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Environmental biotechnologies for phosphogypsum recycling : protection of the atmosphere, hydrosphere and lithosphere

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This monograph substantiates biotechnological solutions for recycling phosphogypsum together with other types of waste with the production of useful bio-based products in the framework of environmental protection technologies. The concept of the methodological approach to phosphogypsum utilisation in bioprocesses includes its integrated use in technologies for the protection of atmospheric air, hydrosphere, and lithosphere. The monograph presents the results of research conducted at the Department of Ecology and Environmental Protection Technologies at Sumy State University.

The book is addressed to scientists, university professors, graduate, and postgraduate students, as well as to all those who are interested in the problem of waste recycling, in particular phosphogypsum, and the development of technological solutions for the protection of environmental components based on synergetic approach.

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Annotation

Environmental biotechnologies for phosphogypsum recycling: protection of the atmosphere, hydrosphere, and lithosphere : monograph

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Waste dumps of industrial processing of raw materials are constantly being replenished and are taking on scales that threaten the sustainable functioning of the ecosystem. The current world growth rate of phosphogypsum is estimated at 200 million tons per year, with a mass utilization rate of 10–15 % according to the most optimistic forecasts. Currently, 15 mineral fertilizer production facilities in the EU, following by 8 industrial sites are producing phosphate mineral fertilizers or possessing phosphogypsum waste handling facilities. This monograph substantiates biotechnological solutions for recycling phosphogypsum together with other types of waste with the production of useful bio-based products in the framework of environmental protection technologies. The concept of the methodological approach to phosphogypsum utilisation in bioprocesses includes its integrated use in technologies for the protection of atmospheric air, hydrosphere, and lithosphere.

The monograph presents the results of research conducted at the Department of Ecology and Environmental Protection Technologies at Sumy State University in the framework of Scholarship Program of French government (2016), National Scholarship Programme of the Slovak Republic (2018), Scholarship of the Cabinet of Ministers of Ukraine (2018–2020), Research Fellowship Program of Matsumae International Foundation (2019), German Academic Exchange Service (DAAD) Scholarship Programme (2019) and under joint Ukrainian-Czech R&D project “Bioenergy innovations in waste recycling and natural resource management” (2021-2022).

CONTENT

Introduction

The risk entering environment pollutants during the placement of waste dumps from chemical enterprises causes need to understand ways of contamination, distribution profile of migration in the soil, impact on plants, and ultimately, on human health. The main source of environment contamination in the areas of placement mineral fertilizer production are arrays of phosphogypsum dump.

Phosphogypsum is a by-product from the processing of phosphate rock by the “wet acid method” of fertilizer production. Millions of phosphogypsum tons is stacked worldwide every year and is progressively considered as an asset more than an environmental burden. Phosphogypsum consists mainly of calcium sulfate dehydrate ($CaSO_4 \cdot 2H_2O$) and contains impurities of not decomposed phosphate, phosphates and silicates.

“The environmental concerns associated with phosphogypsum stacks include fluoride uptake, ground and surface water pollution if located nearby. Main vectors for their transport into the environment are wind and water erosion, infiltration, leaching into surface and ground water and airborne emissions of gaseous and radioactive elements. Fine particles of phosphogypsum can be picked up and transported by wind and vehicular traffic on stacks into adjacent areas. Dust particles containing fluoride is a concern for operational and non-operational stacks. Elevated levels of fluoride have been found in soil/vegetation adjacent to the stacks. Disposal of phosphogypsum on land may pose seepage problems beneath the repositories or the process water holding ponds if not lined or controlled properly. Phosphogypsum stacks up to a height of 20 m are in operation in the country. The water stock from phosphogypsum cannot be discharged as such as it contains significant quantities of fluorine and phosphates as P_2O_5. The outer dikes are generally earthen dikes designed to prevent the escape of contaminated water into nearby streams. Fluoride contaminant present in phosphogypsum may attack silicate minerals and dissolve them” (Guidelines for Management and Handling of Phosphogypsum Generated from Phosphoric Acid Plants, 2012).

Thus, the timeliness and urgency of the search for ways to solve the problem of accumulation of chemical industry waste and reducing the technogenic impact on the environment from the storage sites of phosphogypsum and its use as a secondary material resource is determined. Ecologically safe biochemical processes of phosphogypsum utilization in environmental protection technologies is the promising direction to formulate an integral concept of phosphogypsum processing on the basis of synergistic approach to stimulate the processes of natural restoration of components of ecosystems.

Chapter 1

Methodological approach for phosphogypsum recycling study in environmental protection biotechnologies

1.1 New opportunity of support mediums developing for bioconversion processes

The need for microorganisms in these or those compounds is determined by their physiological characteristics. The very first approximation of the need for microorganisms in nutrients can be determined by the chemical composition of the microbial cell biomass, but in this case the number and composition of metabolites removed by the cell into the external environment are not taken into account, as well as the fact that the chemical composition of the cell depends on the composition of the habitat and varies greatly.

In this monograph, the possibility of using a chemical by-product as an additional source of macro and trace elements is considered.

Phosphogypsum is a by-product of the chemical reaction called the “wet process,” whereby sulfuric acid is reacted with phosphate rock to produce the phosphoric acid needed for fertilizer production (Gennari et al., 2011). The quality and quantity of phosphogypsum generation depend upon the quality of the phosphate rock, the process used to produce phosphoric acid, calcium sulphate generated either in di-hydrate ($CaSO_4.2H_2O$) or the hemi-hydrate ($CaSO_4.1/2H_2O$) form. Production of 1 tonne of phosphoric acid generates 5 tonnes of phosphogypsum (Ministry of Environment & Forests, 2014). The presence of harmful impurities and their concentration depends on the composition of the phosphate rock. The reaction of the interaction of fluorapatite and acid mixture:

$$Ca_5(PO_4)_3F + 5H_2SO_4 + nH_3PO_4 + mH_2O \rightarrow$$
$$\rightarrow (n+3)H_3PO_4 + 5CaSO_4{\cdot}mH_2O + HF$$

Fluorine compounds and sulfur to be primary pollutants in industrial emissions generated by the production of wet-process phosphoric acid and phosphate fertilizers. Possible heavy metal pollution in areas of phosphorus-containing fertilizer production is caused by the presence of metals in phosphorite raw materials in the form of ballast elements, which is described in many works (Nemecek et al., 2011; Muravyov et al., 2007 and 2010; Derimenci et al., 2007; Savoyskaya, 2017; Villa et al, 2009; International Financing Corporation, 2007).

Phosphogypsum is stocked in large open areas or accumulated in lakes resulting in a major environmental problem due to the presence of the toxic elements, dispersed by wind (dust) and water. The metal contamination may affect the functionality, sustainability, and biodiversity of ecosystems (Medina, 2013). At the same time, estimates on the severity and frequency of acute exposures deriving from old generation facilities would be important, to estimate the extent of local health impacts. Similarly, much better and more complete data are needed on informal waste management activities and illegal operations, given the likely substantial magnitude of the health burden suffered by the people involved (WHO Meeting Report, 2005).

The main challenges regarding phosphogypsum management are:

– high fluoride concentration (in the range of 0.5–1.5 %) which can be leached and contaminate the groundwater;

– presence of radionuclides;

– the occurrence of heavy metals (Cd, Cr, Pb, etc.) that may enter into the food chain through potable water and agriculture products.

According to Perez-Lopez et al (2010), the amounts of mobile contaminants that could be released for every tonne of phosphogypsum are approximately $7 \cdot 10^2$g Sr, $1.1 \cdot 10^2$g Fe, 55g Y, 30g Ce, 12g Cr, 11g Ti, 5g Zn, 4g each of Cu and Pb, 3g each of V and Cd, 2g each of As and Ni and 1g U. Multiplying these amounts by 100 Mt and 20–25t/ha, it is possible to calculate risk assessments of phosphogypsum for both estuarine zones, e.g. in a hypothetical stack collapse and waste spilling, and agricultural soils, respectively (Perez-Lopez et al 2010). Tóth et al. proposed that in some cases (e.g. Hg and Cd) the high concentrations of soil heavy metal attributed to human activity can be detected at a regional level. While most of the European agricultural land can be considered adequately safe for food production, an estimated 6.24% or 137,000 km^2 needs local assessment and eventual remediation action (Tóth et al., 2016).

Thus, an important task is to justify the possibility of using phosphogypsum as a mineral carrier for different groups of microorganisms, considering the mechanisms of biochemical detoxification.

1.2 The expediency of phosphogypsum use in environmental protection biotechnologies

Phosphogypsum is not a commercial product and it is stocked in large open areas inducing an environmental problem. The solid waste is generated in the process of sulfuric acid decomposition of natural phosphate raw material and the solid phase (calcium sulfate) separation from phosphoric acid solutions.

The precipitate consists mainly of calcium sulfate dihydrate ($CaSO_4 \cdot 2H_2O$) and contains impurities of phosphate, which is not decomposed phosphates and silicates. The quantitative content of impurities depends on the mineral composition of the feedstock, smooth flow of production, serviceability of equipment and process discipline, etc.

Calcium sulfate in the process of crystallization captures in its structure various impurities (heavy metals, phosphorus, fluorine, rare-earth elements, one and a half oxides), which is why it received its name - phosphogypsum.

$CaSO_4 \cdot 2H_2O$ is a colorless crystal, molecular weight 172.17, density 2320 kg / m3, solubility in water - 0.206 kg / 100 kg, soluble in glycerine.

Studies have shown that the fluorine content in phosphogypsum is no more than 0.1 - 0.4%. The volumetric weight of wet friable phosphogypsum varies from 531 to 581 kg/m^3 and averages 556 kg/m^3; of dried phosphogypsum to constant friable weight - from 508 kg/m^3, averaging 517 kg/m^3. Phosphogypsum has a specific odor, texture disordered structure monomer.

According to sieve analysis data, the predominant fraction of phosphogypsum is particles of $1.6 \cdot 10^{-3}$ - $0.4 \cdot 10^{-3}$ and $0.16 \cdot 10^{-3}$ - $0.1 \cdot 10^{-3}$ m. The content of fractions is less than $50 \cdot 10^{-3}$ m - 2 - 3%. The proportion of phosphogypsum dried in natural conditions is $2.34 \cdot 10^{-3}$ - $2.36 \cdot 10^{-3}$ kg/m^3, as in natural double-water gypsum. The specific surface varies from $310 \cdot 10^{-3}$ to 350 · 10-3 m^2 / g.

The maximum allowable concentration in water is 0.3 m/m^3, although the flavor can be already felt at 0.25 m/m^3.

The existing modern technologies of fertilizer production do not pay enough attention to the purification of raw materials from toxic elements, so the solid waste often contains fluorine, traces of unwashed phosphoric acid and its salts, rare-earth metals (REM), arsenic, strontium, heavy metals - cadmium, lead, vanadium, may contain radioactive elements. Fresh dumps are, first of all, sources of air pollution by fluorine compounds. Low pH values (from 3 to 5 units depending on the age of the dump) cause mobility of toxic components and the possibility of their migration into soils around dumps and underground waters.

Disposal of phosphogypsum on land may pose seepage problems beneath the repositories or the process water holding ponds if not lined or controlled properly. The water stock from phosphogypsum cannot be discharged as such as it contains significant quantities of fluorine and phosphates as P_2O_5. The outer dikes are generally earthen dikes designed to prevent the escape of contaminated water into nearby streams. Fluoride contaminant present in phosphogypsum may attack silicate minerals and dissolve them.

Depending on the quality of the raw material used and the technology adopted, 1 ton of acid (100 % H_3PO_4) produces 4.5 to 8.4 tons of wet phosphogypsum (or 3.6 to 6.2 tons in terms of dry matter). When processing 1 ton of apatite concentrate into extraction phosphoric acid (EPA), 1.6 tons of phosphogypsum is formed, and 1 ton of P_2O_5 produces 4-5 tons of phosphogypsum.

As can be seen from the above data, the amount of phosphogypsum that is formed exceeds the amount of phosphorites taken for the reaction, from which it becomes clear that the problem of its use is quite urgent and important.

Thus, in the mid-90's the world production of EPA was about 20 million tons in terms of P_2O_5, and about 90 million tons of phosphogypsum were obtained. In the future, the production of EPA will attract phosphorites with a reduced content of phosphorus and the amount of waste will increase two to three times.

The Group's mineral fertiliser production facilities generate tens of millions of tonnes of large-scale waste.

The hypothesis of combining digestate and phosphogypsum was studied. Dehistat has an alkaline pH of 7-8 and increases soil pH. On the contrary, phosphogypsum has acidic pH 3-4 and decreases the pH of soil.

This work proposes a biochemical approach to the use of phosphogypsum in environmental protection technologies. For example, the addition of phosphogypsum at the stage of preliminary preparation of raw materials for anaerobic digestion. Microbiological treatment can accelerate the rate of substrate degradation in anaerobic digestion. It is at the stage of acidogenesis in anaerobic digestion of organics pH 5.5, then there are the release of hydrogen, pH system changes and at the stage of methanogenesis becomes about pH 7. Pre-treatment with separate stages of hydrolysis and homogenization with phosphogypsum will improve the results of fermentation products as well as biomass productivity. Accordingly, this will affect the qualitative and quantitative characteristics of the initial digestate, which can be used to improve the ecological condition of the soil.

Microorganisms in fermentation processes need nutrients for bacterial activity and growth. Thus, nutrients such as nitrogen, phosphates, metal ions, and other trace elements are needed in the fermentation processes for fermentation activity and biomass growth, which also affects the production of hydrogen and methane.

In other words, it is possible to additionally produce biohydrogen from biological processes that are less energy intensive and more environmentally friendly in terms of the global reduction of CO_2. These technologies of reducing biohydrogen have the potential to become competitive, as they can use low-level biomass waste as feedstock (municipal, agricultural, industrial organic waste, and wastewater). Besides, the production of biohydrogen is also affected by the types of substrates and methods of their pre-treatment, bioreactor configurations, and sources of enrichment.

As studied in the work of Geheimer et al. (2015) biomass is rich in carbohydrates, as wheat waste and palm oil waste has a lack of nutrients (nitrogen, phosphorus) or minerals (trace elements). Therefore, nutrients or trace elements should also be provided as a supplement for the optimal action of the microorganisms to convert biohydrogen from substrates rich in carbohydrates (Ghimire et al., 2015). We consider phosphogypsum as an acid-resistant mineral carrier, and in addition, it is a source of macro- and microelements for the development of necessary ecological and trophic groups of bacteria. Phosphogypsum entering the environment of life activity of microorganisms becomes a source of nutrition and stimulates metabolic processes of a bacterial cell due to a list of elements necessary for its life activity.

The model of the influence of the main components of phosphogypsum on the vital functions of microorganisms is presented in Fig. 1.1. For its formation, we used data from previous studies (Yakhnenko, 2017) and electronic bioinformation databases of the Kyoto Encyclopedia of Genes and Genomes (KEGG) and Metabase of Bacterial Diversity (BacDive).

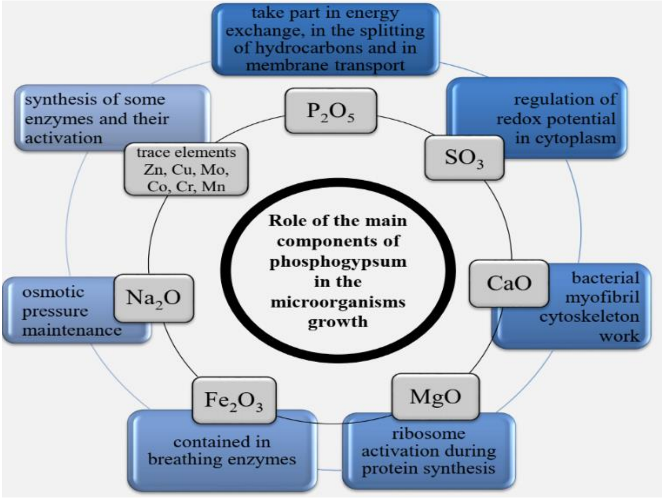


Figure 1.1 – Role of main phosphogypsum components in microbial activity

Nitrogen is of great importance for the production of biohydrogen as it is an important component of proteins, nucleic acids, and enzymes. Similarly, phosphate serves not only for nutrients but also for buffering biochemical reactions. Studies by Ghimire et al. (2015) have proven that Fe also regulates C/N and C/P ratios and can increase H2 production. Microelements of some metal ions increase the reactor productivity, e.g., iron and nickel (Ghimire et al., 2015).

Taking into account the previous studies, we have formed a technological scheme of anaerobic fermentation of organic waste with the production of useful bio-based products involving phosphogypsum as a mineral resource (Fig. 1.2).

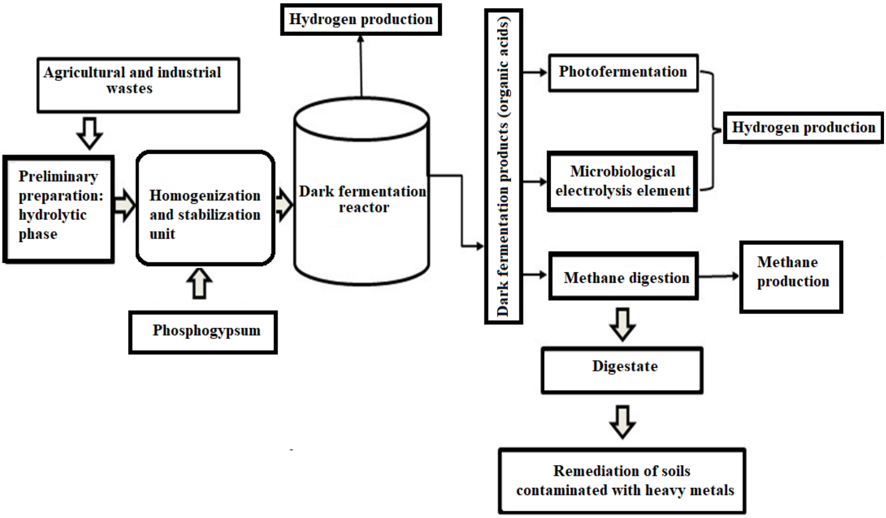


Figure 1.2 – Technological scheme of anaerobic fermentation of organic wastes

Agricultural and industrial wastes get to the preliminary preparation - hydrolytic phase, at this stage with the help of hydrolytic bacteria enzymes the processes of hydrolysis of complex organic compounds begin. The next stage is homogenization and stabilization of organic raw materials, where we propose to add phosphogypsum as an additional source of mineral substances, here the mixture to acquire homogeneous properties of the organic-mineral mass. Then the mixture is fed to the stage of anaerobic dark fermentation, where the transformation of simple organic compounds in acidophilic conditions with the production of volatile fatty acids, biohydrogen, and carbon dioxide. The next stage is the treatment of dark fermentation end products (mainly organic acids). Several possible options have been identified: photofermentation, microbiological electrolysis element, and classical methane digestion. In the process of methane fermentation, methane and anaerobic digestate are formed, which can be used to restore soils contaminated with heavy metals.

1.3 Environmental impact of the manufacturing processes of phosphogypsum by-product

The negative influence of dumps exploitation and, first of all, of non-treated phosphogypsum, may be revealed in contamination of subterranean and surface waters, soil-vegetation cover by the substances that penetrate the screen, or, as a result, of their evaporation and flushing from the dump walls by atmospheric precipitation due to weathering and dusting.

Phosphogypsum wastes contained in the dumps have a certain amount of free water. Its part under the condition of semi hydrated storing is used for chemical and mineralogical transformation of semi-hydrate into dihydrate. The other part of moisture together with the infiltration of atmospheric precipitation results in the formation of aquifers in the man-made deposits, the regime of which significantly determines the conditions of slope stability due to hydrostatic and hydrodynamic forces (Filatov and Ivochkina, 2012). Accordingly, waste dumps are a source of hydrodynamic impact on the environment, causing a change in groundwater level, which can lead to a negative effect in the adjoining building area. But, first of all, the alienation and pollution of large land plots and the landscape transformation take place.

All of the above factors can adversely affect the functioning of the adjacent ecosystems, result in a complicated environmental situation in the region. From the environmental point of view, the peculiarity of such geotechnical systems is that their negative impact on the environment is much wider than their actual size.

The study subject is the process of phosphogypsum recycling in environment biotechnological protection systems.

Fig. 1.3 presents the developed model of the influence of phosphogypsum as a secondary resource on the environment within the concept of environmental safety according to the traditional application of phosphogypsum and materials on its basis.

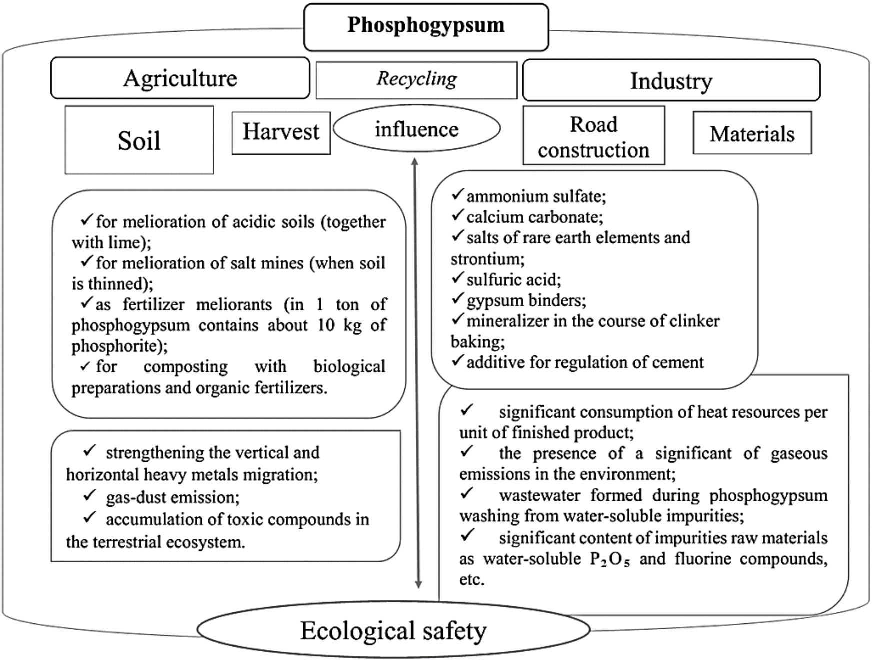


Figure 1.3 – Block diagram of environmental safety analysis of phosphogypsum application in traditional technologies (Chernysh and Plyatsuk, 2017)

At the same time, the influence of phosphogypsum as a raw material on the environment has been taken into account. In particular, the possibility of toxic components migration while applying phosphogypsum as an ameliorant in agriculture and the influence of the manufacturing cycle of phosphogypsum processing with the extraction of useful elements and the obtaining end products have been considered (Fig. 1.2). It should be noted that phosphogypsum is characterized by the high concentration of calcium, sulfur, silicon, phosphorus, and trace amounts of iron, fluorine, barium, manganese, chromium, which are the elements of mineral nutrition for bacteria. These useful components of phosphogypsum have been used while developing the biochemical direction and its application in environmental protection technologies.

According to the provisions of environmental safety, the formalized model of phosphogypsum influence as a secondary resource at its use in environmental protection biotechnologies (Fig. 1.4) as a carrier for the development of various ecological and trophic groups of microorganisms has been developed.

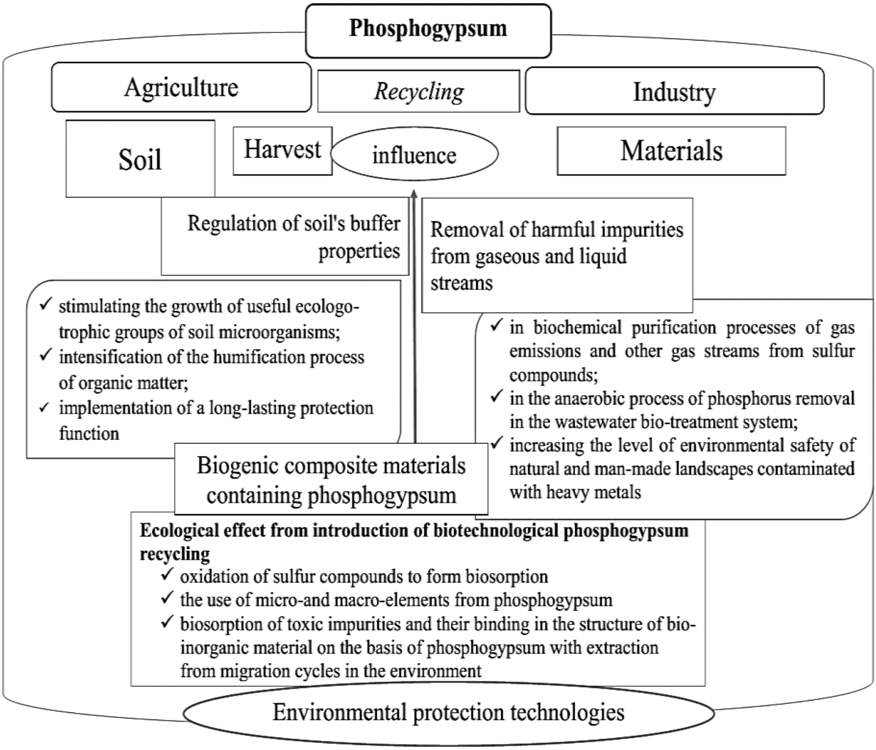


Figure 1.4 – Block diagram of phosphogypsum application as a secondary resource in environmental protection biotechnologies (Chernysh and Plyatsuk, 2017)

At the same time, the application of phosphogypsum based materials have been considered and, accordingly, the technology solutions for the application of these materials in such environmental protection technologies have been considered:

– the process of biodesulfurization of gas streams in the atmospheric air protection technologies while applying phosphogypsum mineral carrier (Chernysh and Plyatsuk, 2014-2017; Chernysh and Yakhnenko, 2016-2017; Patent of Ukraine for utility model 103687, 2015);

– the biochemical process of anaerobic extraction intensification of biogenic elements from wastewater and sludge with the help of dehydrate phosphogypsum in technologies of aquatic ecosystems protection (Chernysh et al., 2018; Patent of Ukraine for invention 103087, 2013; Patent of Ukraine for utility model 87422, 2014);

– the application of modified phosphogypsum biocomposites in the technology for soil remediation (Chernysh and Plyatsuk, 2014-2018; Patent of Ukraine for invention 122820, 2021).

Thus, the biochemical transformation of toxic compounds under the condition of biotic component development on phosphogypsum carrier has determined the possibility to achieve a systematic long-term environmental effect consisting in the extraction of useful biogenic components with their subsequent recycling in agriculture (for example, biosulfur and phosphorus compounds) and with toxic substances immobilization through the limitation of their introduction into land ecosystems (in particular, precipitation of heavy metals in the complex of low-soluble / insoluble compounds).

