabolism, bacteria stimulate arrival of easily digestible forms of sulfur to plants. Dead cells contribute to composition of soil humus without harm to the environment. Biosulfur is not toxic, there is the possibility of dosing this product depending on the use (for example, foliar or root treatment).

6. Discussion of results of studying biosulfur to enhance effective S-nutrition of plants

The obtained results make it possible to form an integrated concept for comprehensive solution of the problem of effective S-nutrition of plants and biosulfur disposal.

Based on the established characteristics of biosulfur, an important scientific and applied problem is substantiation of the biochemical mechanisms of its influence on the process of S-nutrition of plants. Analysis of existing metabolic models of transformation of various forms of sulfur in biotic systems makes it possible to solve this problem.

In fact, the resulting biosulfur is a biocomposite, which contains biogenic substances (primarily sulfur organic and inorganic, phosphorus, calcium) and bacterial biomass. Transition of sulfur compounds to accessible for the plants form is possible by using metabolic interactions in association of thiobacteria. The examined consortium is removed as excess biomass from the system of biochemical gas cleaning. Biosulfur as the product of treatment also includes metabolites (elemental sulfur) and transformed part of phosphogypsum.

Fig. 6 shows analysis of the basic ecological-trophic groups of microorganisms that take part in sulfur oxidation. In this case, autotrophic, mixotrophic and heterotrophic bacteria were taken into consideration. It was found that the correlation between auto-, hetero- and mixotrophic groups, involved in the sulfur oxidation, is at the level of 79:6:15 %.

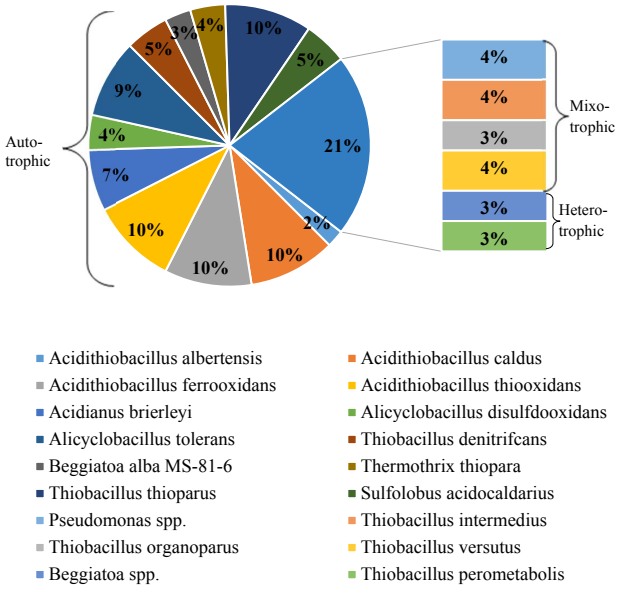

Fig. 6. Diagram of ecological-trophic groups involved in sulfur oxidation

Fig. 7 shows a diagram of metabolic pathways of transformation of sulfur compounds by sulfur oxide bacteria that was created using the information of KEGG database resource. Sulfur ability for oxidation is widespread among bacteria and archaea, including phototrophs and hemolithoautotrophs. The system of SOX (sulfur oxidation) [MD: M00595] is a well known method of sulfur oxidation and occurs in both photosynthetic and non-photosynthetic sulfur oxidizing bacteria [25].

Green sulfur bacteria and purple sulfur bacteria produce anoxygenic photosynthesis with reduced sulfur compounds, such as sulfide and elemental sulfur, as well as thiosulfate (in some species with the SOX system) as an electron donor for photoautotrophic increase. It was found that for some hemolithoautotrophic sulfur oxidizers (such as *Thiobacillus denitrificans*), the enzymes that reduce sulfur sedimentation, operate in the opposite direction, forming the way of oxidation of sulfur from sulfite to the APS and then to sulfate (Fig. 7).