Ukrainian phosphogypsum stock case

Currently, over 50–65 million tons of phosphogypsum are accumulated in Ukraine. Currently, over 14 million tons of phosphogypsum are accumulated in Sumy region. Phosphogypsum is formed in an amount of about 100 tons annually in PJSC “Sumykhimprom”(Sumy region).

The phosphogypsum dump is located behind town Tokari of Sumy region (Ukraine) at a distance of 800 (direct measurements) – 1.300 m (along the road) from the residential area and continues to take a new turn of dihydrate phosphogypsum formed at PJSC “Sumykhimprom” (Fig. 1.5). Within the city of Sumy, dumps of phosphogypsum began to be organized in 1972, and today they account for about 15 million tons of the substance.

Figure 1.5 – Phosphogypsum terraces from the southeast side of dump, Sumy Region (Ukraine), 2021

The sampling sites, located on the dump and in different distances from the dump, were noted as the points in the Fig. 1.6. Their GPS coordinates are listed in the Table 1.1.

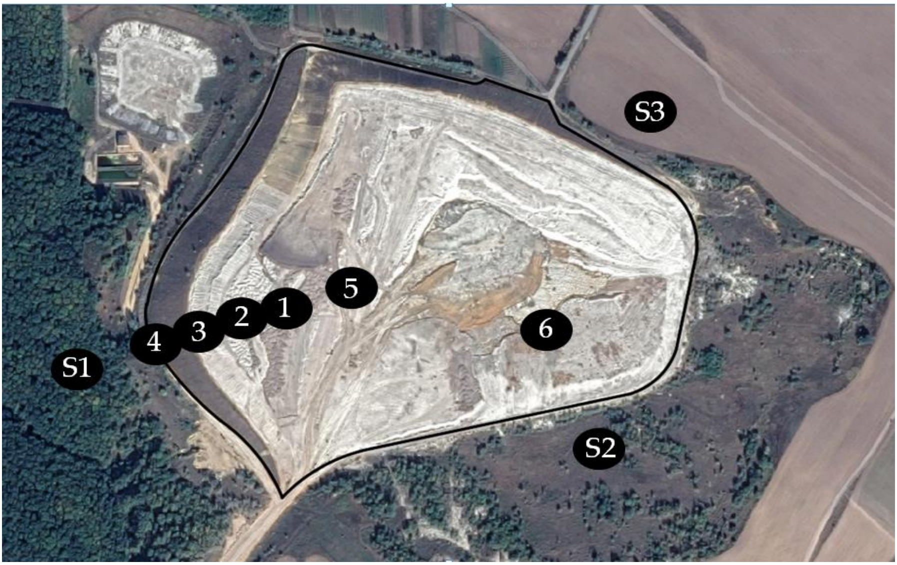


Figure 1.6. – Location of sampling sites in the vicinity and terraces of the dump in Sumy Region (Ukraine)

Table 1.1 – GPS coordinates of sampling points

Sampling point number	GPS coordinates
S1	50°55'03.3"N 34°57'33.2"E
S2	50°55'01.1"N 34°58'05.0"E
S3	50°55'14.3"N 34°58'07.1"E
1	50°55'07.3"N 34°57'46.3"E
2	50°55'06.6"N 34°57'44.1"E
3	50°55'06.1"N 34°57'42.3"E
4	50°55'05.6"N 34°57'39.8"E
5	50°55'08.6"N 34°57'51.0"E
6	50°55'05.5"N 34°58'03.0"E

The Fig. 1.7 shows the profile of grey forest soil near the phosphogypsum dump from which the soil samples of different horizons were collected. In the profile of Haplic Luvisols soils near the phosphogypsum dump distinguished following horizons (Fig. 1.7):

– S1 is dark brown turf (capacity up to 2 cm). This horizon can also be called a surface organogenic horizon, consisting of the remains of plants from the past years. It contains at least 70 % by volume of organic matter of varying degrees of decomposition;

– H is humus cumulative with dark gray turf with silica powder (7–11 cm);

– E1 is eluvial grayish-white color, structural composition of powdered-lime with carbonate inclusions, contains many plants roots (18–20 cm);

– E2 is transient eluvially illiual plated nougat with carbonate inclusions, compacted, transition gradual (6–10 cm);

– I is iluvial brown color lobed-prismatic (32–35 cm);

– P is soil forming rock with a depth of 60–80 cm.

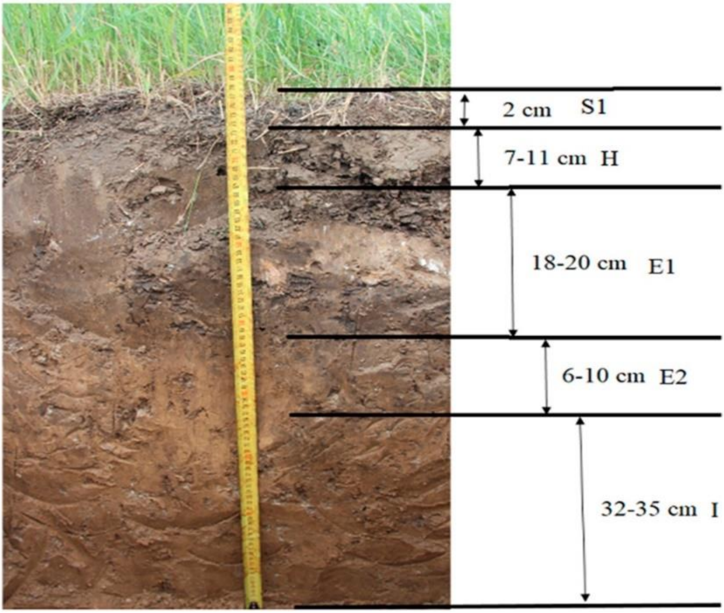


Figure 1.7 – Profile of Haplic Luvisols soil near the phosphogypsum dump

Point samples were taken at a test site from several layers or horizons using the envelope method so that each sample represented a part of the soil typical of genetic horizons or layers of a given soil type.

The combined sample was made by quartering, i.e. mixing point samples taken at one test site. For chemical analysis, the pooled sample was composed of five spot samples taken from one sample site. The mass of the combined sample was at least 1 kg. To control pollution with heavy metals, point samples were taken in layers from a depth of 0–5 and 5–20cm, each weighing no more than 200 g. To control soil contamination of territories adjacent to the dump, point samples were taken at genetic horizons along the depth of the soil profile. Spot soil samples designed to determine heavy metals were taken with a plastic spatula. All combined and spot samples were recorded in the journal and numbered (Interstate council for standardization, metrology and certification. GOST 17.4.3.01-2017).

Soil samples for chemical analysis were dried to an air-dry state. Air-dried samples were stored in glass containers. The soil was ground in a mortar with a pestle and sieved through a sieve with a hole diameter of 1 mm. The selected neoplasms were analyzed separately, preparing them for analysis in the same way as a soil sample. To determine the gross content of mineral components from a sifted sample, a representative sample of no more than 20 g was taken and ground in a mortar to a powder state (GOST5180-2015 Soils. Laboratory methods for determination of physical characteristics).

Initially, the dump was organized on the site of the natural ravine Glubokij yar about 40 m deep. The presence of the ravine led to the formation of a dump with a displacement of the terraces. As can be seen in Figure 1 and 3 terraces form a single frontal surface from the southeast side. The area of the dump is 492 m^2, with a sanitary protection zone of 637 m^2, the perimeter of the active dump is about 1,900 m. The most of the phosphogypsum dump is conserved by loamy soil and on the west side forms 4 terraces, 12–15 m high, which can be easily seen. A new line of waste forms the upper phosphogypsum area. In order to protect from drains, a protective reservoir-accumulation is built near the dump from the north-west side.

In the body of the phosphogypsum dump there is also a burial of iron sulfate, which is a waste of the work of the workshop for the production of titanium whitewash PJSC “Sumykhimprom”. Such burial promotes the sealing of iron sulfate in the body of phosphogypsum waste. However, it does not solve the problem of the accumulation of chemical waste in a given territory, which requires new opportunities and support for an additional man-caused strain on the environment.

The phosphogypsum wastes contained in the dumps contain a certain amount of bound and free water. Part of the free water in the process of hydration of stored phosphogypsum goes to the chemical-mineralogical transformation of hemihydrates into dihydrates. Partly moisture together with atmospheric precipitation, which are infiltrated into the lower layers of the body of phosphogypsum, forms anthropogenic aquifer. When interacting with water, phosphogypsum changes its state and properties, which affects the process of accumulation and migration of compounds, both in the dump itself and in the components of the natural environment. In water-saturated massifs, as the sealing load increases, when a newly formed mass of phosphogypsum is accumulated, a long process of compaction and deformation takes place with a structural redistribution of the material, its destruction and the formation of new structural bonds. These physicochemical processes are important in the organization of further processing of phosphogypsum wastes and the development of appropriate technological solutions to reduce the level of man-made environmental impact from dumps.

The dam of the dump was constructed from natural clay soil, then it was built up by dried phosphogypsum with the help of bulldozers, forming original terraces. An anti-filter screen with a clay base, forming a filling and compacting with sand, protects the ground under the dump.

There is no vegetation on fresh slopes of the dump, on the slopes freshly covered with loamy substrate the mosaic of the vegetation cover is observed: from single specimens over a larger area to the overgrowth of one third of the projective covering of the area.

On the old reclaimed slopes of the canopy, the covering of the grassy perennial vegetation is complete, on the lowest terrace, in addition to the herbaceous one- and perennial vegetation, young trees are found, which indicates ongoing successional changes in plant communities. On the three lower slopes of the dumps, the projective covering of the vegetation is maximally complete, to the upper part of the terraces, especially recently subjects of reclamation, the quantity and species diversity of plants decrease.

The powder X-ray diffraction was used to characterize the phosphogypsum samples. The diffraction pattern of the representative sample is shown in Fig. 1.8. All samples from different horizons (terraces I - IV) exhibit almost identical composition, excluding of quartz (SiO_2) presence in the sample from the second terrace. The results confirmed major presence of calcium sulphate dihydrate ($CaSO_4.2H_2O$) together with brushite ($CaPO_3(OH).2H_2O$). As mentioned above also silicon oxide (SiO_2) is present. The dehydrate or hemihydrate phase of sulphate is missing so it can be stated that the samples from all horizons are completely hydrated.

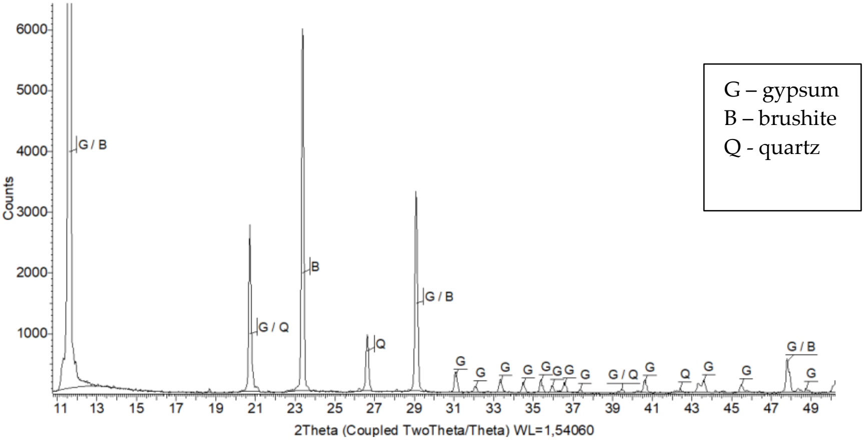


Figure 1.8 – The X-ray diffraction pattern of phosphogypsum (terrace I)

X-ray fluorescence analysis of the samples from all horizons of the phosphogypsum dump was also performed. The overall results are listed in the Table 1.2. The results of the analyses support the statements form X-ray diffraction, thus the samples exhibit relatively identical composition and the presence of silicon oxide in the second horizon is several times higher than in the other three horizons, which can be probably caused by inflation of soil particles.

Table 1.2 – Composition of phosphogypsum in terms of oxides, %

Sample	**Al2O3**	**SiO2**	**P2O5**	**SO3**	**CaO**	**Fe2O3**	**SrO**	**F**
1.terrace	< 0.004	1.54	0.78	60.9	34.37	0.257	1.782	0.56
2. terrace	0.167	19.87	0.31	51.8	27.63	0.078	0.059	0.21
3. terrace	< 0.004	2.68	1.19	60.5	35.46	< 0.0009	0.112	0.9
4. terrace	< 0.004	1.23	0.72	61.7	35.99	0.183	0.123	1.33
5. fresh	< 0.004	2.59	1.28	60.2	35.75	< 0.0009	0.112	0.66
6. internal	< 0.004	1.22	0.7	61.5	36.39	0.023	0.093	2.27

The largest amount of strontium is found in raw phosphogypsum from terrace 1, which has just entered production. Its presence is associated with the process of production of phosphoric acid, and more precisely with raw materials (phosphorites), changes in the quality of raw materials affect the change in the content of impurities in phosphogypsum, in particular in strontium.

The study of the soil from different horizons in the territory near phosphogypsum dump

A subtype of grey forest soils, characterized by a large, deep, fairly loose, granular, densely permeated plant roots humus-accumulative horizon (humic content 3 – 4% to 6 – 8%), formed over a larger area of the area around the dump, which is explained by the predominance of salinisation process. Salinization of the soil with neutral salts (for example, chlorides, sulfates) leads to the formation of a neutral and slightly alkaline reaction of the medium (pH=7.2), but when exposed to phosphogypsum, which has an acid reaction, the soil pH changes to the acid side. Soils are characterized by a less acidic pH value, on average, from 5.8 to 6.5 units, the absence or masking of transitional horizons along with the soil profile, weakly expressed by the eluvial and well-defined illuvial horizon of brown-brown colour.

The group composition of humus in grey forest soils is characterized by an insignificant predominance of humic acids in the composition of soil humus, the saturation of the bases with 18-30 mg-eqv per 100 g of soil; In the illuvial horizon, the absorption capacity is markedly increased. The eluvial horizon is fairly dense, mostly of a nut-prismatic structure.

Behind the sanitary protection zone of the dump are agricultural lands, where the upper horizons of the soils, primarily the humus-accumulative horizon and part of the eluvial horizon, are fed to plowing. Although grey forest soils differ from light grey by slightly better agronomic indices, they are united by acid reaction in the upper horizons, unsaturation with bases, and low content of nutrients.

Soil humus possesses high sorption properties, forming complex compounds in soils with a high content of heavy metals. An essential factor determining the behavior of chemical elements in soils is the presence of acidic water-soluble, and therefore mobile, fulvic acids, which cause the processes of intensive leaching out of the soil profile of many trace elements - Fe, Mn, Zn, Cd, Pb, Sr, including heavy metals. This process is also characteristic of grey forest soils.

The carried out the diffractometric analysis of these soils did not reveal the presence of compounds coming from the phosphogypsum dump. The samples from different horizons (H, E_1, E_2, and I) exhibit comparable results, except for the missing phase of calcium carbonate in the horizon “I”. The representative pattern is shown in Fig. 1.9.

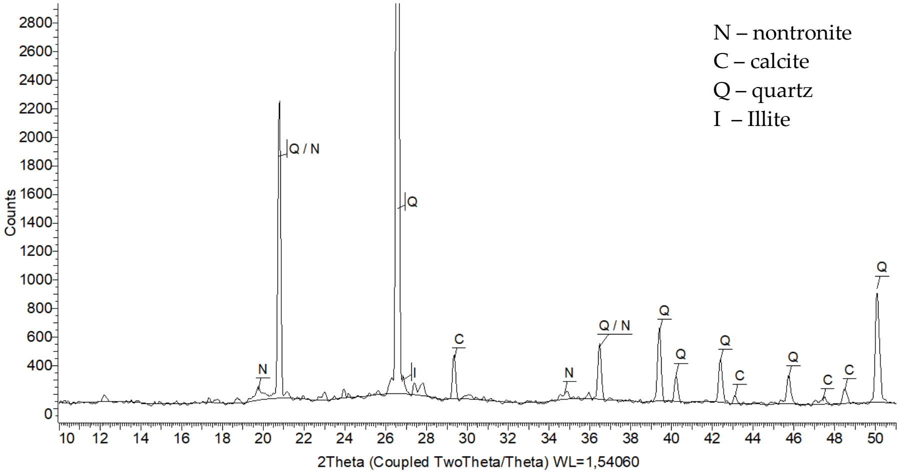


Figure 1.9 – The X-ray diffraction pattern of soil sample (sampled point S1) (horizon “H”)

The patterns confirmed the major presence of silicon oxide (SiO_2) together with alumino-silicates compounds (illite and nontronite). As mentioned above also calcium carbonate is present in horizons H, E_1, and E_2. The finding of dominant compounds listed in the diffraction pattern of phosphogypsum (calcium sulphate dihydrate and brushite) was unsuccessful.

Based on the results from XRF analysis (Table 1.3) it can be stated that the samples of soils that come from the profile of Haplic Luvisols soil mostly are not significantly affected by the phosphogypsum dump. From the table can be observed higher concentration of phosphorus and sulphur in the upper horizons. Moreover, the same results were also achieved in the soil samples located around the phosphogypsum dump (samples S1, S2, and S3). The analysis of soil samples from sampled points S2 and S3 was taken from the horizon “H”, generally it’s most susceptible to anthropogenic influence and the accumulation of pollutants in it.

Strontium is ubiquitous and one of the most abundant of the trace elements in surficial deposits and rocks acts as a proxy for Ca because both are alkaline earth elements with similar ionic radius and the same valence (Capo et al., 1998). Strontium is easily mobilised during weathering, especially in oxidising acid environments, and is incorporated in clay minerals and strongly fixed by organic matter. The Sr content in soil is highly controlled by parent rocks and climate and, therefore, its concentrations range from 50 to 1000 $mg.kg^{-1}$ (Taylor, 1964). Nevertheless, the content of strontium can be associated with the influence of phosphogypsum dump in Sumy region, which requires further monitoring. The soil fluoride levels measured in this study were comparable to the average soil fluoride of worldwide soils (Dartan, 2017).

Based on the results from XRF analysis (Table 1.3) also from diffraction patterns, the chemical compositions soil horizons indicate the presence of alumino-silicates in studied samples.

Table 1.3 – Chemical composition of soil horizons from Haplic Luvisols soil (sampled point S1) and soils around phosphogypsum dump in %

Sample	**MgO**	**Al_2O_3**	**SiO_2**	**P_2O_5**	**SO_3**	**K_2O**	**CaO**	**Fe_2O_3**	**SrO**	**F**
S1 (S1)	1.231	9.19	49.98	0.17	0.31	1.71	0.50	4.07	0.019	< 0.05
H1(S1)	1.048	7.65	56.09	0.42	0.53	1.34	3.19	2.33	0.018	0.06
E1(S1)	1.104	8.25	57.4	0.46	0.55	1.47	4.13	2.61	0.022	0.05
E2(S1)	1.094	8.159	55.22	0.44	0.35	1.52	3.77	2.66	0.021	0.05
I1(S1)	1.715	10.79	61.63	0.16	0.10	1.90	0.96	3.61	0.012	0.05
H2(S2)	1.067	8.54	58.85	0.16	0.17	1.64	0.74	2.66	0.016	< 0.05
H3(S3)	1.073	8.83	64.66	0.28	0.20	1.81	1.13	2.77	0.018	< 0.05

Fig. 1.10 shows the infrared spectra of soil horizons.

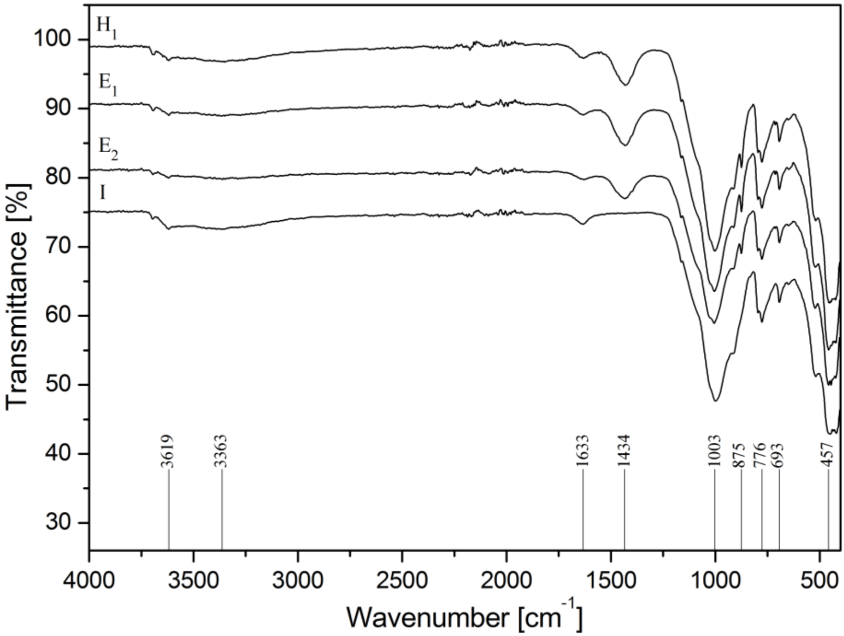


Figure 1.10 – Infrared spectra of soil horizons (sampled point S1)

The absorption peaks at wavenumber 3620, 3363, and 1633 cm^{-1} are assigned to H–O–H stretching vibrations of water molecules weakly hydrogen bonded to the Si–O surface (Madejova, 2003). The spectra of the soil horizons show the intensive band at 1003 cm^{-1} attributed to the Si–O stretching vibrations and at 693 and 453 cm^{-1} could be assigned to Si–O–Al (octahedral Al) and Si–O–Si bending vibrations, respectively. Another characteristic band of amorphous silica was observed at 776 cm^{-1} (Farmer et al., 1974). Also, in 3 horizons were revealed the presence of absorption band at 1434 and 875 cm^{-1} referred to stretching vibrations of CO_3 groups (Krol, 2016).

1.4 The study of biochemical transformations of the technogenic array of phosphogypsum

It has been established that for all phosphogypsum samples, irrespective of the residence time in the dump, and increased acidity is characteristic (Plyatsuk et al., 2010). Thus, the pH of the aqueous extract of hydrolysed phosphogypsum at a temperature of 24 ^{0}C was 2.3–2.8 for fresh phosphogypsum of a new dump turn taken from above the platform. For phosphogypsum stored in the dump, depending on the storage terrace, the pH value was from 3.8 to 5.8 depending on the age of the terrace. The changes in the pH of the aqueous extract of phosphogypsum, depending on the terrace, and hence the residence time in the heap, is shown in Fig. 1.11.

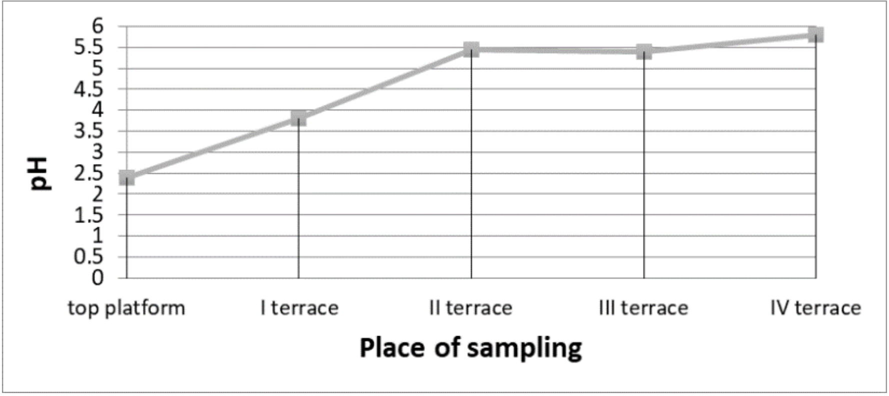


Figure 1.11 – The pH values of aqueous extract of phosphogypsum samples depending on the place and time of stay in the dump

The low pH level of fresh phosphogypsum samples can be due to the presence of water-soluble fluorine compounds (possibly H_2SiF_6, Na_2SiF_6, K_2SiF_6, HF), traces of non-void phosphoric acid and its salts, sulfuric acid. The decrease in acidity in older terraces is explained by the gradual elution or evaporation of acidic compounds from the phosphogypsum stored earlier in the terrace under the influence of various natural factors.

The concentration of phosphorus compounds decreases with the storage time of phosphogypsum, which is mainly due to the gradual transition of its compounds into water-soluble forms and washing out into the dump or the soil of the adjacent territories under the influence of hydraulic forces or atmospheric precipitation.

Open areas of the phosphogypsum dump act as a substratum for settling on their surface, primarily algae and moss, and in the future, gradually, the higher herbaceous vegetation (Fig. 1.12 and 1.13).

a)

b)

Figure 1.12 – Surface layer of non-cultivated areas of phosphogypsum dump with settlements of algae (a) and mosses (b)

It should be noted that the inclusion of silicon in the phosphogypsum structure occurs during the deformation and fracture of mineral components as the sealing load on the phosphogypsum layers increases in the dump. The change in the Sr content in the phosphogypsum samples (Table 1.2) can depend both on the composition of raw materials used for the production of fertilizers and, possibly, the tendency to migrate its compounds is traced. The conducted observations on the territory of phosphogypsum dump showed that over time (more than 10 years) as a result of natural processes of soil formation and passage of successions, a vegetative crust occurred with a thickness of no more than 3-4 mm on the surface of non-cultivated phosphogypsum embankments (Philippot et al., 2013).

Figure 1.13 – *Calamagrostis epigejos*, which dominates in restorative plant groups on the surface of the phosphogypsum dump

The walls of the heap, after falling asleep with a mixture for reclamation, unlike open areas, are easily and quickly covered with light-loving grassy vegetation. The surface roughness of phosphogypsum in the form of depressions, cracks, and also its significant moisture capacity, contributes to the settlement of the heap by plants, so phosphogypsum also contains a sufficient amount of residual phosphorus, sulphur, calcium and other substances that act as macro and microelements, becomes a good substratum especially for ruderal and meadow vegetation.

On freshly formed open slopes of the phosphogypsum dump vegetation is absent, on the slopes of freshly loamy substratum mosaic vegetation is observed from single specimens to overgrowth of one-third of the projective covering of the area. On old, reclaimed loams, slopes of the canopy, the projective covering of the herbaceous perennial vegetation is complete, on the lowest terrace, apart from the grassy one- and perennial vegetation, young trees start to settle, which indicates the slow progressive succession changes in plant groups. On the three lower slopes of the heap, the projective covering of the vegetation is as complete as possible, in the upper part of the terraces, especially recently subjected to reclamation, the quantity and species diversity of plants decreases. The one- and perennial herbaceous plants are dominant on the reclaimed slopes of the phosphogypsum dump of the PJSC "Sumykhimprom"(Table 1.4).

On the lower terraces (third and fourth) tree forms begin to settle, represented mainly by *Populus tremula*, *Populus alba, Betula pendula, Robinia pseudoacacia*.

The stage of the formation of a permanent herbaceous cover with a full projection of the substrate coating lasts at least a decade on the extreme substrates, including phosphogypsum.

The acidity of the environment affects the plant growth and species dominance and this effect is limited by a number of factors:

- the features of the technogenic relief of the dump,
- the high concentration of certain chemical elements (calcium),
- the inconstant availability of water,
- a small length along the depth of the recultivating substrate.

The formation of tree stands is observed mainly 20 years after dumping the dump. This process of self-growth is affected by the high acidity of the substrate, the high concentration of chemical elements, which is mainly determined by the intake of chemical elements and their compounds from the dump (Chernysh, 2019). Fig. 1.14 shows a general diagram of the process of self-growth vegetation in the dump.

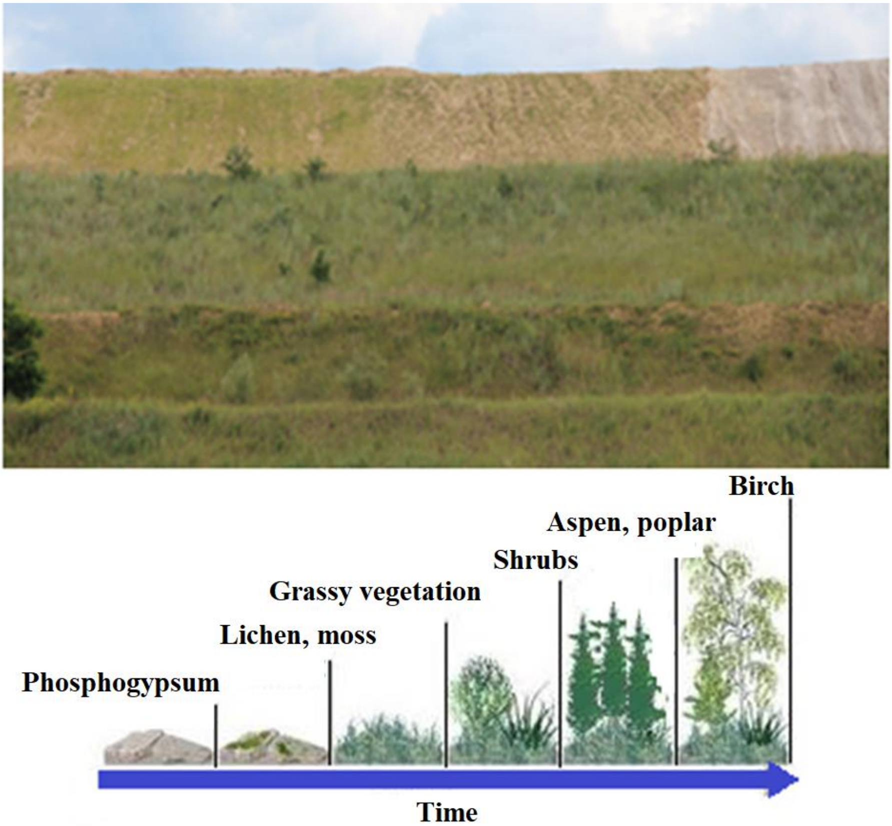


Figure 1.14 – Plant formation occurred naturally in the process of succession.

As a result of reclamation of the surface of the embankments of the dump, loamy soil with a layer of 20 to 40 cm, which, to a certain extent, is mixed with mineral particles of phosphogypsum. A substrate is formed, which is to be populated with living organisms, primarily microorganisms and plants (Philippot et al., 2013; Xu, 2017). According to Yakhnenko (2017) and Shulipa et al. (2018) microorganisms carry out the transformation (oxidation, reduction) of a number of inorganic compounds of the substrate, including mineral particles of phosphogypsum, transferring their components to more or less conversely, depending on the conditions (pH, Eh).

Table 1.4. – Herbaceous plants that dominate the reclaimed slopes of the phosphogypsum dump of PJSC “Sumykhimprom”.

Named family of plants	**Representatives**
Cereals	*Calamagróstis epigéios*
	Elytrigia repens
	Echinochloa crusgalli
	Setaria viridis
	Poa annua
Beans	*Lotus corniculatus*
	Trifolium pratense
	Trifolium repens
	Trifolium ochroleucum
	Melilotus officinalis
Compositae	*Tussilago farfara*
	Erigeron canadensis
	Achillea millefolium
	Erigeron annuus
	Tanacétum vulgare
	Artemisia vulgaris
	Sónchus arvénsis
	Cirsium arvense
Buckwheat	*Polygonum aviculare*
Kippine	*Onagra biennis*
Figwort	*Linaria vulgaris*
Willow wood forms	*Populus tгemula*
Birch wood forms	*Betula pendula*
Bean wood forms	*Robinia pseudoacacia*

The roots of plants gradually settle on the surface of the substrate of the dump, absorb substances directly from the soil solution, and in contact with the particles of the soil absorbing complex. Cations and anions that are in the absorbed state on colloidal particles of the soil are exchanged for ions adsorbed on other colloidal particles or the surface of the root cells (Philippe et al., 2018). Thus, the intake of cations K^+, Ca^{2+}, Na^+ are exchanged by micelles for protons H^+, as well as anions of NO^{3-}, SO_4^{2-}, PO_4^3- and others are exchanged for anions of organic acids (malic, citric, oxalic) directly by the cells of the root. Particularly effective is absorption by contact exchange, in which there is an exchange of ions between colloidal soil particles and plant roots without entering them into the soil solution. Ions are adsorbed on the surface of the cell membranes of the root rhizoderm and are absorbed by the plant (Roy, 2006).

The removal of a certain element from the minerals of the substrate is carried out in the process of biological and physic-chemical (exchange) absorbency. As a result, either the complete dissolution of the mineral or the replacement of the mineral ions by hydrogen ions or ions occur, it releases the roots in the composition of organic acids without destroying the crystal lattice of the mineral.

Thus, organic compounds directly or indirectly interact with mineral particles of phosphogypsum, destroying the crystal lattices, transferring elements from one form to another with a change in valence and mobility. The chemical elements that are part of the phosphogypsum, or associated with it, are not necessarily withdrawn in proportion to their content and the relationship in the material. Biological weathering can lead to the transformation of this mineral into another due to a change in the chemical composition with selective absorption of elements (Zirnea et al., 2013; Schwilch et al., 2018).

The reaction of the soil solution, the concentration, and the ratio of salts in it cause a particularly strong influence on the supply of nutrients (Yakhnenko, 2017). For most plants, the weakest acid (pH = 5-6) or neutral reaction is most favorable. On slightly acidic soils, which include the substrate of the reclaimed terraces of this dump, due to the lower pH values of phosphogypsum, nutrients such as phosphorus, iron, zinc, manganese, boron, and others become more assimilable for plants.

We suggest that the large concentration of calcium due to its receipt from phosphogypsum allows plants to adapt to the conditions and continue to populate the surface of the dump. It should also be taken into account that Ca^{2+} in high concentrations can inhibit the excess intake of K^+, Na^+ or Mg^{2+} into the plant and vice versa.

1.5 Integrated methodology of phosphogypsum recycling research

Fig. 1.15 below schematically shows how the disparate areas under the heading of by-products and bio-based products can be linked via the life cycle analysis approach.

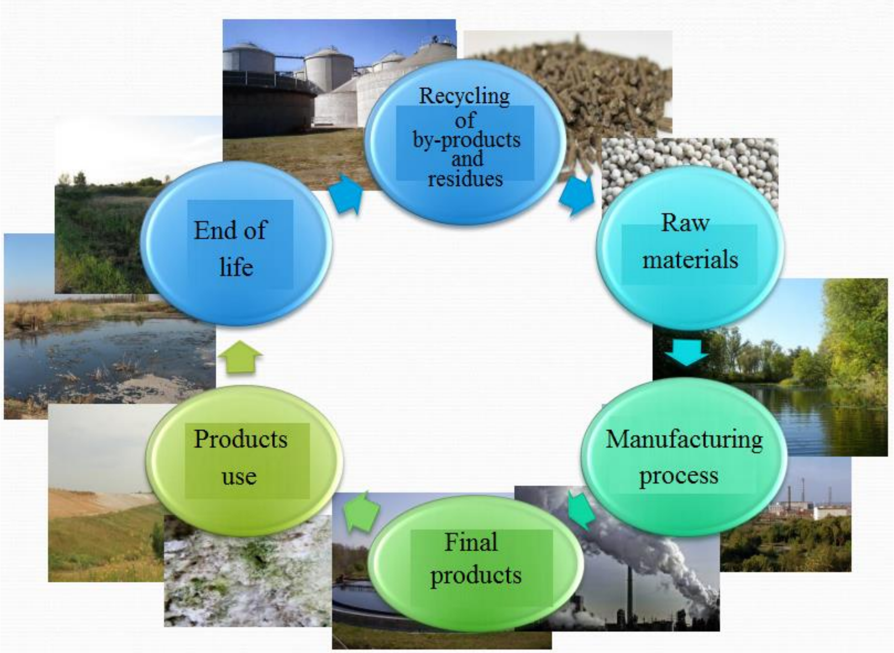


Figure 1.15 – Life cycle approach to biotechnological solutions for using and processing of secondary resources

The concept of research is the development of biotechnological solutions for using and processing secondary resources (organic and inorganic waste) with the production of biofuels and other useful bio-products.

This concept is based on the use of municipal sewage sludge as carbon substrates and phosphogypsum as the source of nutrients and microelements for cultivated useful groups of microorganisms for biofuels and bio-based products. Tests on inorganic support based on phosphogypsum, microbial transformation of wastes, biochemical basic research on substance detoxification as well as the technical optimization of waste anaerobic degradation and aerobic bio-desulfurization system are being carried out.

The principle diagram of the integrated research methodology, shown in Fig. 1.16, has been developed.

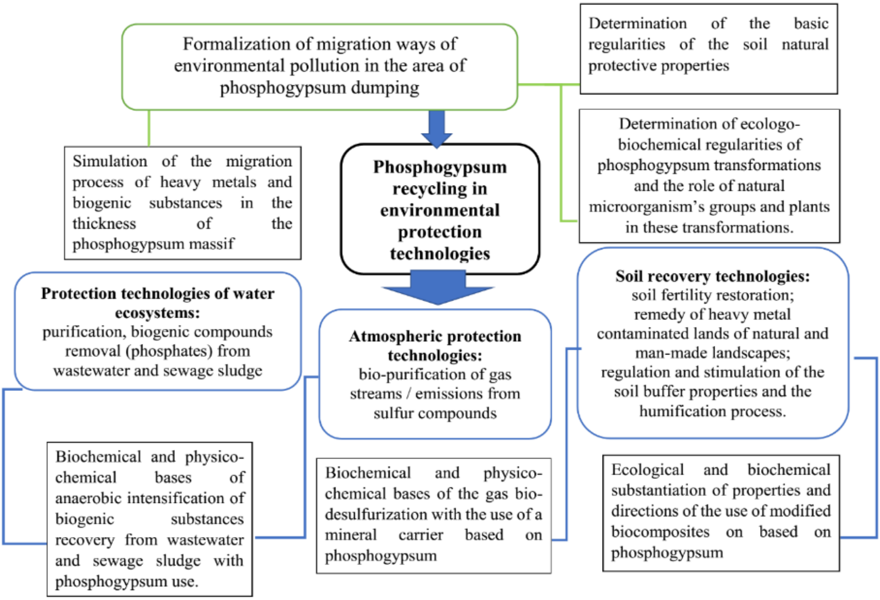


Figure 1.16 – Integrated scheme of the sequence of experimental and theoretical research. Based on (Chernysh, 2017)

According to the developed scheme of research sequence, it is worth noting the integration marked by the connection lines between the research blocks for separate technological solutions for the protection of aquatic ecosystems, atmospheric air, and soil remediation technology. This relation can be found in the related spheres of the application of phosphogypsum based mineral carriers. Thus, the modified phosphogypsum granules can be used for the gas purification systems and the biological treatment systems of wastewater and sewage sludge of wastewater treatment plants. In this case, the secondary products are formed:

- biosulfur from the biodesulfurization systems of gas flows;

– organic and mineral product of the separation of solids from liquids in technologies of aquatic ecosystems protection under the condition of dissimilation sulfate reduction.

These products contain separate components of phosphogypsum and new mineral and organic compounds, which are formed into complex structures during the biochemical transformation and can bind toxic substances. It is expedient to recycle such products in soil remediation technologies (recycling will be discussed in the following chapters).

The sequence of experimental research is as follows:

– development of the methodology and scheme of research, at that it is necessary to determine the optimal conditions for research with least cost and high accuracy;

– series of experiments with the predetermined values of technological and operating parameters (factors) for each experiment;

– processing of measurement results, their analysis, and decision making.

The sequence of theoretical research is as follows:

–to carry out biochemical and mathematical formalization taking into account the optimization criteria based on the kinetics of phosphogypsum recycling;

–to determine the values of the process constants taking into account the influence of the biotic component of the ecosystem components;

–to formalize the synergetic patterns of phosphogypsum influence as a secondary resource on the ecosystem components.

The subject of theoretical and experimental research is to determine the functional links for the studied systems and the optimal functioning parameters of the proposed biochemical systems of phosphogypsum recycling in environmental protection technologies.

The factors determining the efficiency of modified granules formation include:

– initial humidity of the dumped phosphogypsum, %;

– composition of phosphogypsum impurities;

– physical and chemical properties of phosphogypsum;

– duration of phosphogypsum pelletizing, min.;

– time of granules hardening, h.

Table 1.5 shows the main factors influencing the efficiency of phosphogypsum recycling and phosphogypsum based products in environmental protection technologies.

Table 1.5. – Factors of determining the efficiency of phosphogypsum recycling in environmental protection biotechnologies

Parameter	**The effectiveness of the use of phosphogypsum and products based on it (modified granules, biocomposite)**		
	Bio-desulfurization systems of gas streams	**Biochemical treatment of wastewater and sewage sludge**	**Bioremediation of soils contaminated with heavy metals**
1	2	3	4
The microbiological system attitude to the oxygen	Aerobiosis	Anaerobiosis	Change of aerobic processes on anaerobic soil complex
The dose of phosphogyp-sum and products based on it	The loading dose of modified granules, g (granules) / dm^3gas)	Phosphogypsum dose, g/dm^3	The dose of biocomposite containing phosphogypsum, per 1 ha of land
Chemical composition of media to be treated	Chemical composition of the gas stream	Chemical composition of wastewater and sewage sludge	The form of the biogenic elements in the mineral structure of the biocomposite; the content of heavy metals (general and mobile form) in soil
Humidity	The moisture of the modified phosphogypsum granules,%	The humidity of sewage sludge, %	Soil humidity

Continuation of the table 1.5

1	2	3	4
pH	Acidophilic conditions	Neutrophilic conditions	According to the acid-base conditions of the soil complex
Temperature regime, К	The mesophilic range	The mesophilic range	The mesophilic range
Duration of treatment	Contact time, h.	Hydraulic time, days	Time of contact of the soil complex with biogenic composite, months
Development of the necessary ecological-trophic groups of microorganisms	Sulfur-oxidizing bacteria growth, CFU / g.	Growth of sulfate reduction bacteria, denitrification bacteria, and phosphorus-metabolizing bacteria, CFU / dm^3.	Biochemical activity of the biogenic composite, growth of rhizosphere microorganisms, CFU / g.
Environmental safety	Environmental safety of phosphogypsum products recycling (bio-sulfur)	Environmental safety of products of the utilization of phosphogypsum (biocomposite)	Integrated indicators for assessing the processes of aggregation, bio-sedimentation, selective fixation, and sorption

Fig. 1.17 shows the combination of research methods for all phosphogypsum recycling processes in environmental protection biotechnologies.

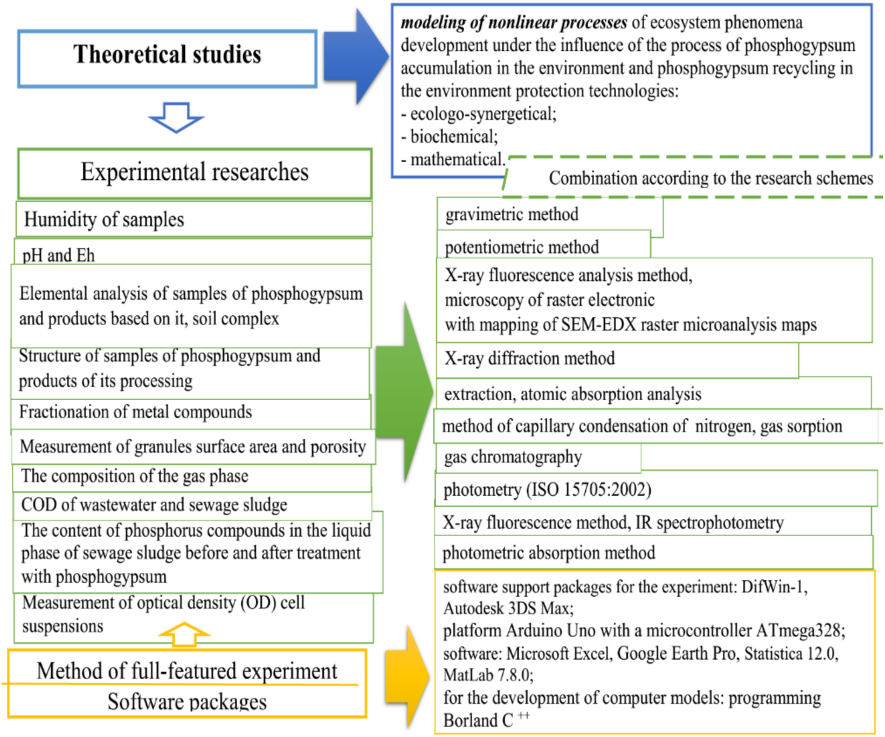


Figure 1.17 – Research methods combination

The method of full factorial experiment has been used to assess the deviation impact of the main operation and technological parameters (factors) on phosphorus reduction from wastewater and sewage sludge with heavy metal precipitation. It has been also used to evaluate the deviation impact of the basic physical and chemical parameters (factors) on the formation of modified phosphogypsum granules with optimal environmental, biochemical, physical, and chemical properties for the stimulation of the development of sulfur-oxidizing microorganisms in the system of gas flows biodesulphurisation and the efficiency of gas flow purification in the atmospheric air protection technologies. The parameters have been calculated using the nonlinear estimation with the help of software Statistica 6.0. MS Excel has been used for dispersion and regression analysis of data, calculation of correlation coefficients, and graphic illustrating of the experimental material. Fisher’s criterion has been used to evaluate the quality of regression models in general and according to the parameters.

The diagram of microbial attributes (Fig. 1.18) has been developed to evaluate the application efficiency of phosphogypsum as a mineral substrate for various groups of microorganisms in environmental protection biotechnologies.

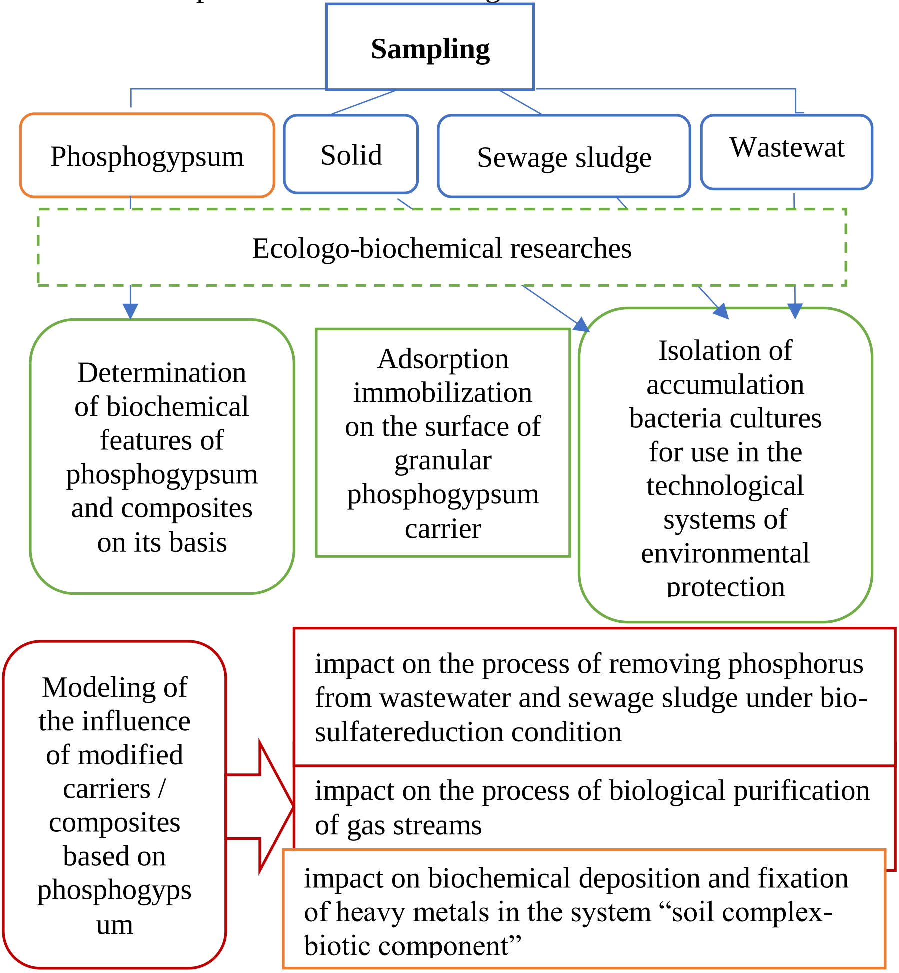


Figure 1.18 – Principle diagram of ecologo-biochemical researches

The extrapolation method has been used to evaluate the influence of phosphogypsum based biogenic composites on stimulating the natural protective properties of the soil and the remediation of affected natural and anthropogenic landscapes.

According to the diagram, the samples for primary inoculation are taken from natural (soil) and natural and anthropogenic ecosystems (system of biological treatment of waste treatment plants).

Using phosphogypsum as a mineral substrate for sulfate-reduction bacteria (SRB) growth has the following advantages: low-cost raw material base; enrichment biogenic elements (fluorine, phosphorus, etc.) of sewage sludge; sulfur compounds contained in the waste can be freely used by SRB as a mineral substrate for their growth, which is due to the high sulfate/sulfite ions affinity of microbial cells; reducing of chemical waste development pressure on the environment.

Isolation of sulfide oxidizing bacteria was done from aerobic sewage sludge collected from the municipal wastewater treatment plant. The aerobic sludge samples were collected and screened for the removal of big particles.

Then the sludge is kept in aerobic conditions by continuous aeration in order to prevent the growth of any anaerobic bacteria for 10 days at a temperature of 35 ^{o}C.

The enrichment medium for *Thiobacillus sp.* cultivation has the following composition: NH_4Cl, 1.0 g; K_2HPO_4, 0.6 g; $CaCl_2 \cdot 2H_2O$, 0.2 g; $FeCl_3 \cdot H_2O$, 0.02 g; we tacked such sulfates: $ZnSO_4 \cdot 7H_2O$, 40 mg; $CaSO_4 \cdot 2H_2O$, 70 mg; $MnSO_4$, 15 mg; $Na_2B_4O_7$, 10 mg; distilled water, 1000 cm^3; pH, 5,0.

The enrichment medium for nitrifying bacteria (*Nitrosomonas sp.*) cultivation has the following composition: $(NH_4)_2SO_4$, 2.0 g; K_2HPO_4, 1,0 g; NaCl, 2.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; $CaCO_3$, 0.001 g; distilled water, 1000 cm^3; pH, 5.0.

Enumeration and Characterization. Five days old cultures of the two isolated strains are studied under a scanning electron microscope for morphological characteristics like size and shape and also gram stain. The standard plate count method is used for the colony count at different serial dilutions ranging from 10^{-1} to 10^{-10}.

Photomicrographs microbial preparations are also processed by a digital image output system “SEO Scan ICX 285 AK-F IEE-1394” and morphometric program “SEO Image Lab 2.0” (Sumy, Ukraine). Identification of bacterial culture produced by Burge based on morphology, physiology, and biochemical properties of microbial cells.

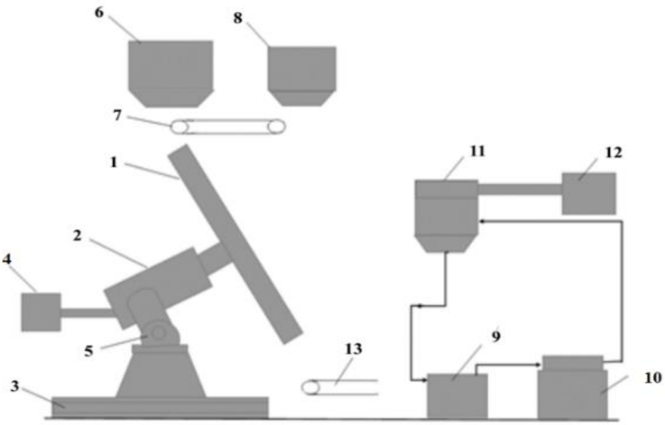
Samples of phosphogypsum granules were taken from the biofilter and made crop on enrichment mediums in Petri dishes under the procedure described above.

1.6 Development of experimental facilities and description of research methods for phosphogypsum recycling in environmental protection biotechnologies

1.6.1 Laboratory experimental plant for modified phosphogypsum granules formation

The selected dump phosphogypsum with humidity exceeding 12–16 % has been preliminary dried-up. Further phosphogypsum has been transferred to the pelletizing plant (rotary plate granulator) to form the granules of the required size and properties. The laboratory plant, designed to study the optimal parameters of the formation process of modified phosphogypsum granules, is shown in Fig. 1.19.

The laboratory plant with a plate granulator (Fig. 1.19) consists of a rotational plate (1) with an electric motor (2) fixed on the bed frame (3) and a voltage regulator (4) to which an alternating current of 220 V has been supplied. The rotational plate (1) with a diameter of 250 mm and sides height of 65 mm has been made from stainless steel. The plate speed has changed from 50 to 80 rpm. The plate angle of the granulator plate has been adjusted



1 – rotational plate; 2 – electric motor; 3 – bed frame;
4 – voltage regulator; 5 – equipment adjusting the plate angle;
6 – phosphogypsum dispenser; 7 – conveyor of source material;
8 – lime dispenser; 9 – tank for an aqueous solution; 10 – flow-control pump; 11 – disk-pulverizer; 12 – power supply unit;
13 – conveyor of ready-made granules

Figure 1.19 – Diagram of phosphogypsum pelletizing plant

with the help of a mechanism for changing the plate angle (5).

During the experiments in periodic duty mode, phosphogypsum has been supplied from the phosphogypsum dispenser (6) with the help of the conveyor (7) to the rotational plate (1) of the phosphogypsum pelletizing plant. Lime from the phosphogypsum dispenser (8) has been supplied to the conveyor (7) and has been mixed with phosphogypsum.

The aqueous solution has been supplied continuously from the tank (9) using the flow-control pump (10) through the disk-pulverizer (11) on the layer surface of phosphogypsum protogranules that has been in motion. The disk-pulverizer (11) has been connected to the power supply unit (12). The formed granules have been obtained through the conveyor of ready-made granules (13).