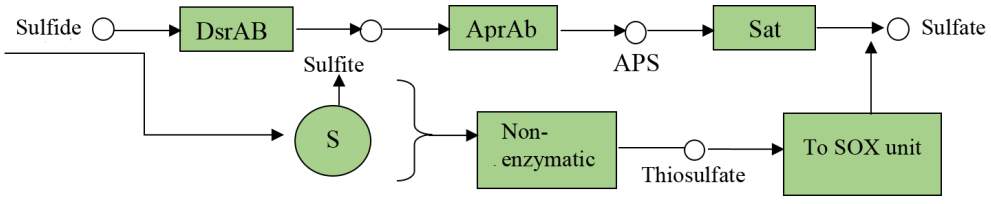

Fig. 7. Combined scheme of pathways of bacterial oxidation of sulfide to sulfate (with the block of transformations by elemental sulfur): Sulfide; DsrAB is dissimilative sulfite-reductase alpha subunit, Sulfite, AprAb — adenylsulfate, subunit A, APS — Adenosine 5'-phosphosulfate, Sat is sulfate adenyliltransferase, Sulfate; SCcR is sulfide-cytochrome-c reductase SoxA, SoxX, SoxY, SoxZ, SoxB — sulfur-oxidative proteins

Fig. 8 shows a metabolic pathway of sulfide-oxidation which is performed by bacteria *Acidithiobacillus ferrooxidans*. In the genome of these strains, the genes, encoding enzymes and proteins-carriers of electrons, which are supposed to participate in oxidation of the reduced inorganic sulfur compounds, were found [26, 27]. Biochemical mechanism of the metabolic pathway of *Acidithiobacillus ferrooxidans* indicates that sulfur oxidation to sulphate is possible due to the reduction-oxidation reaction, accompanied by simultaneous reduction of iron, in this case, sulfur is a source of a donor of electrons. The obtained alternative model of the process involves the transfer of electrons from S^0 to Fe^{+3}, through the respiratory chain, which includes a complex of bc1 and cytochrome c [28].

The model was created in the environment by the tools of database of metabolic pathways MetaCyc.

Thus, different ecological and trophic groups of microorganisms have different mechanisms, and accordingly, the systems, which are provided by oxidation of sulfur-containing compounds to sulfate that are accessible to plants. Based on the comparison of the metabolic pathways and ecophysiology of three strains of bacteria *Acidithiobacillus ferrooxidans, Acidithiobacillus Thiooxidans and Acidithiobacillus caldus,* it was found that the first one has *SQR* system, and the last two have *SOX* system for sulfur oxidation. In addition, only *Acidithiobacillus ferrooxidans* is capable of sulfur-reduction [29, 30].

Fig. 9 shows a review of the main pathways of sulphur dissimilation by plants.

Sulfate (SO_4^{2-}) is one of the most oxidized and, therefore, stable sulfur forms of that exist in the soil. Sulfur absorption by the plants' root system from the soil occurs almost exclusively at the expense of sulfate absorption. The form of sulfur, found in xylem and the juice from the phloem, is mainly sulfate, which is why sulfur translocation throughout a plant occurs mainly through non-metabolized sulfate. Then sulfate is exposed to activation by 5'-phosphosulfate adenosine (5'-adenylilsulfate [APS]) for further conversion. The main assimilation pathway is reduction of APS to sulfite (SO_3^{2-}), and then to sulfide (S^{2-}). Complete reduction of sulfate to sulfide requires one ATP molecule and eight electrons. Then sulfide combines with O-acetyl-Ser (OAS), which was formed with Ser, obtaining Cys. A relatively small point of deviation in this way is transformation from the APS to 3'-phosphoadenosin-5-phosphosulfate (PAPS), which is limited for sulfation.