The obtained pelletized granules have been transferred to an exicator for hardening (8 – 9 kg/cm^2) and drying up to the moisture content of 10%

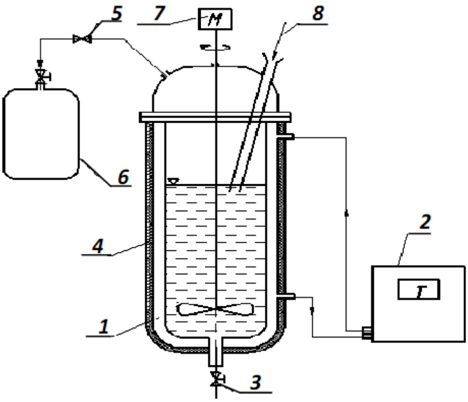
Research methods. The sieve analysis has included counting the granules number of the specified size and measuring the mass fraction of different granules fractions.

The measurement of surface area and porosity of modified phosphogypsum granules has been carried out using the capillary nitrogen condensation method with the help of Quantachrome NOVA 4200e gas sorption analyzer.

The hardness of phosphogypsum modified granules of the same size (kg/cm^2) has been determined by the effort required for compression crushing. The average value obtained as a result of 10 parallel measurements has been taken.

1.6.2 Experimental set-up for the study of the process of phosphogypsum recycling in protection biotechnologies for the water ecosystems and atmosphere

Experimental set-up of phosphorus removal from wastewater and sewage sludge by phosphogypsum use. To carry out a series of experiments, the experimental plant (Fig. 1.20), consisting of a bioreactor, (1) has been used.



1 – bioreactor; 2 – thermostat; 3 – port for the removal of the fermented sewage sludge; 4 – insulation; 5 – control valve; 6 – sampler for gaseous phase; 7 – mixer; 8 – charge opening for sewage sludge

Figure 1.20 – Experimental plant for anaerobic phosphorus removal of sewage sludge

It is an anaerobic fermentation cylindrical chamber made from stainless steel with a volume of $5 \cdot 10^{-3} m^3$ and, at that, the working volume does not exceed 7/10 of total volume. The chamber has been coated with a heat insulation film (4), under which the warm water (313 K) has circulated in heat-resistant plastic pipes, which has maintained the temperature of 309 K inside the bioreactor. The volume of circulating water was $3{,}6 \cdot 10^{-3} m^3$. The thermostat (2) Loip LT-108 (TJ-TC-01 / 8-100) has been used to maintain the required temperature.

There has been an opening (3) in the lower part of the bioreactor for discharging anaerobic stabilized sewage sludge. To maintain airtightness the upper part of the bioreactor has been tightly closed with a lid with an opening (8) for loading a new portion of sewage sludge and there has been also a pipe for removal of the gaseous phase with the help of a control valve (5). At that, the substrate has been supplied to the bioreactor using the pump with the flow rate of 0.1 dm^3/h and the hydraulic load has been 0.013 $m^3/m^2 \cdot h$.

The gas sampling has been made with the help of the special sample bags made from inert plastic Teflon® (6) with the volume of $1 \cdot 10^{-3}$ and $6 \cdot 10^{-3} m^3$.

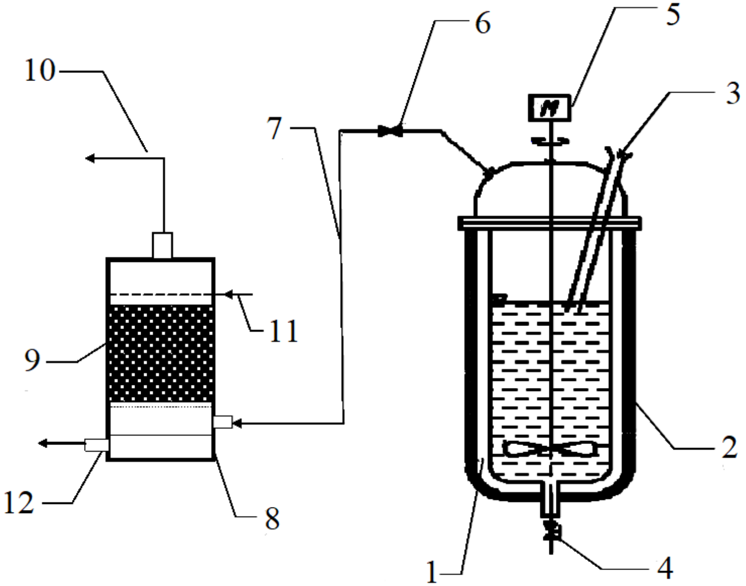
The mixer (7) with an engine power of 11 kilovolt and a nominal speed of 0.2 RPS has been installed to mix the wastes mixture in the anaerobic bioreactor.

The sampling of the fermented sewage sludge together with the residual components of dihydrate phosphogypsum for analysis has been carried out with the help of a graduated cylinder of $0.05 \cdot 10^{-3} m^3$ (1,4 % of the bioreactor displacement). After sampling (once every two days), the lack of liquid phase in the anaerobic bioreactor has been replenished with a new portion of sewage sludge with phosphogypsum addition to the initial volume. It has maintained the constant moisture of at least 86 % and the given liquid volume.

A sample bag with a volume of 1 dm^3 has been blown out three times with the analyzed gas. The sample has been analyzed on the day of sampling. The graduated mixture has been also put in sample bags using a three-way cock connected to a cylinder with a tested gas mixture (TGM).

To study the possibility of modified phosphogypsum granules application as a substrate in the biofilter, a research bench has been designed and assembled and a series of experiments has been carried out to purify biogas. The measurement of the exit concentration of H_2S has been performed at regular intervals (0.5, 1.0, 1.5 hours); the values of pH and concentration of hydrogen sulfide in the biogas entering the biofilter have been also measured.

Experimental set-up of aerobic hydrogen sulfide removal by granular phosphogypsum use. Fig. 1.21 shows the anaerobic bioreactor, a continuous stirred tank reactor with an internal settling zone, that had a working volume of 5 dm^3. The biofilter looked like a plexiglass column with a volume of 2 dm^3, which consists granulated support medium of phosphogypsum.



1 – anaerobic bioreactor; 2 – insulation; 3 – opening for sewage sludge loading; 4 – port for sludge removal; 5 – mix device; 6 – control valve; 7 – biogas inflow; 8 – biofilter; 9 – granulated phosphogypsum; 10 – purified biogas outflow; 11 – a water supply for irrigation; 12 – removal of biosulfur suspension

Figure 1.21 – Experimental set-up

Phosphogypsum granules were inoculated with an enrichment culture of sulfide-oxidizing bacteria. The biogas from the anaerobic bioreactor fed to the bottom of the column through the sleeve. Water was used in the water feed to the biofilter. Air was bubbled into the water that comes from the irrigation system to the biofilter. At the top of the column was positioned to drain fitting gas that was clean, and sampling for analysis.

The concentration of H_2S in biogas and the bacterial population at a regular interval (5, 10, 15 h) of EBRT consisted of 30 tests for each interval. The concentration of H_2S gas and bacterial populations were measured at optimum EBRT for various pH values (4.0, 4.5, 5.0, 5.5, 6.0, and 6.5) of culture. Each pH value was maintained by 15 hours for 1 test. This experiment consisted of 30 tests for each pH value.

A study of the gaseous phase was carried out on a laboratory gas chromatograph SelmiChrom-1 (Ukraine). The thermal conductivity detector (katharometer) was used. Argon was chosen as the mobile phase. Compounds are separated by traveling through a coated column and separate based on size and intermolecular interactions. The sample is injected into the pre-PLOT (porous layer open tubular) column-1 with a porous layer of sorbent PoraPlot Q (styrene-divinylbenzene coating). The gas mixture passing through the column-1 goes to one of the two katharometer cells. The hydrogen, oxygen, nitrogen follow out of column-1 as a single chromatographic strip, then methane, carbon monoxide (IV), and hydrogen sulfide are separated. The ballast column-2 is filled with an inert carrier “Chromaton N-AW-DMCS”. The H_2 and N_2 were separated in the HP-Plot Mole Sieve column-3. Registration and processing of chromatograms were performed by the software Multichrom version 1.52x. Operating temperature range of the thermostat columns from 50 to 400 °C, speed range of linear programming from 5 to 25 °C/min; injection volume (autosampler) was $0,5 \cdot 10^{-3}$ cm^3. Carrier gas of 10 cm^3/min, linear velocity 43 cm/s.

The quality analyses of materials were performed on the scanning electron microscope-microanalyzer REMMA-102 (Ukraine). Measurements were performed at five points on each sample. Processing spectrometric data, perform the necessary calibration measurements, the decryption of x-ray spectra, qualitative and quantitative electron probe analysis were performed using a software microanalysis system. The sensitivity of the measurement was at 1%. Additionally, surveyed the elemental composition of waste by X-ray fluorescence analysis, which made it possible to determine the concentration of elements in the ppm-level. Photomicrographs received and processed using the digital output of image «SEO Scan ICX 285 AK-F IEE-1394» and morphometric program «SEO Image Lab 2.0» and on the scanning electron microscope REMMA-102.

pH was analyzed by pX-meter pX-150 (ionometer) (Belarus).

Sieve analysis included the calculation of the number of granules with different sizes and dimensions of the mass fraction of the granule's different fractions.

1.6.3 Modeling of the transformation processes of components of phosphogypsum and biogenic composite on its basis in the soil

The research of quantitative and qualitative changes in the fractional composition of the soil complex of gray forest soil was carried out at the application of the increased doses of a biocomposite based on sewage sludge and phosphogypsum. The experiment was carried out in the plexiglass blocks with a perforated bottom area of 0.20 m^2 (0.5 x 0.4 x 0.5 m). The blocks were filled with gray forest soil taken from the territory with a high level of man-made load containing 17.6–21.2 mg/kg (total form) of lead.

The perennial grasses were grown in blocks using increasing biocomposite doses. The natural vegetation which grew in blocks was mowed and removed from the soil surface every season. The soil in blocks was dug over a depth of 0–20 cm and partially removed from the blocks, mixed, and put again into blocks at random. In this case, the perennial grasses used in crop rotation (alfalfa, clover, their mixtures with cereals) were applied.

The temperature in the premise was maintained at 22–25 ^{0}C.

The experiment was carried out according to the scheme biocomposite introduction at a rate of 1) 25 t/ha; 2) 50 t/ha; 3) 75 t/ha.

The biocomposite based on sewage sludge and phosphogypsum was mixed with the layer of 0–20 cm. The repeatability of the experiment is three times.

The soil samples were taken after harvesting from the layer of 0–20 cm. They were mixed and dried in the open air after each repetition. The residues of plants and mesofauna were sifted through a sieve with holes diameter of 3 mm. The air-dry samples were used to determine the fractions of heavy metals (HM).

The extraction conditions are given in Table 1.6.

Table 1.6 – The fractionating sequence of metal compounds

Metal compounds	Extraction agent	Correlation soil: solution	Extraction conditions
Exchange	1 M $MgCl_2$, pH 7.0	1:8	Stirring for 1 hour at room temperature
Related carbonates to	1M $NaCH_3COO$, pH 5.0	1:8	Stirring for 5 hours at room temperature
Related to oxides and hydroxides of iron, manganese	0.04 M $NH_2OH{\cdot}HCl$ в 25% CH_3COOH	1:20	Heating for 8 hours at a temperature of 95^0C, periodical shaking
Related to organic matter	0,02M HNO_3+30 % H_2O_2, pH 2.0	1:20	Heating for 5 hours at a temperature of 85 °C, periodical shaking
Residuum (silicates and sulphides)	$HClO_4$ + HF	1:25	Evaporation

The microscopic analysis was also carried out using surface images of an object with high spatial resolution and depth sharpness in reflected (BSE) electrons due to SEM-EDX scanning electron microscopy (using an energy-disperse analyzer) in combination with microanalysis for the creation of a mineral composition map of the soil samples after processing by biocomposite based on sewage sludge and phosphogypsum.

X-ray diffractometry studies of the mineral constituent were carried out. The research was carried out with the help of an automated diffractometer DRON-4-07. DRON-4-07 is an automation system based on a microprocessor controller that controls goniometer GUR-9 and transfers digital data on a PC. The experiment results were transmitted directly to the DifWin-1 experimental software support package. The measurement sensitivity is at the level of 0.1 %. While measuring, CuKα emission (wavelength is 0.154 nm) was used, Bragg-Brentano focusing is θ-2θ (2θ-Bragg angle). The values of current and voltage on the X-ray tube were 20 mA and 40 kV. The sampling was carried out in continuous recording mode (speed 1° / the 60s), angles range was 2θ from 15° to 105°. Moreover, the chemical fractions extraction was carried out according to the corresponding methods (Zemberyova et al., 1998; Chmylenko and Smitiuk, 2013) to study all metals speciation.

1.7 Products of phosphogypsum bioconversion processes: environmental synergetic approach

The ecological synergetic approach is an integrated set of principles and techniques for assessing ecosystem changes under the growing influence of anthropogenic factors, taking into account the synergistic mechanisms for the development of natural and anthropogenic processes to substantiate the directions of environmentally safe waste disposal, for example, phosphogypsum in environmental protection technological solutions.

The analysis of ecosystem processes under the influence of technogenesis, as nonlinear ones are the important stage in the development of a methodological approach to assess the level of anthropogenic pressure from the processes of phosphogypsum accumulation on the environment. The main characteristics of nonlinear systems that are applied at the ecosystem level can be presented in the form of a block diagram (Fig. 1.22), which also includes the factors of the environment.

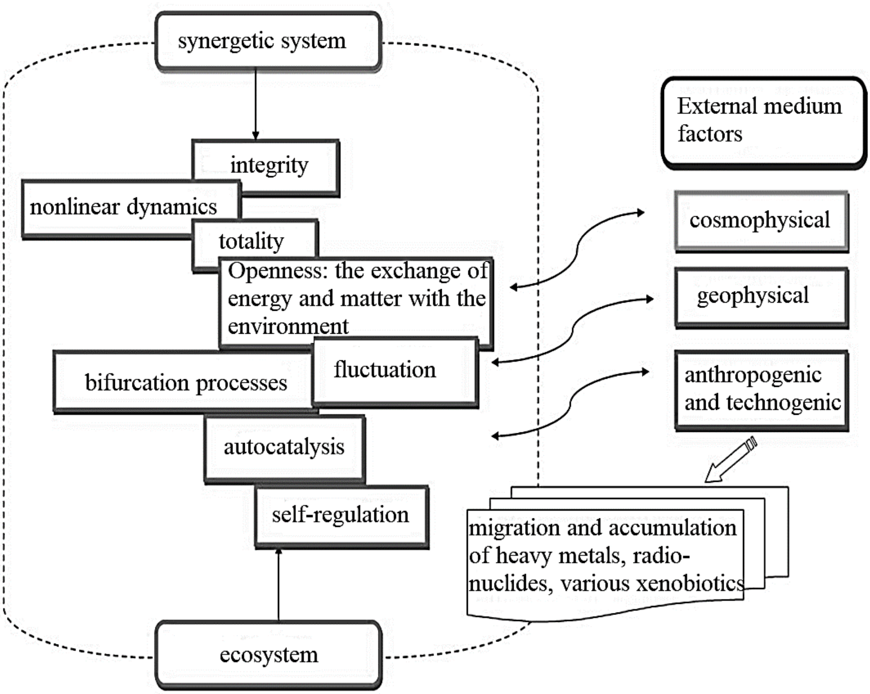


Figure 1.22 – The combination of characteristics of synergistic and environmental systems under the environmental factors influence (Plyatsuk and Chernysh, 2014)

In such systems there is an agreed behavior of the subsystems, which increases the degree of its orderliness, thus, decreases entropy and self-organization. The thermodynamics of nonequilibrium processes, the theory of random processes, and the theory of nonlinear oscillations and waves are the basis of synergetics. As all terrestrial macrosystems (biological, geological, geographic, ecological, etc.) the biosphere is largely open systems, then its development follows the laws of synergetics.

Fig. 1.23 shows the main processes occurring in the ecological nonlinear system. At the same time, the synergetic properties of the ecosystem are manifested, when interacting with the factors of the environment.

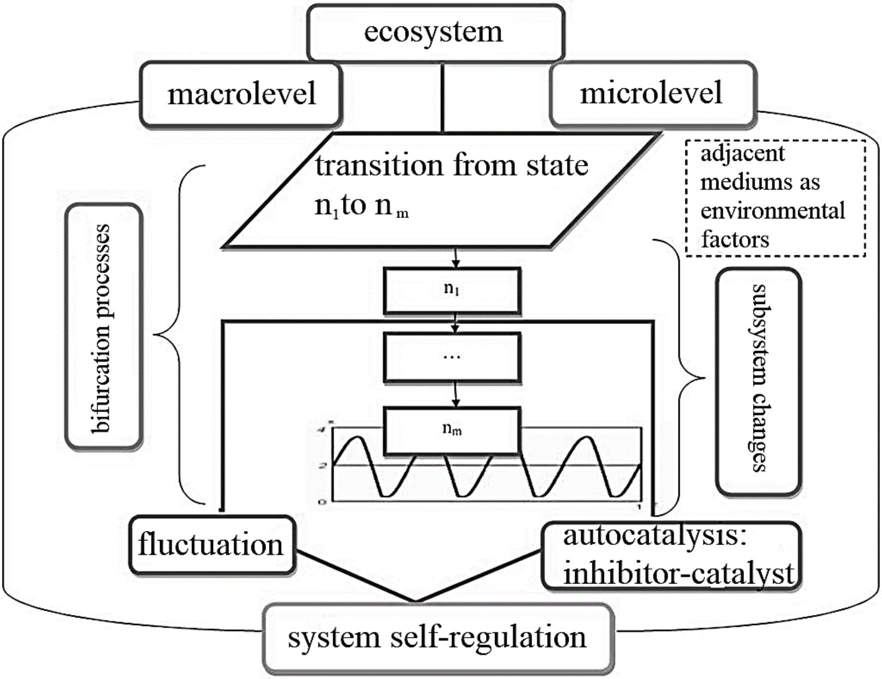


Figure 1.23 – Characteristics of the ecosystem as a nonlinear system (Plyatsuk and Chernysh, 2014)

Thus, the development of the ecological synergistic concept of phosphogypsum utilization consists of such directions:

– determining the directions of spontaneous processes in the system “dump – environment” to obtain a new quality (emergence) ecologically acceptable by them;

– assessing the environmental safety of using phosphogypsum in biochemical systems, which are inherently synergistic, in environmental protection technologies with substantiation of structural qualitative changes in microecosystems for phosphogypsum actions, determining synergistic effects as a stimulating factor and ecological synergistic mechanisms as the basis for the formation and regulation of phosphogypsum transformation processes in the cleaning systems of environmental components.

Nowadays the new direction of technological solutions for the phosphogypsum treatment as a mineral raw material to stimulate the development of the necessary ecological trophic groups of microorganisms is developed. The biochemical conversion will be able to provide efficient purification of environmental components using dihydrate phosphogypsum and products of its processing as supply medium in biotechnological environmental protection systems. In the process of phosphogypsum conversion, a mineral carrier is obtained for the immobilization of microorganisms in the technologies of aquatic ecosystems protection, biochemical gas purification from sulfur compounds, in remediation processes of contaminated soils. Biogenic composites based on the phosphogypsum can be divided into the following groups:

1. organo-mineral composites based on dihydrate phosphogypsum and sewage sludge, obtained in the process of anaerobic stabilization under sulfatereduction conditions. Such biocomposites aren't contained immobilized microorganisms, and directly interact with the biotic component when applied to the soil complex to stimulate the natural processes of humification and mineralization that affected the buffering properties of soils;
2. granules of dihydrate phosphogypsum, modified by additives (lime and bioactive salt of manganese), which can be used as a mineral carrier for microorganisms in various biotechnological solutions of environmental protection;
3. bioorganic systems based on dihydrate phosphogypsum with addition other man-made wastes (fly ash) and alginate as immobilization matrix for the necessary ecological trophic groups of microorganisms depending on the direction of use. For example, phosphor-mobilizing bacteria to stimulate the soils remediation, microorganisms-destructive complexes of hydrocarbons and phenolic compounds, sulfur-oxidizing bacteria for use in the process of gas purification in bio-desulfurization systems, etc.
4. Bio-sulfur is a product of phosphogypsum treatment in biochemical purification systems of gas streams, that contains elemental sulfur, organic sulfur as inclusion in bacterial biomass and

residual gypsum, calcium carbonate, and calcium phosphate compounds and can be used in wastewater denitrification systems.

The unity of all directions of the phosphogypsum use in the concept of ecologically-synergetic approach to the process of its utilization in the environment protection biotechnologies is shown in Fig. 1.24.

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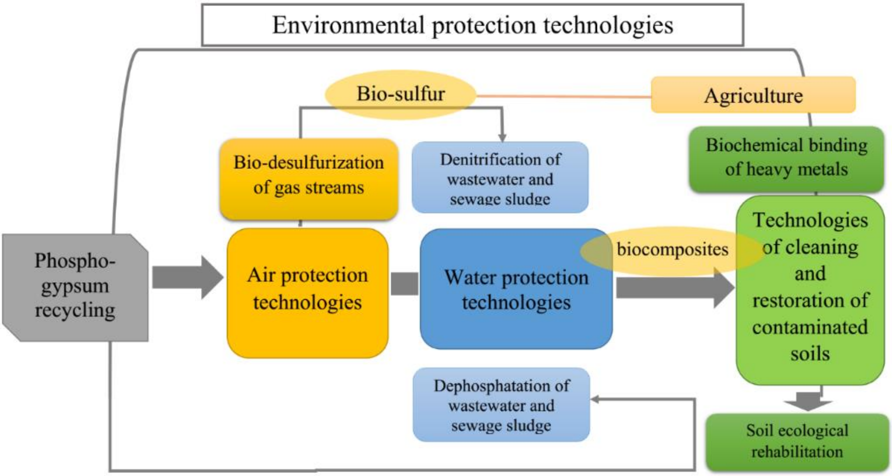


Figure 1.24 – Formalization of the unity of phosphogypsum recycling directions in environmental protection biotechnologies (Chernysh, 2019)

The first type of composite material will be considered in Chapter 4 since its production is directly related to the implementation of technological solutions for phosphogypsum recycling in the biotechnologies of aquatic ecosystem protection (Chapter 3).

Modified phosphogypsum granules (2nd and 3rd types of composite material and bio-sulfur) should have appropriate ecological, biochemical, and physicochemical characteristics for their most efficient and environmentally safe use (Chapter 2):

– the content of biogenic elements that can stimulate the bacterial matrix developed used in the purification system;

– the ability to form a sorption surface for easily develops the necessary eco-trophic groups of bacteria and nutrient accumulation;

–the size of the optimum granules for creating on their surface aerobic conditions for the possibility of the necessary bacteria groups growth;

–expansion of the contact surface between the liquid and gaseous phases and the bacterial matrix;

–stability during operation in acidophilic technological mode.

Consequently, the modified phosphogypsum granules use will contribute to the stable operation of biotechnological systems and will reduce the need for introducing into the system additional doses of nutrients, which will increase the operation efficiency of biochemical environmental protection technologies.

Chapter 2

Formation of modified composites based on phosphogypsum for use in bioengineering technologies for atmospheric protection

2.1 Efficiency criterion for identifying environmentally friendly properties of modified phosphogypsum based composites for environmental protection systems

The values of the physical and chemical parameters of pelletizing to form modified granules or phosphogypsum based composite materials with optimal environmental and biochemical, physical, and chemical properties to stimulate the development of the necessary ecological and trophic groups of microorganisms in technological systems of environmental protection have been varied during the series of experiments.

The mathematical expectation of the optimization criterion Y is a function of vector X of the input dependent variables (variation factor):

$$M(Y) = f(\bar{X}) = f(X_1; X_2; ...; X_n), \qquad (2.1)$$

where n is the factor number, then the problem is reduced to the search of such factors values $\bar{X}^* = f(X_1^*; X_2^*; ...; X_n^*)$ at which the objective function reaches the extremum.

Thus, the mathematical expectation can be expressed by the regression equation of the form

$$M(Y) = a_0 + a_1 X_1 + a_2 X_2 + a_3 X_1^2 + a_4 X_2^2 + a_5 X_1 X_2 + ..., \qquad (2.2)$$

where a_0, a_1, a_2, ... is a regression coefficient at correspondent variables.

Thus, the task of the experiment has been to determine the function extremums of the biochemical activity (BIO_x) in the variables domain of physical and chemical parameters of the formation process of modified phosphogypsum granules *X*(*f*) according to the formula (2.2) for their application in the biochemical gas purification systems. At that, while planning the experiment has been carried out on two levels of variation: maximum (+1) and minimum (–1). The coded notation of variables is given in Table 2.1.

Table 2.1 – Substitution of parameters name by coded notation

Factors		Variability Interval, Δ	Variation Levels		
			–1	0	+1
Parameter Name	Coded Notation				
Pelletizing Time, min.	X_1	5	5	15	25
Humidity, %	X_2	6	26	32	38
Lime, % of phosphogypsum	X_3	1	3	5	7
Bioactive salt (manganese sulphate), % phosphogypsum	X_4	1	2	3	4

The mathematical data processing of the results of the experiment has been carried out using the software Microsoft Office Excel and Statistica12.0

The results of statistical data processing of various factors (X_1-X_4) influencing the obtaining of modified granules with optimal properties (size, additives content, etc.) to increase biochemical activity, which corresponds to the growth of thiobacteria matrix on the surface of the granules.

It should be noted that while pelleting based on the organic and mineral product of sewage sludge treatment together with phosphogypsum, bioactive salt and lime have not been added. Its composition and properties will be considered and the application of this biogenic composite in soil remediation technologies will be considered separately in Chapter 4.

2.2 Specific features of the biofilm growth on the surface of the granulated carrier based on phosphogypsum for environmental protection biotechnologies

Following the task of research and the use of phosphogypsum granules, the rationalization of their size should be based on the determination of the penetration depth of the bacterial matrix.

The aerobic groups of sulfur-oxidizing bacteria were used for the process of gas stream purification from sulfur compounds. The mineral components of phosphogypsum (calcium, phosphorus, sulfur, potassium, magnesium, etc.) can be effectively used as aerobic and anaerobic microorganisms of various ecological trophic groups. Therefore, it is important to determine the optimal size of the granules and to limit the diameter following the nature of the thiobacteria development to stimulate their dominance among other types of microorganisms.

According to the previous research (Chernysh and Plyatsuk, 2018), the granules of optimal size are considered the granules surrounded by aerobic groups of sulfur-oxidizing microorganisms (Fig. 2.1).

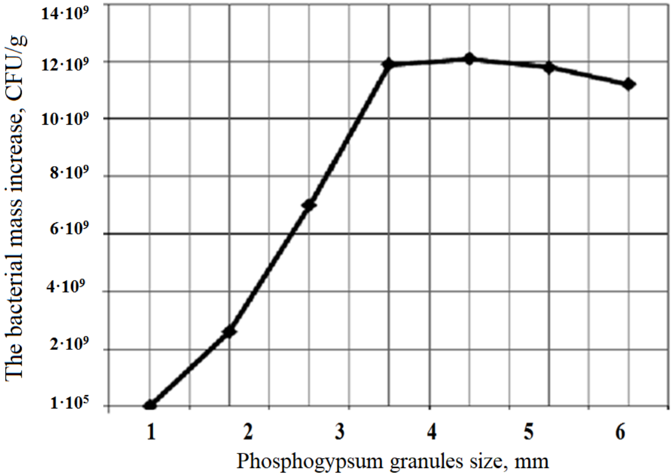


Figure 2.1 – Dependence of the growth of thyobacteria bacterial mass on the size of modified phosphogypsum granules

According to the obtained results (Fig. 2.2), the effect of the phosphogypsum granules size ($d_φ$) introduced at the stage of pellet formation on the biochemical activity *(BIO_x)*, which corresponds to the growth of thiobacteria matrix (CFU/g) on the surfaces of the granules has been approximated by the regression equation:

$$BIO_x = -\,6.0 \cdot 10^8\, d_φ{}^2 + 7.0 \cdot 10^9\, d_φ - 7.0 \cdot 10^9 \qquad (2.3)$$

$$R^2 = 0.9608$$

While forming the biofilm on granules, the depth of bacterial matrix penetration inside the granules has not exceeded 1.5–2.5 mm (Fig. 2.2 and 2.3). At that, the biotransformation of phosphogypsum components has occurred. The anaerobic growth region should not exceed 2.5 mm.

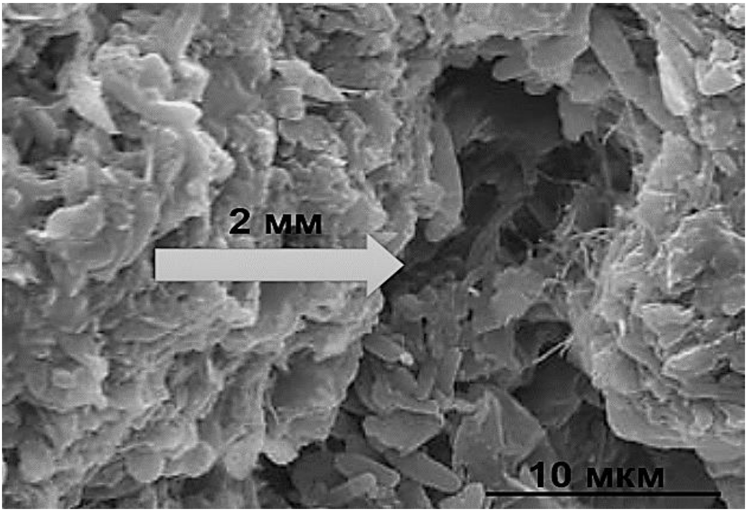


Figure 2.2 – Microphotograph of the structure of phosphogypsum modified granules after immobilization of the enrichment culture of thiobacteria, enlargement 10 μm: the arrow indicates the depth of the bacterial matrix.

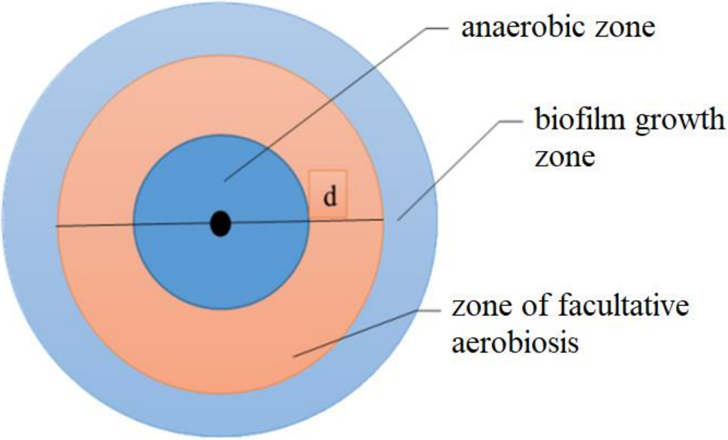


Figure 2.3 – Diagram of the modified phosphogypsum granule structure

Based on the above stated, the size of the optimal granules can be empirically determined with the help of a scanning electron microscopy of phosphogypsum granules of various sizes with immobilized thiobacteria on the surface.

Accordingly, their optimal diameter is 4–5 mm at such physical and chemical parameters, such as pH = 4.5–5.0, contact time 1.5 hours, the humidity of the nutrient medium 60%, mesophilic temperature condition.

2.3 Effect of pelletizing time and phosphogypsum humidity on the formation of modified phosphogypsum granules

While pelletizing, the increase in the average size of spherical granules has been observed with an increase in the time interval of 10–25 minutes. The particles grow as a result of adhesion while pelletizing of calcium sulfate dihydrate with phosphogypsum with the formation of the granules and also as a result of particles adhesion because of adhesive forces has taken place. The best option variant with the diameter of the granules of 4–5 mm has been observed at pelletizing of a half product at humidity 32% (Table 2.2).

Table 2.2 – Weight part of the granules fraction depending on humidity

Fraction $d_{aver.}$, mm	Weight part of the fraction at various humidity		
	w= 38 %	*w*=32 %	*w*=26 %
<1,0	–	–	–
1–2	0.09	<0.01	<0.01
2–3	0.2	0.2	0.1
3–4	0.5	0.3	0.2
5–6	0.3	0.4	0.4
6–7	–	0.1	0.3

The three-dimensional surface diagram has been developed while trying to find the optimal combination of factors such as pelletizing time of phosphogypsum and its humidity value (Fig. 2.4).

It should be noted that too high humidity (38 %) increases the percentage of cake-like clumps, which adhere to the plate walls, while at humidity less than 22 % the granules of spherical shape larger than 1 mm are practically not formed.

According to the obtained results (Fig. 2.4), the effect of the factors X_1 (granulation time) and X_2 (phosphogypsum humidity during processing) on the size of the granules (Y_1) is approximated by the regression equation:

$$M(Y_1) = 215.34 - 190.27X_1 + 65.21X_2 - 20.46X_1^2. \quad (2.4)$$

The determination coefficient has been 0.9987, the standard error of estimation has been 0.22120.

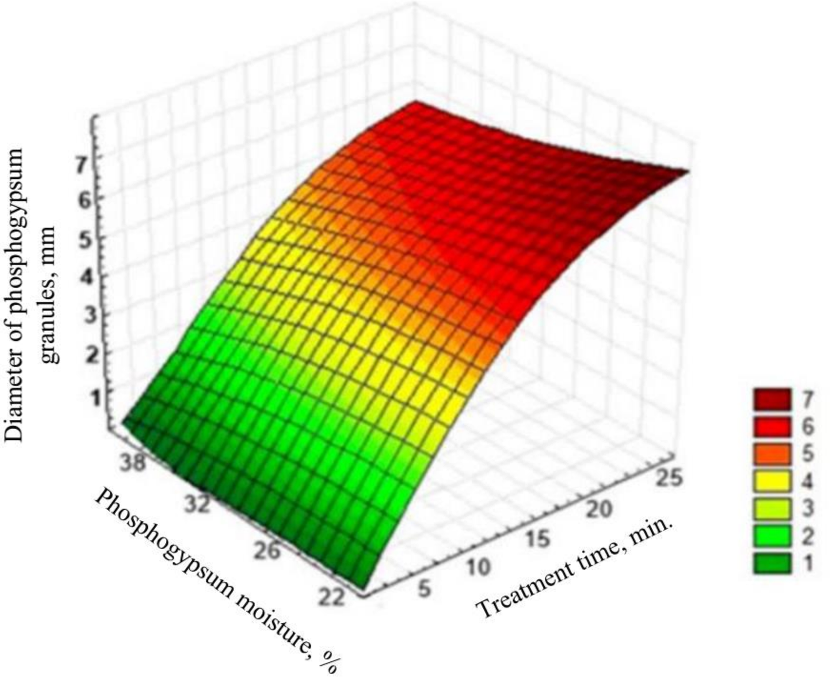


Figure 2.4 – Diagram of the dependence of optimal size formation of modified phosphogypsum granules on processing time and phosphogypsum humidity

2.4 The effect of lime on the biochemical activity of modified phosphogypsum granules

Fig. 2.5 shows the relation between the increase in the bacterial mass and the amount of lime additive at phosphogypsum pelletizing. At that, there has been no inhibition of the microorganisms development at lime addition in the range from 3 to 5 % of phosphogypsum (Chernysh et al., 2015–2017).

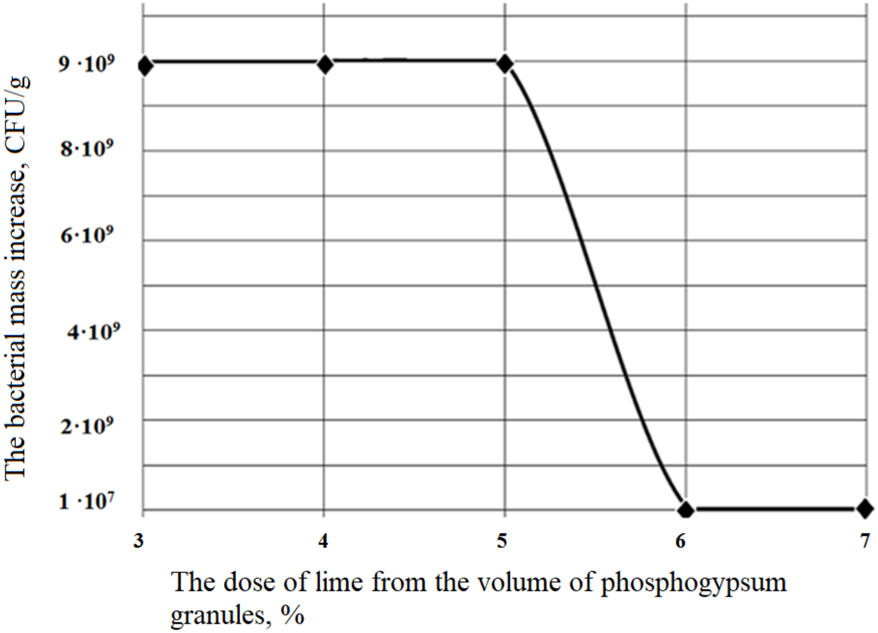


Figure 2.5. – Dependence of bacterial mass growth on the amount of lime additive at obtaining modified phosphogypsum granules

According to the obtained results (Fig. 2.5), the influence of lime amount *(X_3)* introduced at the stage of pellets formation on the biochemical activity *(BIO_x)* which corresponds to the bacterial matrix growth (CFU/g) on the surfaces of the granules is approximated by the regression equation:

$$BIO_x = -7.0 \cdot 10^8 \cdot X_3^2 + 4.0 \cdot 10^9 \cdot X_3 + 4.0 \cdot 10^9 \qquad (2.5)$$

($R^2 = 0.8234$)

The increase in a lime amount up to 6 % or more has resulted in the decrease in the development of sulfur-oxidizing bacteria on the surface of modified phosphogypsum granules by 10^2 CFU/g comparing with the optimum value (Fig. 2.5). This is connected with the formation of carbonate film on the surface of the granules because of calcium hydroxide carbonization according to the equation:

$$Ca(OH)_2 + CO_2 + nH_2O = CaCO_3 + (n+1)\cdot H_2O \quad (2.6)$$

The interaction mechanism of lime and phosphogypsum is to increase the hydrophobic properties of granules. During pelletizing, the consolidation of phosphogypsum aggregates has taken place under the influence of the inner aggregate crystallization pressure. The formation of calcium sulfate dihydrate crystals from calcium sulfate hemihydrate and calcium sulfate monohydrate, contained in phosphogypsum, with which the pores of modified phosphogypsum granules are overgrown. The existing phosphogypsum aggregates of calcium sulphate dihydrate have been partly hardened. We should mention that the small amount of the additive around 3–4 % has not allowed the necessary physical and chemical processes described above. The increase in the weight part of lime has resulted in an increase in hydrogen in the mixture. So, when lime has increased up to 7 %, pH has reached 11 units. Therefore, lime value not more than 5 % at the initial pH value of granules around 9 units have been determined as expedient to reduce the time of microorganisms adaptation and to stimulate spatial-temporal dominance of exactly acidophilic groups that purify the gas. Further, this initial pH value of the phosphogypsum granules has been adjusted in the biodesulphurisation system with the help of synergistic mechanisms for the development of complex micro-ecosystems at the excretion of microorganisms metabolism products. For example, the weak solution of sulfuric acid not more than 0.5–1.2 % of the metabolites of sulfur-oxidizing microorganisms has taken place at complete oxidation of hydrogen sulfide according to the reaction:

$$H_2S + 2O_2 \rightarrow 2H^+ + SO_4^{2-} \quad (2.7)$$

Accordingly, the pH of phosphogypsum granules with bacterial matrix developed on the surface during the operation in the biofilter ranged from 4.0–5.5 units. It has been explained by the gradual domination of acidophilic ecological and trophic groups.

Thus, the best option for the bacterial matrix development of modified phosphogypsum granules with a diameter of 4–5 mm has been observed at the treatment of phosphogypsum with humidity of

32 % and the addition of hydrated lime in the amount of 5 % of phosphogypsum weight and the pelletizing time of 15 minutes. The finished granules have been used in the system at an initial humidity of not less than 30 %. But for efficient transportation at considerable distances at different temperatures they have been dried up to the humidity of 10–15 %. The topochemical transition of phosphogypsum from dihydrate to semihydrated form has taken place. Both options of the application of modified phosphogypsum granules (dihydrate and semihydrate) have not shown any significant changes in the efficiency of gas purification; it has been determined by the biofilm development on their surface formed during the water film formation on the granules when the water has been supplied from the irrigation system. Accordingly, the biochemical transformations of dihydrate and semihydrate phosphogypsum have been carried out according to the same principles of the microecosystem autocatalysis, which is scientifically grounded in the paper.

2.5 Effect of bioactive metal salt on biochemical activity and duration of modified phosphogypsum granules hardening

Manganic sulfate dissociates into manganese cation Mn^{2+} and (complex anion) SO_4^{2-}in the solution. Manganese cation is absorbed by the bacterial matrix as a result of metabolic processes. There has also been passive magnesium adsorption on the surface of phosphogypsum protogranules. At that, manganese forms a number of simple and complex ions and several oxides of various compositions. SO_4^{2-}ion is a mineral form of sulfur that is easily absorbed by the bacterial matrix. Fig. 2.6 shows the relation between the bacterial mass increase and the amount of $MnSO_4$ additive at phosphogypsum pelletizing. The development stimulation of the necessary ecological and trophic groups of microorganisms has started at 2 % $MnSO_4$ of phosphogypsum volume. The increase in the amount of bioactive salt has resulted in the stable development of thiobacteria at the level of 10^{10} CFU/g with maximum growth up to 10^{11} CFU/g.

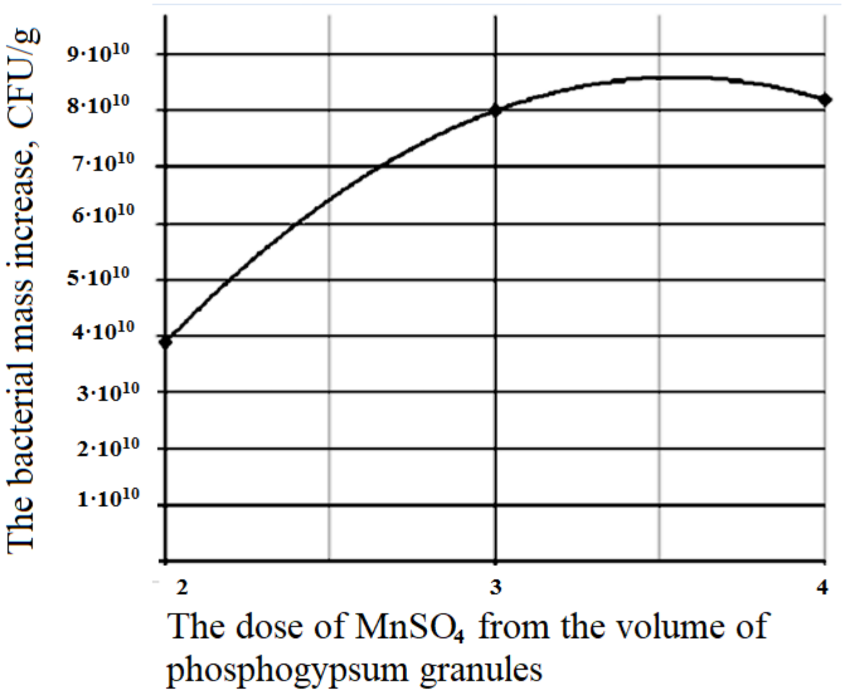


Figure 2.6 – Dependence of bacterial mass growth on the amount $MnSO_4$ at the treatment

According to the obtained results (Fig. 2.6), the influence of bioactive salt amount (X_4) introduced at the stage of formation of modified phosphogypsum granules on the biochemical activity (BIO_x) which corresponds to the growth of the bacterial matrix (CFU/g) on the surfaces of the granules is approximated by the regression equation:

$$BIO_x = 2.0\cdot10^{10}\, X_4^2 + 1.0\cdot10^{11}\, X_4 - 2.0\cdot10^{11} \qquad (2.8)$$

$(R^2 = 0.9987)$

The effect of the salt of bioactive metal ($MnSO_4$) on the duration of phosphogypsum granules hardening has been shown in Fig. 2.7.

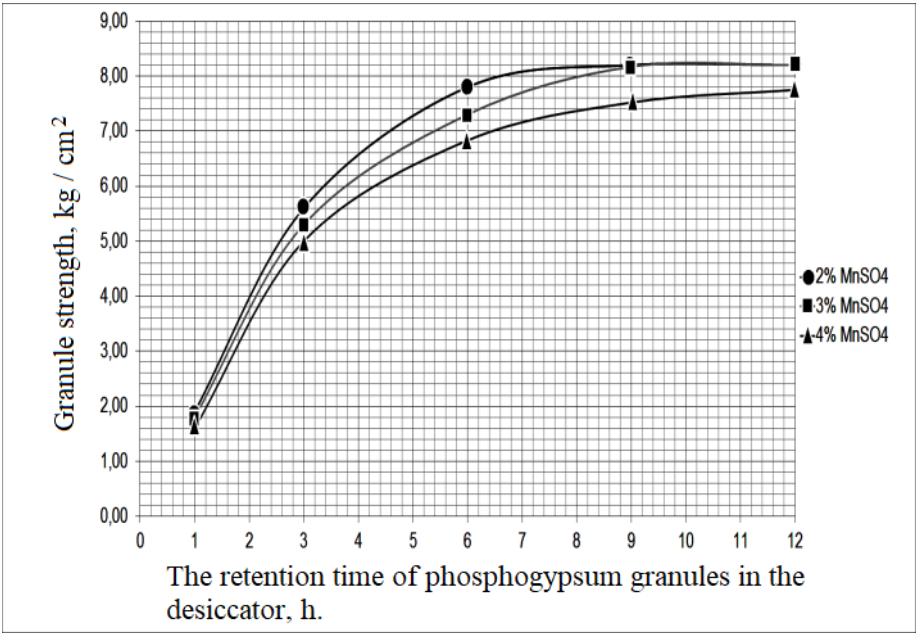


Figure 2.7 – Dependence of modified phosphogypsum granules hardening on the retention at various concentrations of $MnSO_4$ at pelletizing

According to the obtained results (Fig. 2.7), the effect of retention time at various concentrations of the bioactive salt *(X_4)* introduced at the stage of pelletizing on the granules hardness *(M_G)* is approximated by the regression equation:

– at 2 % $MnSO_4$

$$M_G = -0.096 \cdot X_4^2 + 1.7657 \cdot X_4 + 0.5947; \qquad (2.9)$$

($R^2 = 0.9733$);

– at 3 % $MnSO_4$

$$M_G = -0.0858 \cdot X_4^2 + 1.6524 \cdot X_4 + 0.5579; \qquad (2.10)$$

($R^2 = 0.9813$);

–at 4 % $MnSO_4$

$$M_G = -0.079 \cdot X_4^2 + 1.5347 \cdot X_4 + 0.5051. \qquad (2.11)$$

($R^2 = 0.9734$)

Thus, it has been determined that it is expedient to introduce 3 wt. % of manganese sulfate (II) into the aqueous solution, which will provide additional nourishment of the bacterial culture, and at the same time it will slightly influence the duration of granules hardening. The increase in weight part of this salt has resulted in the decrease in the hardening rate of modified phosphogypsum granules in the exiccator (Chernysh et al., 2015–2017).

2.6 Characteristics of modified phosphogypsum granules

Figure 2.8 shows granules (diameter 4–5 mm, humidity 10 %) that are made on the phosphogypsum basis. The formation of spherical granules was observed and their average size increase with increasing time of granulation in the range of 0–25 min.

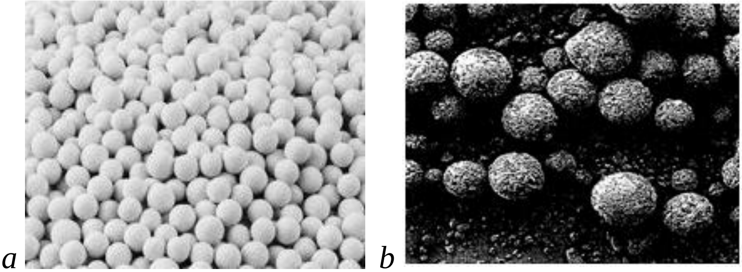


Figure 2.8 – The granulated phosphogypsum: a – general view; b – granules, magnification: x 100

The optimum granules size with a diameter of 4–5 mm was observed under such parameters of granulation: phosphogypsum moisture content of 38 % and the addition of hydrated lime in an amount of 5 % by weight of PG (Fig. 2.9). Phosphogypsum was neutralized by lime to pH 5 and phosphogypsum granules became more hydrophobic.

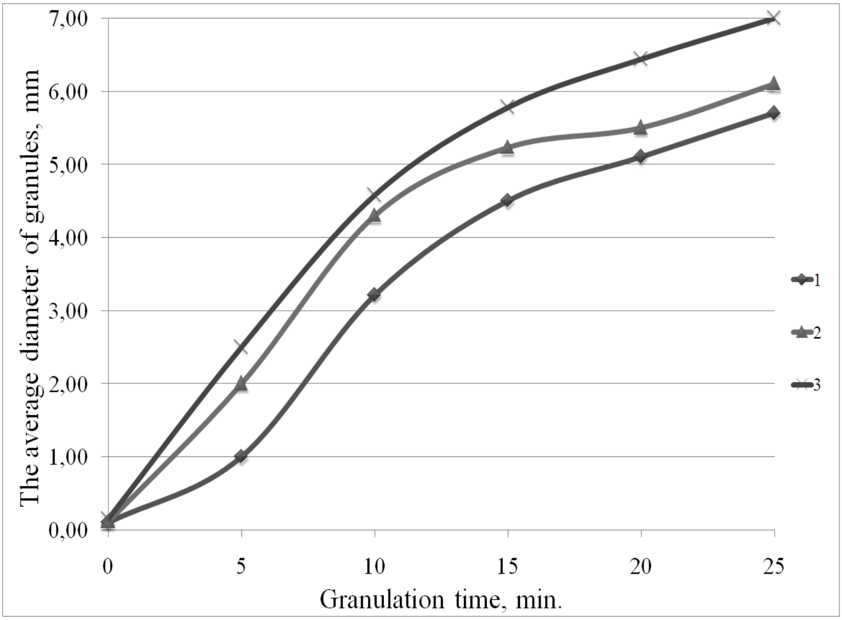


Figure 2.9 – The average diameter of granules according to granulation time and humidity of phosphogypsum 1 – w = 38 %; 2 – w = 32 %; 3 – w = 26 %

The changing of the average radius of granules during contact time depended on the process of drusen buildup on proto-granule by adhesive forces. The interaction of lime with phosphogypsum was for the seal of assemblies of phosphogypsum under the influence of the intra-pressure crystallization of the resulting gypsum hemihydrate and monohydrate, which contained in phosphogypsum. Pores overgrown crystals of gypsum dihydrate and partially cemented existing units of gypsum dihydrate in phosphogypsum. The particle size distribution was depended on the degree of hydration of phosphogypsum and residence time in the granulator (Fig. 2.9). The results obtained are also shown in table 2.3, the physical properties of the granules are described in table 2.4.

Table 2.3 – Mass part of granules fractions depending on humidity

fraction d_{av}, mm	Mass part of fractions under different moisture of phosphogypsum		
	w = 38 %	*w* = 32 %	*w* = 26 %
<1,0	–	–	–
1–2	<0.01	0.09	<0.01
2–3	0.2	0.2	0.1
3–4	0.3	0.5	0.2
5–6	0.4	0.3	0.4
6–7	0.1	–	0.3

Table 2.4 – The physical properties of granulated phosphogypsum

Parameters	Size
Specific surface area (m^2 / g)	215–325
Specific pore volume (cm^3 / g)	0.20–0.35
Density (kg / m^3)	635–789
pH with biofilm (5% aqueous solution)	4.0–5.0
Humidity,%	10–15 %
Average particle size (mm)	4–5

As a result, series of experiments, optimum content (in %) of components in modified granules of phosphogypsum (in terms of metal oxide) was determined (Table 2.5).

Table 2.5 – The main chemical components of modified phosphogypsum granules (samples dried at 333 K)

Granules component	CaO	SO_3	P_2O_5	MnO	SiO_2	Fe_2O_3	K_2O + Na_2O	CuO + ZnO
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 modified granular phosphogypsum, the mass fraction of the basic substance ($CaSO_4 \cdot 2H_2O$) is at least 80 % in terms of dry two-water gypsum.

Phosphogypsum is a source of calcium, phosphorus, sulfur, and trace elements, so it's used for chemical soil reclamation, for example, salt mines, while the introduction of phosphogypsum improves the soil structure due to the high content of Ca^{2+} cations.

2.7 Immobilization of *Thiobacillus sp.* on granular phosphogypsum

Phosphogypsum granules were suggested to implement the method of adsorptive immobilization of microorganisms. The humidity of the granulated support medium of biofilter was increased, which at the beginning of the experiment was in the process of desulfurization unit. The humidity was 10 % at the beginning of the experiment, at 30 days was 15 % and at 50 days was 17 %. This indicates the saturation of its moisture, not only from irrigation systems but also moisture contained in the sludge formed during sludge digestion biogas. The water film was formed on the surface granulated support medium of phosphogypsum. There absorbed hydrogen sulfide and ammonia with subsequent transformation of the association of aerobic microorganisms that due process autoselection changed its species composition (Table. 2.6).