Reduction of sulfates can occur both in the assimilation pathway that absorbs energy, and in the process, which ensures release of energy through dissimilation. Assimilation pathway, which is found in a wide range of organisms, leads to the formation of reduced sulfur compounds for biosynthesis of S-containing amino acids and does not lead to direct release of sulfide. In the dissimilation pathway, which is limited by compulsory anaerobic bacterial and archeal lines, sulfate (or sulfur) is a terminal electron acceptor of the respiratory chain that produces a large amount of inorganic sulfide. Both pathways begin with the activation of sulfate by the ATP reaction with the formation of adensilylsulfate (APS). In the assimilation pathway [MD: M00176], APS are transformed into 3'-phosphoadenylilsulfate (PAPS), and then reduced to sulfite, and sulfite is subsequently reduced to sulfide by assimilation sulfitereductase. In dissimilation pathway [MD: M00596], APS are directly reduced to sulfite, and sulfite is subsequently reduced to sulfide by dissimilation sulfitereductase (Fig. 10).

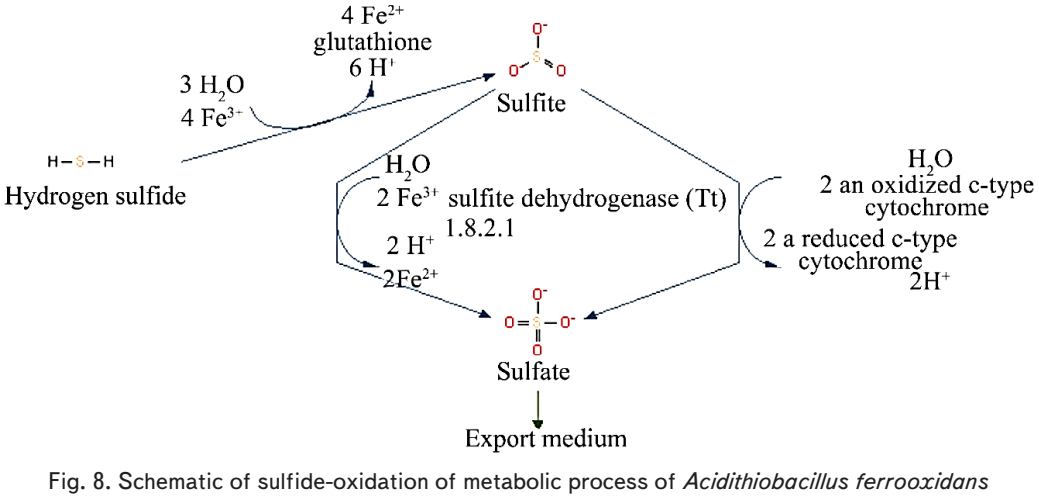

Fig. 8. Schematic of sulfide-oxidation of metabolic process of *Acidithiobacillus ferrooxidans*