Table 2.6 – Microorganisms immobilized on granulated support medium of the phosphogypsum in the biofilter of desulfurization unit

The duration of experiments	CFU / g (granules)	
	Sulfideoxidizing bacteria	Nitrifying bacteria
10	10^6	10^5
20	10^7	10^4
30	10^8	10^3
40	10^9	10^2
50	10^{10}	0

In Figure 2.10 we can see the clusters of bacteria of *Thiobacillus sp*. (including by *T. thiooxidans* and *T. ferrooxidans*) immobilized on the surface of the support medium (phosphogypsum) and biosulfur deposits. The total immobilized biomass at the end of batches was 1.9–$3.7 \cdot 10^{10}$ CFU/g of phosphogypsum. During the period operated desulfurization system, this material didn't require regeneration.

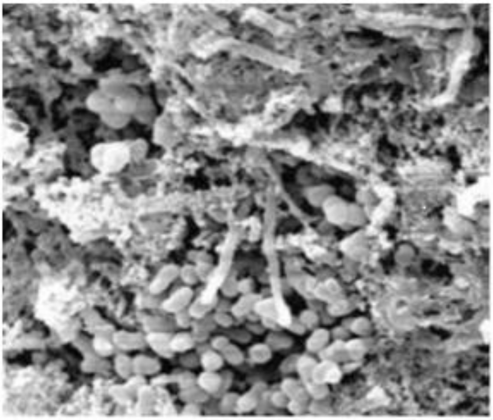


Figure 2.10 – Scanning electron micrographs of the structure of phosphogypsum granules with *Thiobacillus* biomass during immobilization process. Magnification: 10 μm

The use of modified granular phosphogypsum has the following advantages: low cost; is the source of the necessary macro- and microelements for microorganisms; stimulates the growth of the necessary ecological trophic groups; creates convinces conditions for the biofilms formation; extends the contact surface of bacteria with "gas – water" flow; resistant to high the medium acidity (pH = 4.0); performs a protection function (Chernysh et al., 2015-2017).

The excess positive charge of the matrix is compensated by the addition of negatively charged microbial cells during immobilizing microorganisms, which partially use the mineral components of granular phosphogypsum as a substrate. Accordingly, transport of nutrients within the cell of sulfur-oxidizing bacteria is carried out, which contributes to a more stable system operation with a uniform biofilm distribution on the surface of the loading from phosphogypsum and an internal layer of the bacterial matrix is formed (Fig. 2.11).

It should be noted that the bacterial matrix penetrated through the fine pores (compared with the size of cells) deep into granules, the cells were subjected to enzyme transformation of mineral components. Thus, sulfur was detected on the surface of the granules and subjected to removal. This elemental sulfur some different in composition and properties from gaseous sulfur produced using the Claus reaction. Biosulfur contains contaminants in the form of dead biomass of microorganisms and phosphogypsum particles. This sulfur can be used in agriculture.

Fig. 2.11 schematically shows the structure of the granules based on phosphogypsum with the formed bacterial matrix. The metabolic reactions of the cell come through the layer of support material based on phosphogypsum, where was occurred the diffusion-controlled transport of nutrients and discharge metabolites.

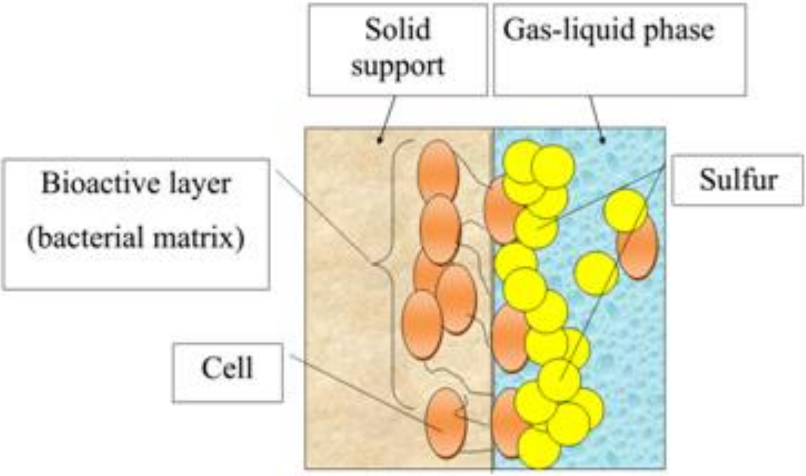


Figure 2.11 – The structure of the mineral carrier based on phosphogypsum with an internal bacterial matrix formed in the process of gas bio-purification

Bacterial matrix closely associated with the support matrix, thereby minimizing removal of the active mass of the sulfide-oxidizing bacteria of the biological filter during washing with loading and removing the surface bio-sulfur. Thus, the bioactive inner layer in a matrix of PG during its interaction with the bacterial cells was formed.

There has been a layer of the carrier material, which is modified phosphogypsum granules, between the external medium and a cell because of the cell's immobilization on the carrier. The metabolism of the "cell-medium" has been carried out through this layer, where diffusion-controlled transport of nutrients and the metabolites removal has occurred.

Moreover, chemotrophic thiobacteria consume CO_2 as a carbon source and chemical energy from the oxidation of reduced inorganic compounds such as H_2S and SO_2. At that, sulfur formed on the surface of the granules can be removed, and the presence of essential bacteria in the composition of phosphogypsum has eliminated the need for additional nutrients in the aqueous solution, which is confirmed by several papers (Chernysh et al., 2015–2018).

The medium study has shown the metabolite formation at the development of sulfurous bacteria on phosphogypsum. Fig. 2.12 shows the overlapping of halftone microphotographs with different enlargement of phosphogypsum granules to determine the areas of biotransformation of phosphogypsum components and thiobacria clump with deposits of their metabolite, which is elemental sulfur, during the oxidation of hydrogen sulfide.

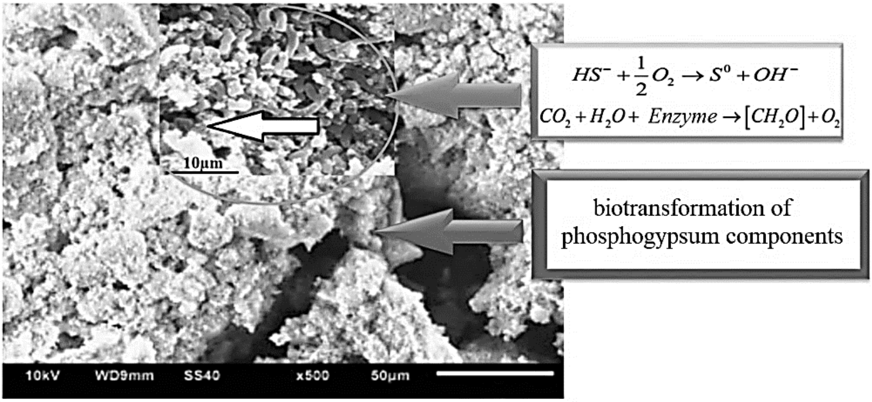


Figure 2.12 – Scanning microscopy of biotransformation of phosphogypsum granules during oxidation of hydrogen sulfide by thiobacteria

Developing on the surface of modified phosphogypsum granules, the biofilm begins to grow in the form of separate cell aggregate, sorbing dissolved compounds from the gaseous phase and transforming them into their metabolites.

Fig. 2.13 shows a general view of biosulfur after drying and rounding.

Figure 2.13 – General view of biosulfur

Based on the diffractometer research and rastrum microanalysis of the structure of biosulfur, it was found that the content of orthomolecular S_8^0 in its composition reaches 60 %. The 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 2.7. 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 phosphogypsum and, accordingly, it was not identified in all samples.

Table 2.7 – Content of basic elements in biosulfur

<table>
<tr><td colspan="8">Content of basic elements in the mineral spectrum of biosulfur, %</td></tr>
<tr><td colspan="3">S</td><td colspan="3">Ca</td><td colspan="2">P</td></tr>
<tr><td colspan="3">55.65–68.21</td><td colspan="3">16.45–34.7</td><td colspan="2">1.8–4.1</td></tr>
<tr><td colspan="8">Organic inclusions of biosulfur</td></tr>
<tr><td rowspan="4">Bacterial cells</td><td colspan="7">$C_{4,40}H_{6,8}O_{1,31}N_{0,86}P_{0,11}S_{0,14}$</td></tr>
<tr><td colspan="7">Elemental composition of biomass, %</td></tr>
<tr><td>C</td><td>H</td><td>O</td><td>N</td><td>P</td><td>S</td><td>Ash</td></tr>
<tr><td>52.9</td><td>6.8</td><td>21.0</td><td>12.0</td><td>3.5</td><td>4.5</td><td>4.0</td></tr>
</table>

Thus, the population of thiobacteria is useful for the environment. In the course of their metabolism, bacteria stimulate the arrival of easily digestible forms of sulfur to plants. Dead cells contribute to the 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).

Fig. 2.14 shows a diagram of metabolic pathways of the transformation of sulfur compounds by sulfur oxide bacteria that was created using the information of KEGG database resource. The sulfur ability for oxidation is widespread among bacteria and archaea, including phototrophs and chemolithoautotrophic. 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 (Grabarczyk and Berks, 2017).

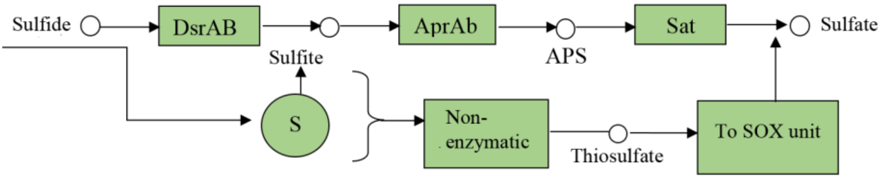


Figure 2.14 – Combined scheme of pathways of bacterial oxidation of sulfide to sulfate (with the block of transformations by elemental sulfur): Sulfide; DsrAB is dissimilative sulfite-reductase alpha subunit, Sulfite, AprAb – adenylsulfate, subunit A, APS – Adenosine 5'-phosphosulfate, Sat is sulfate adenyliltransferase, Sulfate; SCcR is sulfide-cytochrome-c reductase SoxA, SoxX, SoxY, SoxZ, SoxB – sulfur-oxidative proteins (Plyatsuk et al., 2018)

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. 2.14).

2.8 Development of the method for producing a biogenic composite based on phosphogypsum and other technologic wastes containing immobilized microorganisms

The main purpose of *Thiobacillus sp.* immobilization during the oxidation of sulfides is to avoid contact between the cells and sulfur formed during the oxidation of sulfides. In the adsorption technique, the cells are physically attached to the surface of the immobilized matrix, which allows the cells to easily separate even due to gentle stirring by aeration, which causes cells separation from the matrix and conversion of sulfur into sulfates. More intensive sulfate formation and leakage of cells in adsorption techniques indicate that. Therefore, the high-priority task of improving immobilization methods is to preserve the cells during the treatment of polluted environment without losing their viability and with the separation of biosulfer as the final product.

Thus, the composition development of a universal carrier of immobilized microorganism cells of different ecological and trophic groups is aimed at the use of a mineral carrier based on technogenic waste not only in the natural environment for its treatment but also in biotechnological systems of different types and at the reduction of the man-made impact on the environment from accumulated industrial mineral waste.

2.8.1 Method development of the microorganism immobilization in the carrier mass based on technogenic wastes

The method for producing a phosphogypsum-based mineral carrier is protected by the invention patent “Method for producing a granulated carrier containing immobilized microorganisms” (Patent of Ukraine for invention 114664, 2017).

The method includes the microorganism’s immobilization in the form of a suspension in a gel forming reagent, using a 3–5 % solution of sodium alginate with subsequent granulation. Also, phosphogypsum was added to the gelling agent. The granulation is carried out in a rotating plate granulator with the mixing of immobilized microorganisms with mineral powder, which was made based on fly ash from the thermal power plant (TPP) for water resistance increased. The initial ratio of components, mass particles: suspension of microorganisms 10–15; 3–5 % solution of sodium alginate 3–5; phosphogypsum 15–20; fly ash 7–10.

Besides, the concentration of the microorganism's suspension should be 10^{10}–10^{11} CFU / g granules.

The acidophilic association of thiobacillus – *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans* is used as a microorganism suspension of the necessary ecological and trophic group.

The inclusion of microorganisms with a share of 10–15 in alginate gel refers to gentle immobilization methods – the cells stay alive and can perform multienzyme processes. The share of alginate gel makes up 3–5, which is conditioned by the necessity to observe the homogenization mode. Simultaneously, micro- and macroelements from the phosphogypsum addition and oxygen diffuse into the gel with the same speed as in aquatic environment, thereby increasing the microorganism cells viability and further, being introduced into a biological treatment system, make it possible to reduce the period of their adaptation to the environment and to increase the degree of purification of the contaminated environment.

The proposed invention applies phosphogypsum as an additive to the gelling reagent at the mixing stage. Its concentration is determined by the fact that when the additive content is less than 11 pts.wt., it cannot be equally spread in the gelling reagent along with the microorganism suspension and lead to complete homogenization of the semi-product, consequently, the microorganisms are not provided with the mineral substrate in the amount10^{9}-10^{11} CFU/g granules. If the content of phosphogypsum additive is more than 20 pts.wt., the increase of the microorganism growth rate during the contaminated environment purification does not take place. Besides, the humidity of the semi-finished product decreases, which negatively affects the granulation process of the carrier for microorganism immobilization. Therefore, it is optimal to add phosphogypsum in the range of 11–20 pts. wt.

Phosphogypsum has high concentrations of calcium, sulphur, and silicon. Besides, it contains phosphorus, potassium, and traces of such elements as iron, fluorine, magnesium, etc. All of these preconditions the use of phosphogypsum for the proper mineral nutrition of various ecological and trophic groups of microorganisms.

The use of phosphogypsum chemical wastes provides microorganisms with calcium cations (Ca^{2+}) that are gradually consumed by microorganisms as a result of a dihydrate form, which is reasonable for the maintenance of high granule bioactivity over a long period of time.

Phosphogypsum refers to low soluble mineral chemical wastes of the hazard class IV which is low hazardous. Wastes of this hazard class do not contain highly toxic substances that can harm the normal functioning of the microorganism group in the biofilter and, on the contrary, boost them. It is pre-washed with water and dried at $+60^0C$ to increase the content of calcium sulphate dihydrate in it. This additive also performs the function of the gel stabilizer, which provides the slowdown of the gel hardening for the period of time sufficient for mechanical crushing of the gel in the mixture with the microbial suspension to granules.

During the granulation, the semi-product is granulated with the powder based on the fly ash of the thermal power plants that are characterized by increased water resistance. Its concentration is determined by the fact when adding less than 7 pts. wt. of the powder, it is impossible to provide increased hydrophobic properties of the granules. When the content of the powder is more than 10 pts.wt., no further increase of hydrophobicity and frost resistance of the granules is observed. Thus, it is impractical from the point of view of the technological process and energy consumption.

According to the chemical composition, the main components of the fly ash of thermal power plants are oxides of silicon, aluminum, and iron. It also contains underburnt carbon. Besides, oxides of magnesium, potassium, calcium, sulphur, and phosphor (from 0.2 % to 8 %) are present. Consequently, the ash composition is of first-rate importance for its use as a mineral powder for the formation of granules on which microorganisms of different ecological and trophic groups can be immobilized not only at the stage of semi-product formation, but also in the future when exploiting this mineral filling in biological treatment systems.

In particular, it refers to granules with immobilized sulfur-oxidizing bacteria. During their exploitation in the biofilter, when purifying the gas stream from sulfur compounds, a biofilm is formed on the surface of the granules merging with the inner bioactive layer formed at the mixing stage of the gelling reagent – a microorganism suspension – a phosphogypsum additive (that is, the formation of the semi-finished product).

The use of the fly ash of thermal power plants leads to increased strength, hydrophobicity, frost resistance of granules, which expands the scope of their use and facilitates their transportation and storage.

The method of including cells into the announced granulated carrier gives advantages due to such properties as simplicity of preparing granules, relative cheapness, possibility to include cells of any size and in a given quantity, flexible fixation of cells, abrasion and tear resistance of cells (the properties required for mixing reactors), which enables the use of the announced mineral filling/feed not only in the natural environment for its cleaning but also in biological systems for gas purification.

Applying to dehydrate phosphogypsum based additives at the stage of mixing microorganism suspension with the gelling reagent in the combination with the fly ash powder that is applied at the granulation stage gives the possibility to produce bioactive granules that have the following advantages: low cost; are the source of the needed macro- and microelements for microorganisms, stimulate the development of the necessary ecological and trophic groups; create favorable conditions for the biofilm formation on the surface of the granules; extend the surface of bacteria contact with the gas-water flow; are resistant to high acidity (pH = 4.0); perform a protective function binding toxic components, for example, heavy metals (volatile metalorganic forms). Thus, natural sorption mechanisms characteristic of living bacteria cells provide them with metals (micro- and macroelements) in the required concentrations which come from the mineral substrate contained in phosphogypsum and fly ash of thermal power plants. The microorganism cells have specific transport systems that provide the transport of ions inside the cells or their secretion into the extracellular space using the energy of hydrolysis of adenosine triphosphoric acid. Inside the cell, metals are released as ions or in the form associated with various cytoplasmic components.

Thus, the industrial process of obtaining granules containing immobilized microorganisms consists of three stages:

– mixing and homogenization of a microorganism suspension with a gelling reagent and a phosphogypsum-based additive (receiving of a semi-finished product);

– granulation of a semi-product with a fly ash based powder that is made in a plate granulator to obtain granules;

– holding of the obtained granules in the tank for drying granules and hardening of the gel in the granules with immobilized microbial cells.

Herewith, phosphogypsum chemical wastes and fly ash of thermal power plants are a cheap source of raw materials and are widespread. Thus, a large number of by-products appear along with the main products during the manufacturing of chemical plants. At present, large tonnage phosphogypsum wastes are the most spread concentrated secondary industrial raw material. However, the percentage of phosphogypsum recycling in Ukraine remains very low (not more than 10 %). Consequently, its disposal areas are hefty and influence directly the environment causing severe contamination. A similar environmental problem exists with the placing of fly ash of thermal power plants in ash dumps. Therefore, the production process of the proposed biocomposite is aimed at reducing the man-made impact of these wastes on the environment and introducing a new way of their recycling in an environmentally friendly technology.

2.9 Mathematical modelling of the gas stream biodesulfurization process in the air protection technologies while using phosphogypsum filling

In mathematical modelling of kinetics of converting hydrogen sulfide by sulphur-oxidizing microorganisms in the phosphogypsum recycling the following assumptions have been made: the whole effective volume of the biofilter is filled with a homogeneous phosphogypsum filling; the concentration of the mineral substrate (phosphogypsum) and the biofilm is equal at each point of the biofilter; the substrate concentration and the total number of cells are connected linearly, the change in hydrogen sulfide concentration (C_{H_2S}) when passing the maximum number of viable cells N (with $\mu_m = \frac{1}{N} \cdot \frac{dN}{d\tau}$= const) results in an exponential function and reveals the dependence on biochemical properties of the phosphogypsum filling.

The biofilm volume expands with the increasing amount of hydrogen sulphide for purification, or with the reducing filtration rate through the filling layer of the gas stream. The use of phosphogypsum components as a substrate by thiobacteria stimulates the formation of a stable biofilm that represents a community of different ecological and trophic groups of microorganisms.

The rate of the gas stream should not exceed the oxidizing capacity of the biofilm formed either on the surface of the mineral carrier or penetrating its inner layer.

To optimize the assessment of the oxidizing capacity of the biofilm the classical kinetics of the microorganism growth has been taken as a basis. Also, it has been proposed to consider the operating parameters of the system and the physical and mechanical properties of the phosphogypsum filling. Here, the active surface of the biofilter ($S_{s.s.}$.) has been taken into account that considers only the biofilter surface containing the bacterial film, that is, the specific surface area of the biofilm developed on a mineral phosphogypsum carrier [197]:

$$BOC = \left(\frac{S_{\text{s.s.}} \cdot V_f \cdot G \cdot D}{\tau_f \cdot v_f} \right) \cdot \left[\frac{\mu_{m_1} \cdot X_1 \cdot \Delta C_{H_2S}}{Y_{X_1/S_1} \cdot \left(K_S + C_{0_{H_2S}} \right) \cdot K_{pH}} + \frac{\mu_{m_2} \cdot X_2}{Y_{X_2/S_2}} \right] \cdot \alpha_{ad}. \quad (2.16)$$

Where *BOC* is biofilm oxidizing capacity, g/m^3·h; $S_{s.s.}$ is biofilm specific surface area, m^2/m^3; V_f is the amount of filtering phosphogypsum filling, m^3; *G* is filling prill size rate (*100/R*); *R* is the typical size of granules (10^{-3}m); τ_f is the retention time of gas-liquid phase in the biofilter, hour.; v_f is filtration rate of the gas stream, m^2/h;

D is biofilter diameter, m; μ_{m1} and μ_{m2} is the specific growth rate of thiobacteria and nitrobacteria, respectively, h^{-1}; X_1 and x_2 is biomass concentration of thiobacteria and nitrobacteria, respectively, g/10^{-3}m^3; $Y_{X1/S1}$ and $Y_{X2/S2}$ is the economic coefficient of biomass yield factor of thiobacteria and nitrobacteria according to the substrate –hydrogen sulfide and ammonia, respectively; $C_{0_{H_2S}}$ is the initial hydrogen sulfide concentration in the gas stream, g/m^3; K_S is saturation constant according to the substrate, g/m^3; K_{pH} is constant considering the acid-alkaline balance of the environment; α_{ad} is adjustment coefficient.

The formula (2.16) has considered the specific character of the filtration process of the gas stream through granulated mineral phosphogypsum filling. Thus, the first multiplier describes the rate at which the dissolved hydrogen sulfide passes through the phosphogypsum carrier and contacts the biofilm concerning operating conditions of the gas stream input into the system and the influence of the main biofilter characteristics on it. Also, the adjustment coefficient α_{ad} has been introduced which takes into account the intensification of thiobacteria growth with their immobilization on phosphogypsum prills, and it has been proposed to define it as:

$$\alpha_{ad} = \frac{1}{\mu_D \cdot t_{PG}}, \qquad (2.17)$$

where μ_D – the specific rate of the thiobacteria biomass dies away, hour $^{-1}$; t_{PG} – a time of covering phosphogypsum prills with the biofilm, hour.

The second multiplier describes the influence of the microbial kinetics on the conversion process of hydrogen sulfide that passes through the mineral phosphogypsum filling. The stationary phase of bacterial development has been taken as a basis to account for the basic parameters of their growth kinetics when the biomass concentration remains at a constant level, that is, the biomass growth rate is completely compensated by the rate of death and cells lysis. Thus, the ratio $\frac{\mu_{m_1} \times X_1}{Y_{X_1/S_1}}$ is the consumption of the substrate (hydrogen sulfide) during the thiobacteria biomass increase. Also, it has been proposed to enter the ratio $\frac{\Delta C_{H_2S}}{\left(K_S + C_{0_{H_2S}}\right)}$ to account for the dynamics of the hydrogen sulphide concentration change with the saturation of thiobacteria biomass according to the substrate (hydrogen sulfide).

Upon the implementation of the mathematic modelling of the values of the BOA according to the equation (2.16) is described in (Chernysh Y., 2016). The initial data have been determined under the biochemical conditions of the purification process of the gas stream (Tab. 2.8).

Table 2.8 – Initial data for mathematic modelling

Initial conditions	Value	Units
X_1	$112.7 \cdot 10^{-3}$	$g/10^{-3}m^3$
$S_{s.s.}$	200	m^2/m^3
V_f	5	$10^{-3}m^3$
G	0.04	$10^{-3}m$
T_f	1.5	h.
D	0.15	m
A_{ad}	2.52	–

The values C_{0H_2S} were changed within the range of 100, 250, 400, 550, 700, 850 ppm. To develop a computer model, the programming language C^{++} in the IDE Borland C^{++} has been used which is intended to describe a wide range of tasks and provides mechanisms for process control and data managing, which optimally suits the task.

During the biodesulfurisation the oxidation of hydrogen sulfide to elemental sulfur takes place, which corresponds to the decrease in the hydrogen sulfide concentration to a certain level that coincides with the change of dynamics of the biofilm oxidizing capacity at various initial hydrogen sulfide concentrations. So, its initial concentration (850 ppm) drops to the level 54,31–69,52 ppm in a series of experiments for 1 hour at the biofilm oxidizing ability of 743,58 mg / m^3 · hour (Fig. 2.15).

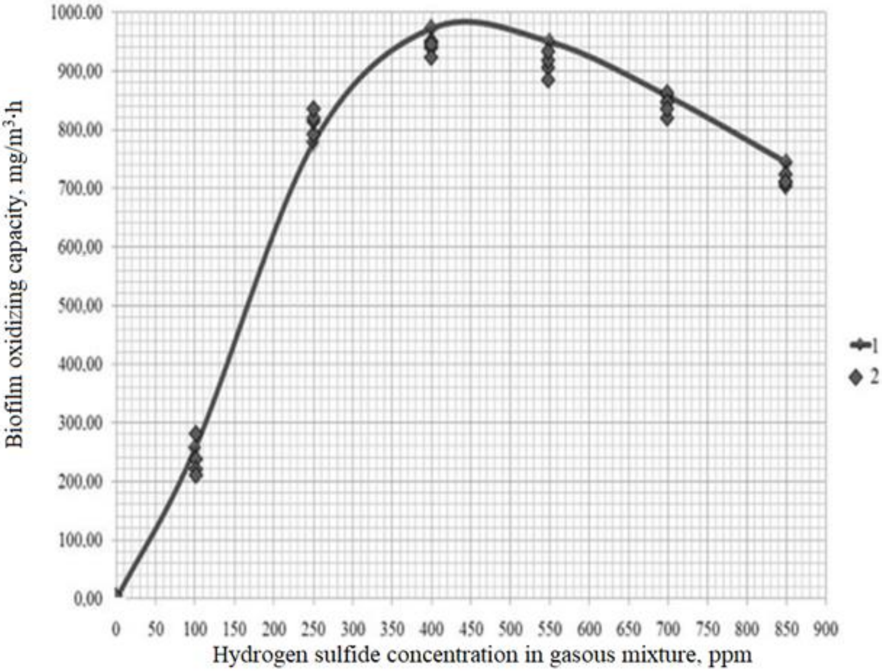


Figure 2.15 – Dependence of the biofilm oxidizing capacity of hydrogen sulfide concentration in gasous mixture: 1 – model; 2 – experimental value (r=0.922)

The curve received as the result of mathematical modelling corresponds to the dynamics of reducing hydrogen sulfide concentration in the time and has a good convergence with the experimental data (Fig. 2.15).

2.10 Development of process flow diagram for gas stream biopurification from sulfur compounds with the use of modified phosphogypsum granules

The development of the process flow diagram of material streamflow based on environmental security principles is a prerequisite for the implementation of the production process.

The main requirements to the process flow diagram include:

- simple design and automated equipment;

- efficient location of equipment following the direction of material streams;
- selection of the optimal operation mode of the technological equipment – batch or constant;
- separation of the final processing product;
- removal of the gaseous phase from the system and its recycling;
- possibility to create recycling systems for material streams;
- by-product recovery.

Based on the processing and analysis of experimental and theoretical data a schematic block diagram of phosphogypsum recycling in desulphurization systems in atmospheric air protection technologies (Fig. 2.16) (Plyatsuk, Chernysh, et al, 2018).

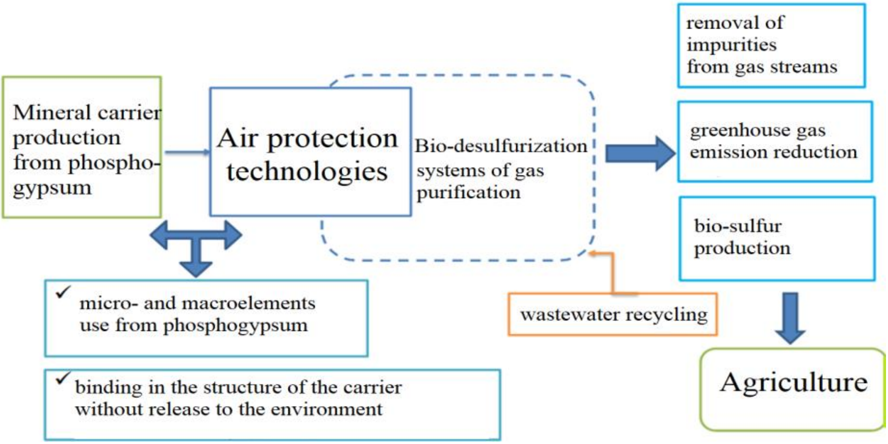


Figure 2.16 – Schematic of ecologically safe processes of recycling phosphogypsum as a mineral carrier in bio-desulfurization systems (Plyatsuk, Chernysh et al., 2018)

At that, it is also important the development of systems and equipment for gas purification with the use of phosphogypsum filling according to the environmental safety requirements (Fig. 2.16). As one can see, the use of modified phosphogypsum granules in the system of biochemical desulfurization is aimed at achieving the environmental effect containing the following components:

1. removal of toxic compounds from gas streams;
2. reduction of greenhouse gas emission;

3. biochemical sorption of toxic agents with their removal from the natural cycle of the ecosystem;

4. reduction of the man-made impact of the phosphogypsum disposal areas.

Meanwhile, the proposed technological solution allows to obtain an environmentally friendly organo-mineral product with a wide range of applications in agriculture (Fig. 2.17) in the process of the metabolic activity of thiobacteria and to carry out recycling of the washing effluent received at the stage of precipitation and drying of biosulfur with the reuse in the biofilter sprinkling system.

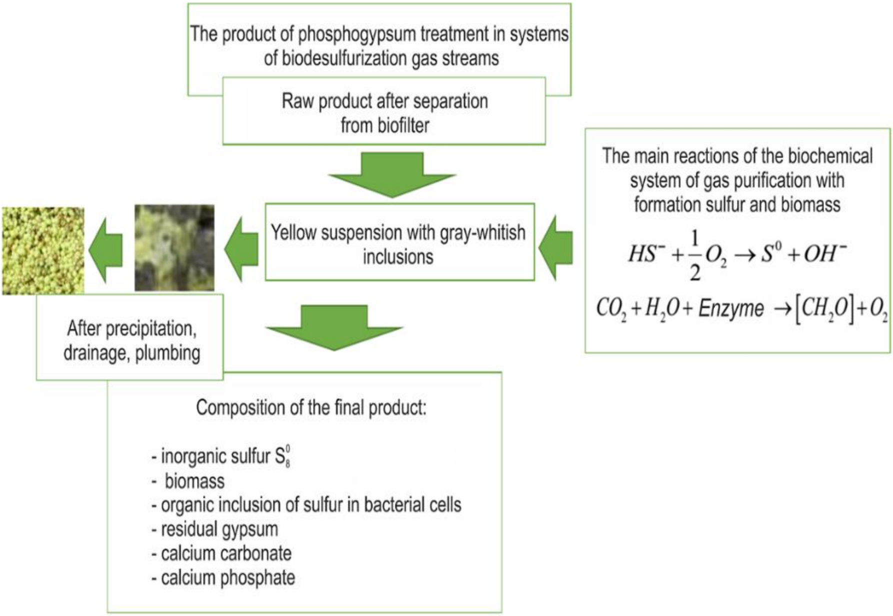


Figure 2.17 – Characteristics of the product of phosphogypsum recycling in bio-system desulfurization of gas streams (Plyatsuk, Chernysh et al., 2018)

Figure 2.18 shows a process flow diagram of the gas stream purification in the biofilter.

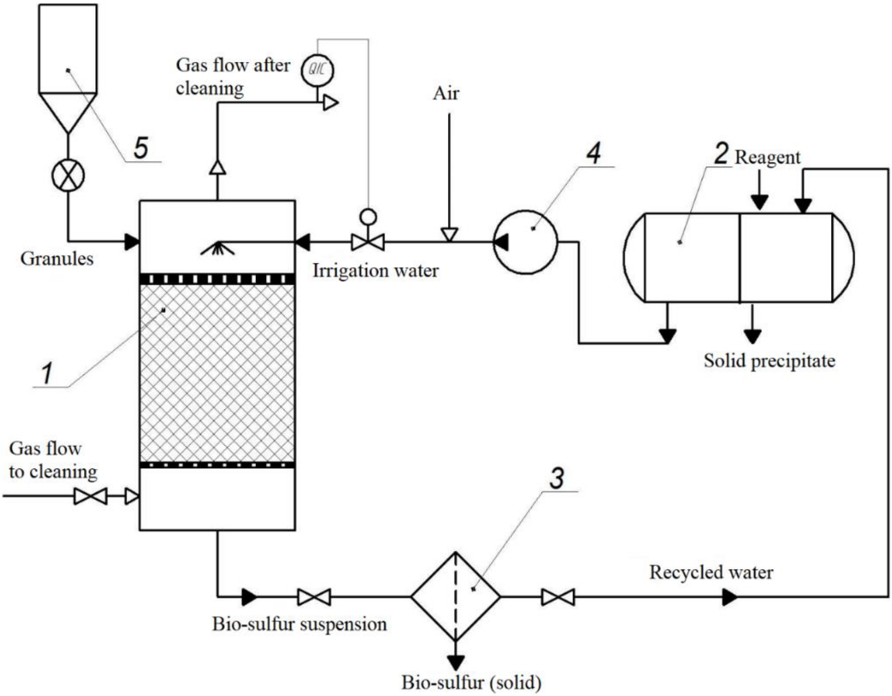


Figure 2.18 – Biofilter for the gas stream purification with the use of modified phosphogypsum granules

The method is carried out as shown in Fig. 2.18.

The gas stream contaminated with sulfur compounds is transmitted through the bottom into the aerobic biofilter made in the form of column 1 with a phosphogypsum filling. The transformation of sulfur compounds (particularly hydrogen sulfide) into biosulfur takes place in the process of the metabolic activity of thiobacteria immobilized on a granulated mineral phosphogypsum carrier.

Besides, the phosphogypsum filling is moisturized with the help of the sprinkling system that consists of an accumulation tank 2 and perforated tubes. Water for sprinkling is pumped by pump 4 into column 1of the biofilter. Before being supplied to the system, the water is aerated, and the air is supplied through the fan.

The recovery of elemental sulphur occurs during the washing process with recycling water from column 1 in the direction opposite to the gas streamflow. As a result, sulfur in the form of a yellow suspension is received through the hole in the lower part of the column.

In block 3, biosulfur is separated from this suspension, dried, and can be reused. The removed water solution (recycling water) is pumped into the accumulation tank of sprinkling system 2. Due to biooxidation with thiobacteria of the mineral elements from phosphogypsum carrier the transition into the liquid phase of metal (Fe, Cu, Zn, etc.) ions takes place. They settle as an insoluble fraction in reagent way in accumulation tank 2 and are removed from the system separately from biosulfur. Hydroxides of calcium and sodium, sodium carbonate, sodium sulfide, etc. can be used as reagents for the removal of metal ions from the recycling water.

The purified gas-water stream passes through the nozzle placed at the upper part of column 1 and then is removed from the system. To control the efficiency of gas purification and the periodicity of biosulfur leaching and to supply additional charge of modified phosphogypsum granules at the outcome from the system of the purified gas stream, a gas analyser detecting hydrogen sulfide concentration in the outcome stream has been installed. When the gas analyser detects reduced efficiency of the gas stream purification at the level of 95 %, automatic washing of the system followed by refilling from hopper 5 following the established optimal volume of phosphogypsum filling occurs.

Chapter 3

Anaerobic conversion under biogenic components removal from wastewater and phosphogypsum in the aquatic ecosystem protection biotechnologies

3.1 The problem of phosphate removal from wastewater

The possibility of co-processing of wastewater, sewage sludge, and phosphogypsum in the anaerobic condition with the precipitation of the heavy metal with biogenic hydrogen sulfide was substantiated in the department of applied ecology, Sumy State University (Chernysh and Plyatsuk, 2014–2016). And the current investigation is conducted with the process of phosphorus removal from sewage sludge and phosphogypsum.

Existing methods of removal of phosphorus compounds from wastewater and its sludge can be summarized as follows: biological removal of phosphorus, chemical precipitation, and thermal treatment of sewage sludge (SS) with the subsequent reagent removal of phosphorus. At present, the possibility of phosphorus removal at the wastewater treatment plants is limited.

One of the ways of raising the efficiency of biological treatment plants is to form highly concentrated quickly settling sludge in bioreactors. Biological removal of phosphorus compounds with active sludge together with denitrification upon alteration of aerobic and anaerobic conditions at wastewater treatment plants is becoming more important. It is important to maintain the normal functioning of anaerobic zones in aerotanks.

To develop an integrated technology for phosphogypsum recycling in the process of biochemical wastewater treatment and sewage sludge purification, it is necessary to carry out the experimental modelling of the autocatalysis kinetics of the anaerobic stabilization after adding dehydrate phosphogypsum into the system to stimulate phosphate ions release from the wastewater and its sludge. The implementation of biochemical technologies of secondary resources management includes fixing of modified phosphogypsum granules in the aerobic-anaerobic mineralization system to stimulate the development of the required ecological and trophic bacterial groups. Besides, the modelling of the phosphorus ion release from sewage sludge and the biochemical binding of toxic components (especially of heavy metals) takes place. This will allow to development of technological systems for integrated waste management with receiving useful environmentally friendly products (phosphate fertilizer, biogas, organic-mineral biogenic composite).

3.2 Transformation compounds of heavy metals (HM) to insoluble form and phosphorus recovery from wastewater under anaerobic stabilization with phosphogypsum

In the mineral composition of the raw sewage sludge were found such compounds: iron phosphate hydroxide - $Fe_4(PO_4)_3(HO)_3$, compounds of aluminum and silicon as a double layer of a hydrated complex of potazium illite-montmorillonite – $KCu_4(Si, Al)_8P_2O_7 \cdot 5H_2O$ (primary sewage sludge) and anorzite – $(Ca, Cr)Al_2Si_2O_8$ (activated sludge), the structure of the crystal lattice which includes atoms HM (impurities).

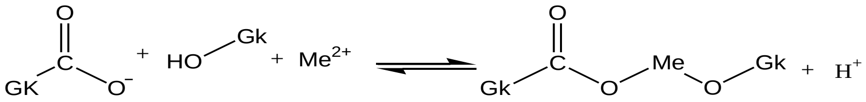
Iron compounds of type $Fe_4(HO)_3(PO_4)_3$ was established by the results of X-ray diffraction analysis of sludge (solid phase) before it processing, that is one of the features of their mineral composition, which can be represented as follows:

$$Fe^{3+} + yH_2O + xPO_4^{3-} \rightarrow Fe(OH)_y(PO_4)_x + yH^+ \tag{3.1}$$

Studies on the SEM structure of sewage sludge samples showed the presence of an iron hydroxide film on the surface of sludge particles, from which the phosphate ions are bound (Chernysh Y., Plyatsuk L., 2018).

In the active sludge (excess and reverse) the mechanism of phosphates sorption is represented by the process of phosphorus-containing compounds accumulation in the microorganism’s cells. Increased phosphorus content in active biomass is since under aerobic conditions bacterial cells absorb phosphorus from the solution and accumulate in themselves. The phosphorus compounds, they are also presented of sludge from silt maps were presented with the compounds of aluminum and silicon in the form of a hydatic complex of potassium $K_2O(Al_2O_3)_{1.73}(SiO_2)_{0.37}(P_2O_5)_{2.98}H_2O$.

Also, various organic complexes with HM was presented in sewage sludge. HM included in the form of completion in the organic matter:



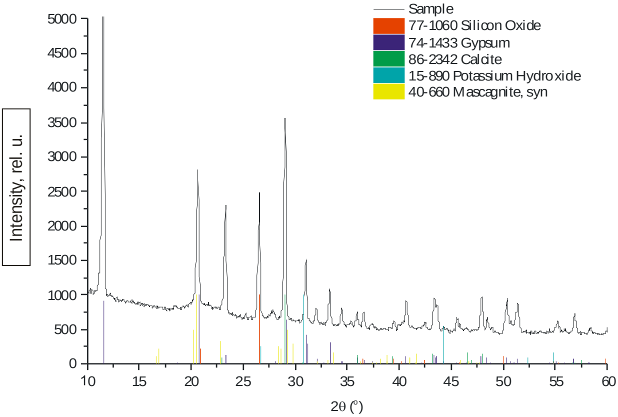
(3.2)

where: Gk is humic acid.

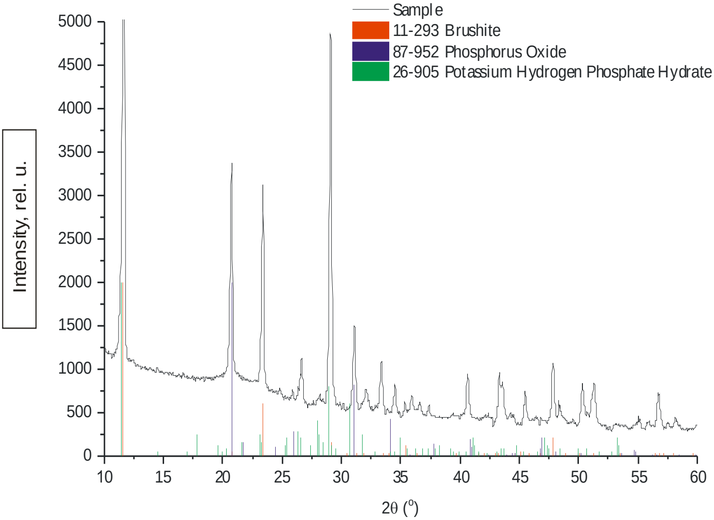
To prepare for cultivation in the aeration absence, sewage sludge was subjected to autocatalytic biodegradation in a container without access to air for 14 days at the temperature of 36 °C. Microscopic studies of the fermented sewage sludge did not reveal the simplest, there are single cysts in the sludge. Subsequently, the sludge was placed in two bioreactors: the control (without sulfates, which is the source of electron acceptors for sulfate-reduction bacteria) and experimentally with the phosphogypsum addition ($CaSO_4 \cdot 2H_2O$) (not less than 2.5 g / dm^3, depending on the primary activity of sulfate-reduction bacteria), the initial concentration of biomass as the sludge dose was 6.5 g / dm^3.

Reactions oxidation of organic matter during AMD provoked output HM to a liquid phase, after that metals ions reacted with H_2S. After biosulfidogenic treatment sewage sludge purchased black color and characteristic odor of decomposed organic matter. By separating the liquid fraction from the solid product was obtained, which in dried form acquired grayish brown.

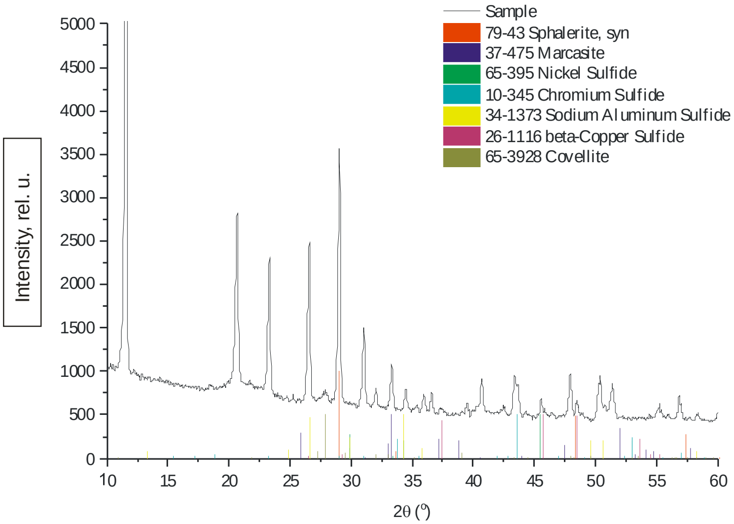
Among the major mineral components of the final product of the biosulfidogenic treatment are: quartz, or amorphous silica – SiO_2, potassium hydroxide (potassium hydroxide) – KOH and potassium hydrogen phosphate hydrate – $K_2H_2P_2O_7 \cdot 1/2H_2O$, brushite – $Ca\,PO_3(O\,H) \cdot 2H_2O$, phosphorus oxide – P_2O_5, calcite – $CaCO_3$, ammonium sulfate (mascagnite), sulfides of HM such as iron sulfide (marcasite), zinc sulfide (sphalerite), copper sulfide (covellite), nickel sulfide, etc. (Fig. 3.1: a, b, c).



a) main compounds



b) phosphorus compounds



c) metals sulfides

Figure 3.1 – The spectrum of mineral compounds of the digested mixture of sewage sludge and phosphogypsum (after treatment)

In general, these processes can be described using such chemical reaction:

$$CaSO_4 \cdot 2H_2O + 2NH_3 + CO_2 \rightarrow CaCO_3 \downarrow + (NH_4)_2SO_4 + H_2O \quad (3.3)$$

This process indicates the ammonia passage, as well as denitrification before the sulfate reduction start, that impact on slows down the sulfate-reduction bacteria growth. But at the same time, it contributes to a more complete primary removal of unbound oxygen, hence the creation of obligate anaerobiosis, which is important for the development of the sulfate-reduction microorganism's association.

We compared the results of diffractometry of raw sewage sludge and the final product of biosulfidogenic treatment during anaerobic conversion. Thus several waste biochemical transformations became such as:

- Biological recovery of phosphates; a significant portion of the liberated phosphate ions chemically bind with calcium and partially into the liquid phase;
- Due to the evolution of carbon dioxide in the system was formed of calcium carbonate;
- Ammonia, which is released during the decomposition of protein compounds, interacted with sulfate ions to form ammonium sulfate;
- Complex compound with HM during anaerobic stabilization destroyed and HM ions passed reacted with biogenic sulfide and came to a stable form of metal sulfides.

Thus, HM mobility was limited by the formation of metal sulfides and caused their minimum flow into the soil pore. So, HM did not migrate to the system “soil – plant”.

Centrifugation of sewage sludge (before and after treatment) showed a change in the phosphorus compounds content in the liquid phase. It was found that the release of phosphate ions into the liquid phase and the destruction of metal-phosphate complexes occurred under the influence of biogenic hydrogen sulfide (Figures 3.2 and 3.3) (Chernysh, 2014).

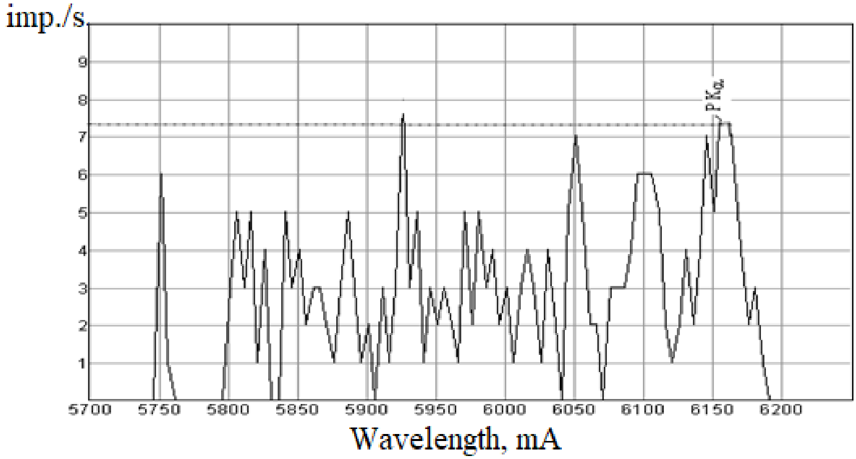


Figure 3.2 – IR spectrum of liquid phase sample of the sewage sludge before anaerobic stabilization together with phosphogypsum under sulfate reduction conditions

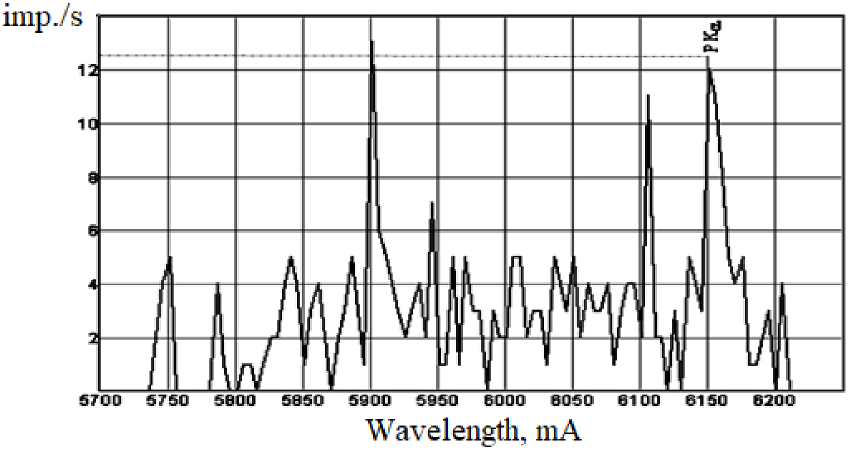


Figure 3.3 – IR spectrum of liquid phase sample of the sewage sludge after anaerobic stabilization together with phosphogypsum under sulfate reduction conditions

Thus, a series of experiments was conducted to determine the mechanisms for the allocation of phosphate ions from sewage sludge under the process of dissimilation sulfate-reduction, which was stimulated by the addition of dihydrate phosphogypsum. This processing can be implemented in technological solutions for the aquatic ecosystems’ protection.

3.3 Investigation of the influence of the phosphogypsum dose and the COD loading on the dephosphation process under sulfate reduction conditions

The dose of phosphogypsum introduced into an anaerobic bioreactor, as a source of sulfate and other macro- and microelements (Ca, P, K, etc.) for the stimulation of sulfatereduction bacteria growth, and affected the amount of phosphate removal. The decrease of phosphogypsum concentration had a close correlation with the release of phosphate ions (Fig. 3.4).

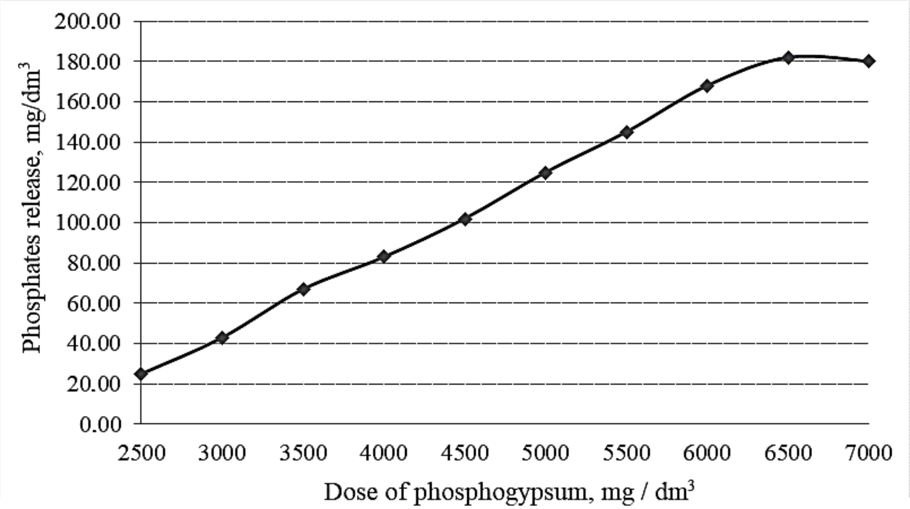


Figure 3.4 – The relationship between phosphates release and the concentration of phosphogypsum decrease under sulfate reduction conditions

It should be noted that this process is conducted with the biochemical conversion of phosphogypsum under anaerobic conditions. The obtained dependence of the process of phosphates release (M (Y)) on the concentration of phosphogypsum (X_1) was approximated by the regression equation:

$$M(Y) = 0.0283X_1^2 + 20.014X_1 + 2137.9 \text{ } (R^2 = 0.984) \qquad (3.4)$$

According to our research (Fig. 3.6) increasing phosphogypsum concentration up to 7000 mg / dm^3 wasn’t impact on phosphate increase in solution was observed. Thus, a rational dose of phosphogypsum was 6500 mg / dm^3.

Phosphate concentration in anaerobic bioreactors varied in a relatively wide range from 32 mg PO_4^{3-} / dm^3 to 67 mg PO_4^{3-} / dm^3. The concentration of phosphate ions in the outflow was observed in the range from 112 mg PO_4^{3-} / dm^3 to 179 mg PO_4^{3-} / dm^3 (Fig. 3.7). This means that as a result of the microorganism’s activity, an increase in the phosphates concentration in the soluble 2.5–5.0 times was observed at the stimulating action of the phosphogypsum additive.

Accordingly, the degree of phosphate ions increases (DI_P) in solution was calculated by the formula:

$$DI_{\mathrm{P}} = \frac{(C_1 - C_0)}{C_1} \cdot 100\ \%, \tag{3.5}$$

where: the concentration of phosphates after (C_1) and before treatment (C_0) in the liquid phase.

In Fig. 3.5 was shown that the degree of phosphate ions increase changed from 62 % to 80 %.