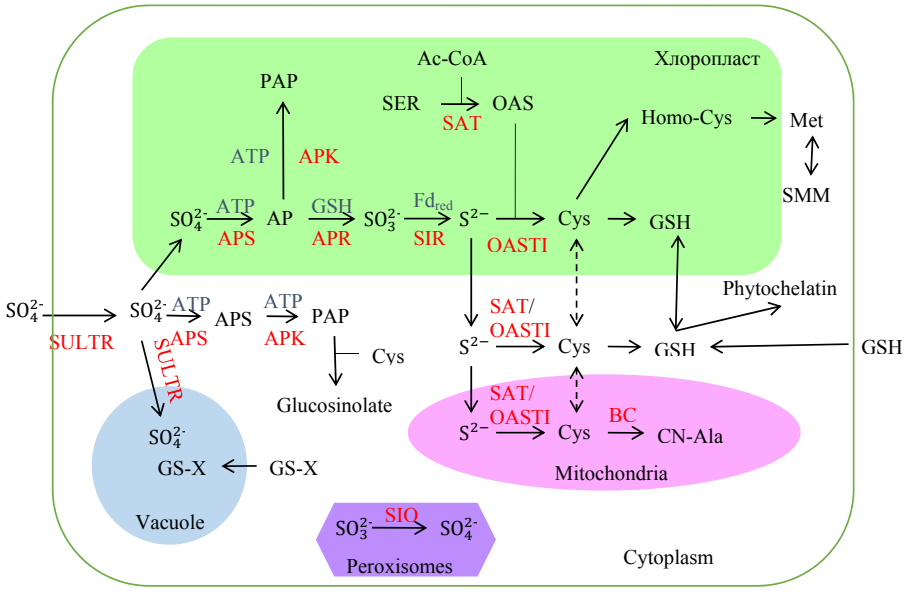

Fig. 9. Assimilation pathway for metabolism of sulphur in the organelles of plant cells. Black color denotes the titles of metabolites: Ac-CoA – acetyl-CoA; APS – adenosine phosphosulfate; CN-Ala – β-cyanoalanine; OAS – *O*-acetyl-Serotonin; PAPS – 3′-fosfoadenozin-5′-phosphosulfate; SMM – *S*-methyl-Methionine. Blue color denotes the titles of co-factors: Fd_{red} – ferredoxin. Red color denotes the titles of proteins: APK – adenozin phospho kinase; APR – adenozin-phospho reductase; BCS – β-cyano sintease; OASTL – *O*-acetyl-serotonin tiolase; SAT – Serotonin acetyltransferase; SIO – sulfite oxidase; SIR – sulfite reductase; SULTR – sulfate transporter

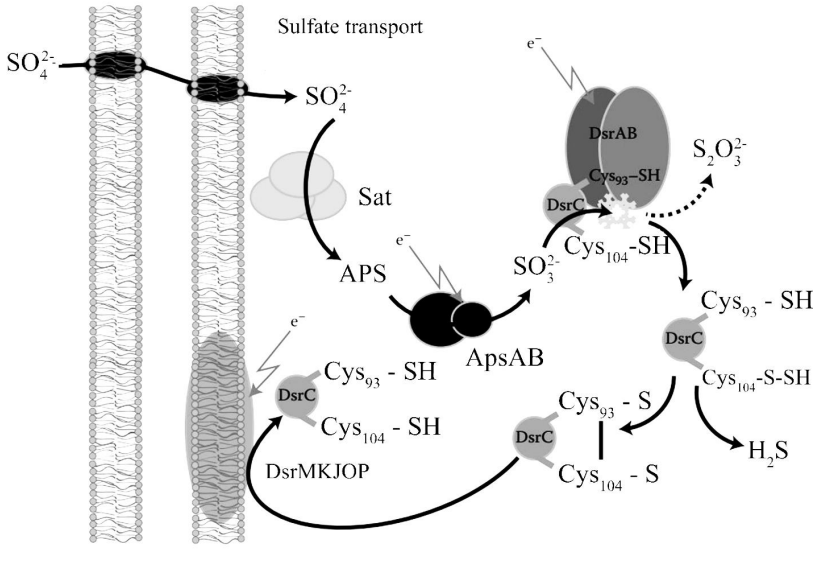

Fig. 10. Sructural diagram of processes, involved in the dissimilation reduction of sulfate

Analyzing the pathways of biotransformations of sulfur compounds, the possible directions of combining processes of biochemical transformations of elementary sulfur by sulfur oxidizing microorganisms were determined. Involvement of sulfur compounds in the form that is easily accessible to plants nutrition makes it possible to substantiate theoretically the feasibility of biosulfur disposal in agroecosystems. In this case, existence of additional biogenic elements (such as calcium, phosphorus) in biosulfur makes it possible to stimulate development of useful resistant groups of microorganisms and is the source of additional macro elements for development of plants.

The general block-diagram of phosphogypsum recycling process with obtaining the mineral carrier for sulfur oxidizing microorganisms with their subsequent disposal in biochemical gas cleaning systems was developed. Production of biosulfur (Fig. 11) occurs according to the developed technological solutions that were described in previous papers [12, 24].

According to the schematic model, disposal of technogenic mineral raw material (phosphogypsum) occurs with the formation of modified granules, which are used as immobilization carrier in technologies of atmospheric air protection in the systems of biochemical cleaning of gases from sulfur compounds. Biosulfur is separated for a suspension from the desulfurization unit, dried, rounded and is subject to disposal. Removed aqueous solution (turnover water) is pumped into the accumulation tank of the irrigation system. As a result of this process, biooxidation by thiobacteria of mineral elements from the media with phosphogypsum, ions

of metals (Cu, Zn, etc.) transfer into liquid phase, are deposited in non-soluble faction by the reagent method and are removed form the system separately from biosulfur.

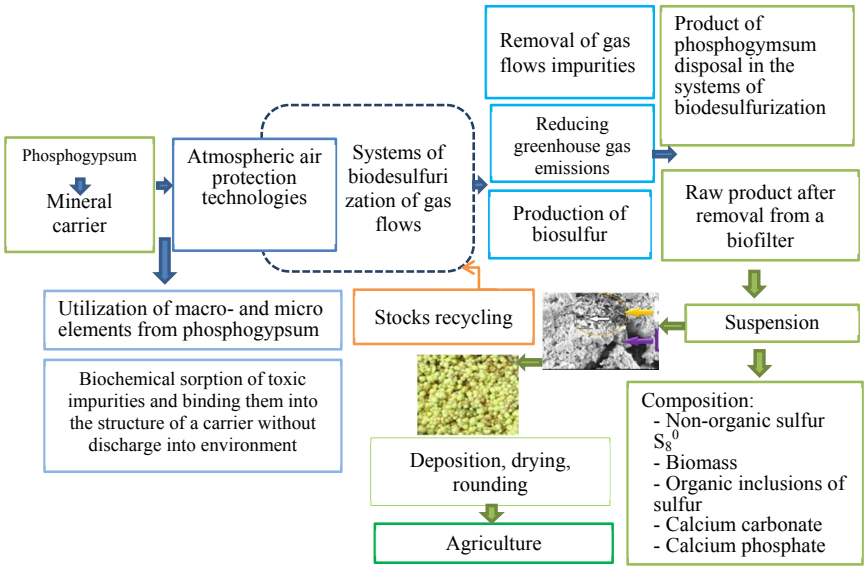

Fig. 11. General block diagram of integrated environmentally safe process of phosphogypsum disposal with obtaining biosulfur as the disposal product

Thus, the environmental effects that are obtained during implementation of such technological system are: removal of impurities (hydrogen sulfide, carbon dioxide) from gas emissions; recycling of dump phosphogypsum; production of biosulfur as the product that is used for improvement of S-nutrition in agroecosystems.

In the further research into the phosphogypsum disposal product in desulfurization systems – biosulfur, field tests will be performed to study its influence on nutrition of different groups of agricultural crops to determine the rational dose for its adding into soil.

7. Conclusions

1. It was found that hemotrophic bacteria of the genus *Thiobacillus* (*thioparus, thiobacilli, denitricans, thiooxidans, ferroxidans)* and other microorganisms (*chlorobiaciae, xanthomonas*) convert H_2S into reduced sulfur compounds, which causes its use in the technologies of biodesulfurization of gas flows. The content of the ortomolecules of S_8^0 in the composition of biosulfur was found to reach 60 %. The existence of organic sulfur inclusions ($S_{org.}$) and a part of the components of transformed granules ($CaSO_4{\cdot}2H_2O$, $CaCO_3$, $Ca_3(PO_4)_2$, CaF_2) was detected.

2. The ratio between auto-, hetero- and mixotrophic ecological groups of thiobacteria, involved in sulfur oxidation, was established at the level of 79 %: 6 %: 15 %. It was found that as a result of metabolic interactions, biosulfur in the form that is readily accessible to plants is formed in association of thiobacteria. A high level of assimilation is caused by transformation of biosulfur by sulfur oxidizing bacteria to sulfates due to existence of elementary sulfur in the composition, disposed phosphogypsum fractions and the dead excess biomass.

3. The technology of integrated environmentally safe phosphogypsum disposal process with obtaining biosulfur as a product of disposal was developed. The following positive environmental effects were obtained: cleaning gas emissions from sulfur compounds, solution of the problem of disposal of such industrial waste as phosphogypsum, restocking agroecosystems with biologically acessible sulfur, which makes it possible to stimulate S-nutrition of plants.

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