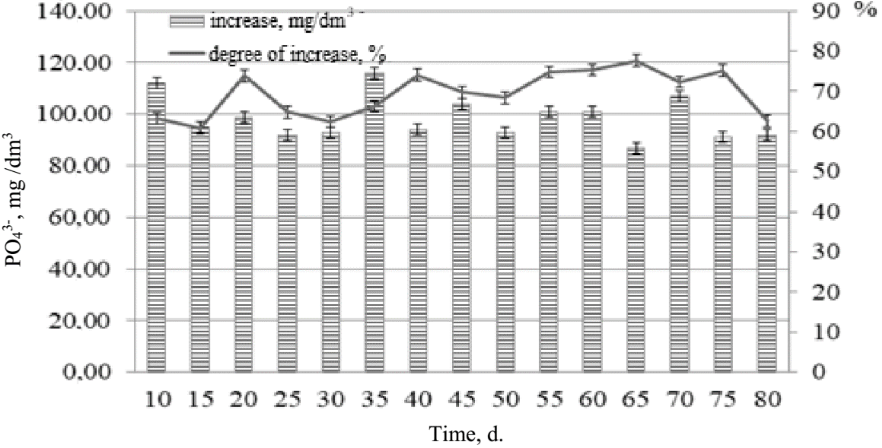


Figure 3.5 – Increase in the concentration of phosphate in the liquid phase after the treatment under sulfate reduction conditions with phosphogypsum processed (Chernysh and Plyatsuk, 2018)

The presence of a source of carbon and energy affects the biochemical process of separating phosphates from wastewater and sewage sludge. Such as in anaerobic conditions, phosphate-membrane microorganisms convert organic compounds and convert slow-acting phosphorus compounds into a soluble form (*Bacillus (B. megaterium), Penicillium*, *Aspergillus*, etc.).

The process of anaerobic phosphate release was enhanced by introducing phosphogypsum into the system and, accordingly, by stimulating the development of sulfatereduction bacteria that secrete products of their metabolism in the medium and indirectly stimulate the activity of phosphate-mobilizing microorganisms and intensify the allocation of phosphate ions in solution.

Together with the release of phosphates, the removal of the organic substrate was measured, which was expressed in changes of the COD. The initial COD of water extraction (1: 1) of sewage sludge treated in an anaerobic reactor was changed from 300 mg O_2 / dm^3 to 376 mg O_2 / dm^3. The final COD of a similar flow filtrate was much lower and was changed of values 96–147 mg O_2 / dm^3.

The maximum COD (181 mg O_2 / dm^3) was measured in the sludge at the initial stage of the dephosphotation. When the induction stage was passing, after 5 days in the stationary mode of operation (10–15 days) all measurements showed a value of COD below 145 mg O_2 / dm^3.

The effectiveness of COD reduction (fig. 3.6) can be calculated by the formula:

$$\mathrm{E}_{COD} = \frac{(COD_0 - COD_1)}{COD_0} \cdot 100\ \%, \qquad (3.6)$$

where the value of COD before (COD_0) and after (COD_1) treatment in the liquid phase.

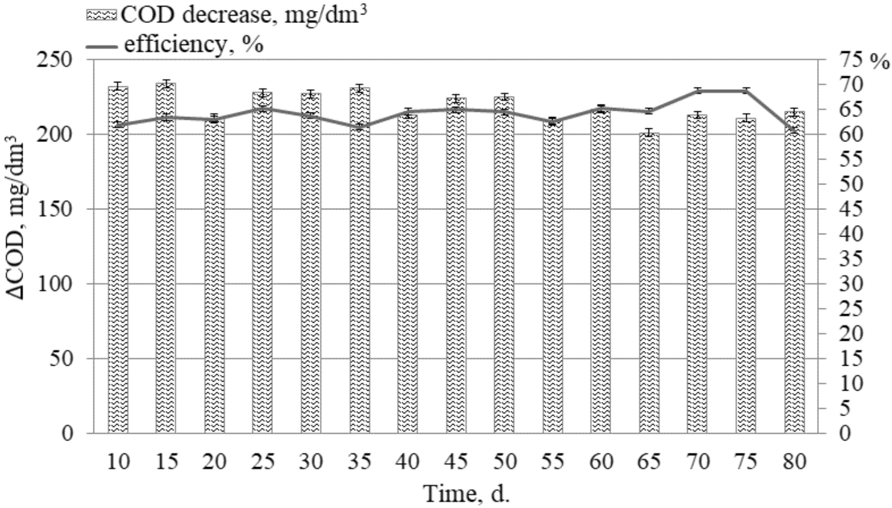
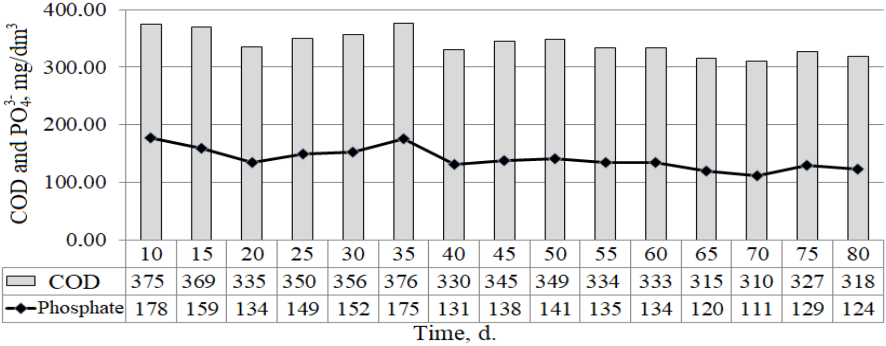


Figure 3.6 – Determination of the level of COD reduction after anaerobic conversion sewage sludge and phosphogypsum

The effect of organic compounds removal was expressed as a decrease in COD after the induction phase and it was not less than 60–70 % (Fig. 3.6). The value of COD in output was 1.52 to 3.19 times lower than the input. The variation of the measured values of the COD in the sewage sludge is reflected in changes in the concentration of phosphates (Fig. 3.7).



	10	15	20	25	30	35	40	45	50	55	60	65	70	75	80
▭ COD	375	369	335	350	356	376	330	345	349	334	333	315	310	327	318
—◆—Phosphate	178	159	134	149	152	175	131	138	141	135	134	120	111	129	124

Figure 3.7 – Dynamics of COD before anaerobic conversion and phosphate concentration after treatment

Fig. 3.7 shows that the amount of CODs of sewage sludge applied for treatment affects the level of phosphate-anion release in the solution.

For the understanding, the synergistic effect of the combined loading effect on the COD and the doses of phosphogypsum (PG) on the metabolic activity of anaerobic microorganism groups in the phosphate release process, the effect of the ratio of COD to the content of phosphogypsum dose on phosphate recovery was investigated (Fig. 3.8).

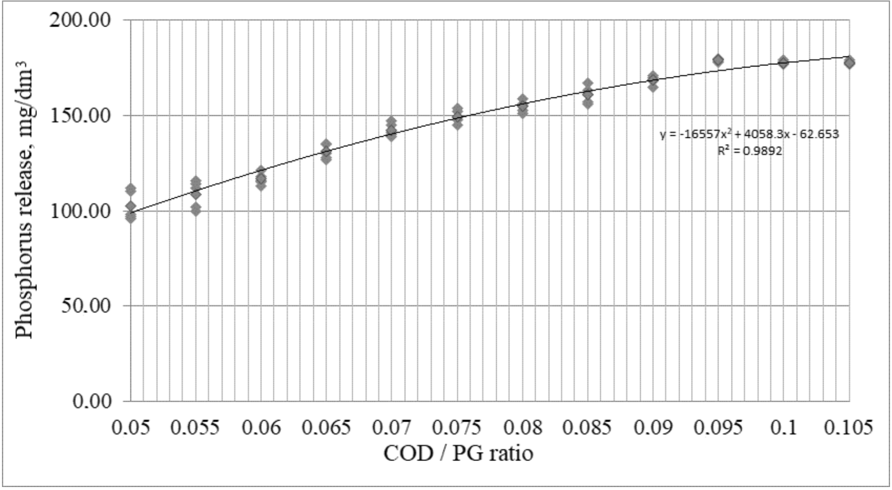


Figure 3.8 – Influence on the process of phosphates release of the COD / PG ratio (Chernysh and Plyatsuk, 2018)

Thus, according to the results obtained (Fig. 3.8), the effect on the release of the phosphate (M (Y)) of the ratio of COD / PG (X_2) is approximated by the regression equation:

$$M(Y) = -16557\, X_2^2 + 4058.3\, X_2 - 62.653 \;(R^2 = 0{,}9892) \qquad (3.7)$$

Along with an increase in the ratio of COD / PG to 0.1, an increase in the phosphate-ions in solution was observed. Further, increase the concentration of phosphate ions isn’t affected by an increase in the ratio of COD / PG.

3.4 Substantiation of biochemical and physico-chemical processes of intensification of anaerobic extraction of nutrients from wastewater and sewage sludge by phosphogypsum use in aquatic ecosystems protection biotechnologies

3.4.1 The biochemical reaction of the anaerobic microbiological degradation (AMD) under biosulfidogenic conditions

The final stage of AMD is determined by the possibility of microorganisms using the various terminal electron acceptors. Preference electron acceptors using determined by thermodynamic factors. The firstly reactions begin which have the greatest energetic effect. Reduction of sulfur to H_2S begins the first then reduction of carbon dioxide to CH_4 as confirmed in (Zavarzin and Kolotilova, 2001). The decomposition reaction of VFA to acetate and hydrogen is thermodynamically unfavorable and can provide microbial growth only with the very low concentration of reaction products. Thus, rapid and complete removal of H_2 must be in the microbial association.

If sulfates are present, SRB such as *Desulfovibrio desulfuricans* multiply. Their multiplication or reproduction often requires the use of hydrogen and acetate - the same substrates used by methane-forming bacteria (Fig. 3.9).

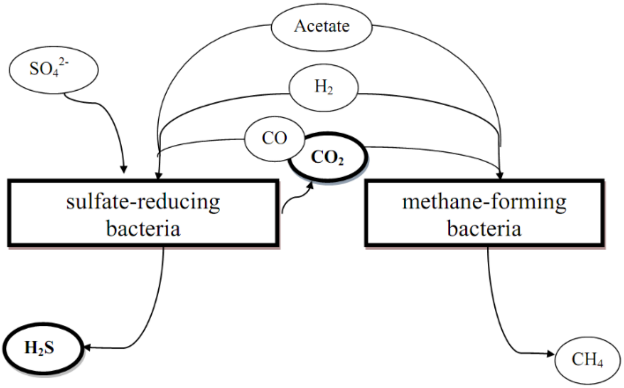


Figure 3.9 – Groups of bacteria within the anaerobic digester which compete for the same substrate and electron acceptor (Plyatsuk and Chernish, 2014)

SRB obtain hydrogen and acetate more easily than methane-forming bacteria. It is due to a higher SRB affinity for the substrate (Gerardi, 2003). In the microbial association SRB has the ability to the acetate and H_2 remove to a lower threshold than methane-forming bacteria (Zavarzin and Kolotilova, 2001):

methanogenic – 100–10 p.p.m. H_2 (gas)
SRB – 10–1 p.p.m. H_2 (gas)

Thus, sulfidogenesis is more energetically favorable for the microbial association than methanogenesis.

Consequently, the process of methanogenesis is not dominant in anaerobic conditions with a constant source of sulphate. The sulfidogenic community of microorganisms is will be developed in the bioreactor.

Note again, the electrons acceptor for SRB were constantly introduced to the medium of the bioreactor during investigations. After that, methanogenesis is not dominated and sulfidogenesis was becoming the fourth step of AMD. This method of sludge treatment is not the basic purpose of biomethane production. But also, methane could be present in the gaseous phase in that process too.

Methanogenesis is usually the last stage of AMD in biogas technology.

The process of AMD was studied for the aim of sedimentation of HM by biogenic hydrogen sulfide. Thus, we studied the AMD under sulfidogenesis. It is not similar to methane fermentation.

Biogenic hydrogen sulfide is the product of SRB belonging to generic taxon *Desulfovibrio, Desulfobacter, Desulfomaculum (Desulfosporosinus), Desulfococcus,* etc. (Karnachuk, 2006).

Thus, according to substratum-product transformation during AMD under bio-sulfidogenic conditions we formed biochemical equations of wastes detoxification on the final digested stage (Plyatsuk and Chernysh, 2016):

$$\begin{cases} CH_3COOH + Y_{X_1/S}Y_{X_1/SO}SO_4^{2-} + Y_{X_1/S}Y_{X_1/NH_3}NH_3 \rightarrow \\ Y_{X_1/S}Y_{X_2/S}C_5H_7O_2N + Y_{H_2S/S}Y_{H_2S/X_1}H_2S + Y_{X_1/S}Y_{CO_2/(X_1+X_2)}CO_2 + Y_{X_2/S}Y_{CH_4/X_2}CH_4 + a1_H\,H_2O, (3.8) \\ C_5H_7O_2N + Y_{X_1/SO}SO_4^{2-} + a2_H\,H_2O \rightarrow Y_{H_2S/X_1}H_2S + Y_{CO_2/X_1}CO_2 + Y_{NH_3/X_1}NH_3, (3.9) \\ CH_3COO^- + \frac{3}{2}H^+ + SO_4^{2-} \rightarrow \frac{1}{2}H_2S + \frac{1}{2}HS^- + 2CO_2 + HCO_3^- + H_2O, (3.10) \\ nMeS + mS^{2-} \rightarrow (Me_nS_{n+m})^{2m-}\downarrow, (3.11) \\ 8HS^- + 9Me^{n+} + SO_4^{2-} \rightarrow 9MeS\downarrow + 4H_2O. (3.12) \end{cases}$$

Here $Y_{X_1/S}$ and $Y_{X_2/S}$ are yield coefficient, the biomass of SRB and methane-forming bacteria, respectively, produced per mass of substrate utilized (g·g^{-1}); $Y_{X_1/SO}$ is yield coefficient, the biomass of SRB produced per mass of sulfates utilized (g·g^{-1}); Y_{X_1/NH_3} is yield coefficient, the biomass of SRB produced per mass of ammonia utilized (g·g^{-1}); Y_{H_2S/X_1} is yield coefficient, the mass of hydrogen sulfide produced by biomass of SRB (g·g^{-1}); $Y_{CO_2/(X_1+X_2)}$ is yield coefficient, the mass of carbon dioxide produced by biomass of SRB and methane-forming bacteria (g·g^{-1}); Y_{CH_4/X_2} is yield coefficient, the mass of methane produced by biomass of methane-forming bacteria (g·g^{-1}); Y_{CO_2/X_1} is yield coefficient, the mass of carbon dioxide produced by biomass of SRB (g·g^{-1}); Y_{NH_3/X_1} is yield coefficient, the mass of ammonia decomposition of biomass SRB (g·g^{1}); *Me* is a generic notation of heavy metal ions, *MeS* is a generic notation of metallic sulfides, $a1_H$, $a2_H$ are stoichiometric coefficients.

The acetate's decomposition process to hydrogen sulfide, methane, and carbon dioxide for the synthesis of microbial biomass ($C_2H_7NO_2$), and decay of the biomass are presented in equations (3.8) and (3.9). Since the AMD was conducted under biosulfidogenic conditions in the equation (3.10) is shown the process of sulfate reduction dissimilation of acetate decomposition without methanogenesis. The equations (3.11) and (3.12) are presented the chemical reactions of HM ions precipitation by biogenic hydrogen sulfide.

Hence, reaction oxidation of the metal compounds to sulfates is limited in conditions of AMD treatment. Heavy metals are fixed in the form of insoluble sulfides in the separated solids fraction of the fermented sludge mixture. This process is interesting in the possibility to use sewage sludge as organic-mineral fertilizer.

We studied the system of AMD within the HM precipitation by biogenic hydrogen sulfide. In that case, mainly biogenic gas consists of hydrogen sulfide. However, methane could be present in the gaseous phase too.

Thus gaseous phase was produced in bio-sulfidogenic treatment we will be called “microbially generated gas” (MG gas) for the prevention of further incorrect comparison.

Hence, after sewage sludge detoxification in the AMD process, HM is fixed in the insoluble sulfides form in the separated solids fraction of the fermented mixture. This process is interesting in the possibility to use treated sewage sludge and phosphogypsum as organic-mineral fertilizer.

3.4.2. Description of mechanisms for the phosphorus release from sewage sludge with phosphogypsum use under anaerobic stabilization

The other way of using sulfatereduction is the biological release of phosphates.

Phosphorus is an essential nutrient with the lowest reserves and phosphorus cannot be substituted by other elements.

Based on the results of the studies, the scientific principles concerning the mechanisms of phosphorus removal from sludge and sewage sludge in the process of anaerobic stabilization under sulfate reduction along with phosphogypsum have been formulated. An important feature of which is not only the phosphate ions release from cells of microorganisms community of the active sludge occurring during anaerobic fermentation but also the destruction of metal-phosphate complexes of the sewage sludge (SS).

It should be noted that a vast majority of heterotrophic microorganisms contained in wastewater and active sludge can accumulate phosphorus. In the process of anaerobic stabilization due to the active release of volatile fatty acids the phosphate ion diffusion from a microbial cell occurs. Besides, the leasing of these microbial cells with the release of polyphosphates in the process of anaerobic destruction is possible, which has been studied in (Playtsuk and Chernysh, 2014).

Fig. 3.10 represents the model of substance flows of matter associated with the process of phosphate removal from SS during joint anaerobic stabilization with dehydrate phosphogypsum. They characterize the dynamics of the development of sulfate reducer community in the bioreactor space, taking into account the process of returning a part of the fermented substrate into the technological system and the behaviour of flocculation processes in SS biomass.

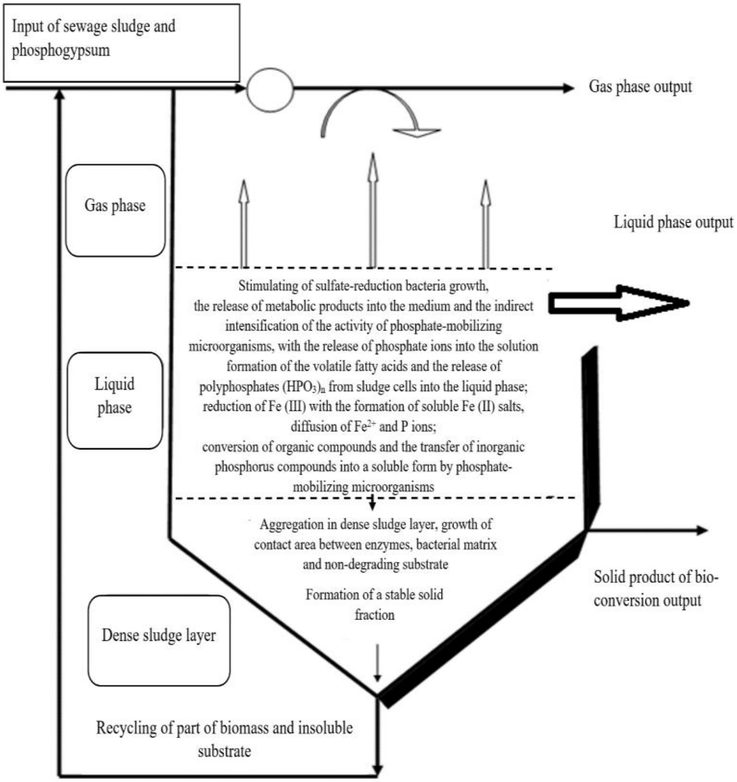


Figure 3.10 – Block diagram of spatial distribution in the bioreactor during dephosphatation of SS together with phosphogypsum under the condition of dissimilation sulfatereduction (Chernysh et al., 2018)

It should be noted that for inorganic compounds the thermodynamic sequence of oxidation-reduction potentials as desired acceptors is strictly kept to. Therefore, the sequence of use of electron acceptors in the process of biochemical reactions is as follows: nitrate reduction or denitrification → recovery of sulphur compounds to hydrogen sulfide, which is associated with phosphorus release from cells of phosphate accumulating microorganisms of the sludge → reduction of carbon dioxide to methane. For the best sulfate reduction, it is necessary to carry out the denitrification of wastewater and sludge in a separate biotreatment unit previously.

In general, the patterns of biochemical transformations of phosphorus in the anaerobic SS and phosphogypsum conversion are as follows:

1. mobilization of phosphorus from its mineral compounds, in particular, iron (III) hydroxide which bound P, appears in the environment deprived of oxygen supply (anaerobic bioreactor), and Fe (III) is reduced by organic matter found in SS in the process of microbial reduction. Since Fe (II) salts including $Fe_3(PO_4)_2$ are soluble then ions of Fe^{2+} and P begin to diffuse;

2. under anaerobic conditions due to fermentation of organic substances the formation of volatile fatty acids and release of polyphosphates $(HPO_3)_n$ from the sludge cells into the liquid phase occur.

It should be noted that phosphate ions having passed into the solution, react with calcium ions forming low-soluble compounds.

The mechanisms of anaerobic stabilization under sulfate reduction conditions with the phosphorus recovery can be described with the following biochemical reactions of phosphate removal:

$$2CH_3COOH + (HPO_3)_n + H_2O \rightarrow (C_2H_4O_2)_2 + a_{PO}PO_4^{3-} + a_H H^+, \quad (3.13)$$

$$Fe_3(PO_4)_2 + 3SO_4^{2-} + 6C_{орг.} \rightarrow 3FeS \downarrow + 2PO_4^{3-} + 6CO_2, \quad (3.14)$$

where $C_{org.}$ – organic substrate; a_{PO}, a_H – stoichiometric coefficients.

The biochemical equations describe the terminal stage of SS organic matter conversion. When introducing macro- and microelements (phosphogypsum) into the source system, the process of sulfate reduction is intensified and, consequently, the development of ecological and trophic groups of bacteria performing it, which corresponds to the auto-selection principle. Herewith, during the sulphate reduction, polyphosphates $(HPO_3)_n$ are released from sludge cells into the liquid phase, which is more simply described in equation

(3.13) where $(C_2H_4O_2)_2$ is a reserve organic matter that is present in SS liquid phase.

Equation (3.14) describes the reduction reaction of iron (III) phosphate under anaerobic conditions to ferrous iron with the subsequent binding of Fe^{2+} ions in the sulphide form. It should be noted that the ions of metals actively interact with hydrosulfide and sulfide ions while phosphate ions are emitted into the liquid phase from which phosphorus can be recovered in the form of its salts with the subsequent use as a fertilizer.

During the anaerobic stabilization under conditions of activity of the sulfate reducing agent the organic matter (carbohydrates, organic acids) is subjected to dehydrogenization; hydrogen is transferred on sulfates, sulfites, or thiosulfates, which are reduced to hydrogen sulfide. Hydrogen sulfide actively interacts both with the solid phase of the sludge and with cations present in the liquid phase. During the anaerobic conversion, the mineralization of the organic component of the sewage sludge and the transformation of chelate complexes with the heavy metals to soluble organic compounds takes place. The increase in the mobility of heavy metals occurs and they pass to the liquid phase of SS with the subsequent precipitation in the form of an insoluble fraction, which can significantly affect the properties of SS. Also, heavy metals forming salts with the compounds of SS organic component, are exchanged for calcium ions according to the mechanism of ion exchange on the surface of phosphogypsum. Thus, in the process of anaerobic stabilization, organic chelate complexes with heavy metals are destroyed and complex isomorphic compounds of sulfides of metals: sulfides of iron (marcasite), zinc (sphalerite), nickel, etc., and carbonates are formed. Comparative elemental and phase analysis of the samples of the end product of treatment is shown in table 3.1.

Table 3.1 – Results of SS samples analysis of solid-phase together with phosphogypsum after anaerobic stabilization

Element	Phase composition
Si P S Ca K	JCPDS 77-1060 (Quartz)
Mg Al Fe	JCPDS 74-1433 (Gypsum)
Ni Cu Zn Cr	JCPDS 26-905 (Potassium Hydrogen Phosphate Hydrate)
	JCPDS 11-293 (Brushite)
	JCPDS 40-660 (Ammonium Sulfate)
	JCPDS 86-2342 (Calcium Carbonate)
	JCPDS 79-43 (Zinc Sulfide)
	JCPDS 37-475, 74-1051 (Sulfides of Iron)
	JCPDS 26-1116, 65-3928 (Sulfides of Cuprum)
	JCPDS 65-395 (Nickel Sulfide)
	JCPDS 10-345 (Chromium Sulphide)
	JCPDS 89-1460 (Calcium Aluminum Silicate)

Precipitation of heavy metals in the form of a complex sulphide fraction is an important factor for the stable functioning of sulfate reducer association in the bioreactor.

The analysis of the biochemical processes proceeding during the anaerobic stabilization of the sewage sludge/wastewater with dehydrating phosphogypsum is incorrect from the point of view of their simple summation. They define a new level of self-organization of the system receiving new qualitative characteristics that determine the environmental safety of the sewage sludge and wastewater processing product. Therein lies the synergistic effect “2 + 2 = 5”, which means the desire to achieve such results that are not “a zero-sum of summands”. And every biochemical reaction proceeds in coordination with various factors and other reactions (chemical, physical, biological) in the system determining each other.

Consequently, the most optimal variant of development of the system on self-organization principles is formed which is appropriate to natural environmental systems.

The process of anaerobic stabilization forms an organo-mineral product which after the separation from the liquid fraction and drying has a grey-brown colour and its consistency reminds of aggregated soil particles (Fig. 3.11).

Figure 3.11 – The general view of the organo-mineral composite material based on phosphogypsum and sewage sludge after the anaerobic stabilization under conditions of dissimilating sulfate reduction (in the biocomposite)

Such components as quartz or amorphous silica – SiO_2; potassium hydroxide KOH; brushite – $CaPO_3(OH) \cdot 2H_2O$; calcite – $CaCO_3$; ammonium sulfate (mascagnite); iron sulfide (marcasite), zinc sulfide (sphalerite), copper sulfide (indigo copper), chromium sulfide, etc., forming a complex sulphide group (Table. 3.1) are among the main components of the mineral constituents of the end product of the mixture anaerobic stabilization. The content of the main elements in the organo-mineral composite based on phosphogypsum and sewage sludge obtained after their joint anaerobic stabilization under conditions of dissimilating sulfate reduction is presented in Table. 3.2.

Table 3.2 – Content of the main elements in biogenic composites expressed as oxides, % (Chernysh, 2018)

SiO_2	**Al_2O_3**	**CaO**	**K_2O**	**MgO**	**Na_2O**
37.65	8.4	31.3	3.2	1.7	3.4
SO_3	**ZnO**	**Fe_2O_3**	**CuO**	**P_2O_5**	**NiO**
9.5	0.01	0.45	0.003	0.5	0.0014

The phosphorus compounds have been released in a liquid fraction and can be applied in combination with the obtained organo-mineral product. The results of the analysis of the mineral component of the end product confirm the main statements of the above mentioned biochemical model of the process.

3.5 Biochemical research of the phosphogypsum recycling in aquatic ecosystem protection biotechnologies

The monitoring of the anaerobic sludge has shown that in the biochemical system the inclusions of semi-transparent fine particles of the crystal structure have been observed in the flakes of sludge (Fig. 3.12), which indicates the formation of a solid mineral phase. Its analysis has shown the presence of calcium carbonate (calcite) (Chernysh et al., 2018).

Bacteria that acidify acetate dominate in the association of microorganisms, which is connected with incomplete oxidation of organic matter to acetates. Therefore, the removal of additionally formed volatile fatty acids (VFA) is of first-rate importance for the system stability. Otherwise, the acidification of the medium and decrease in pH to 4.0 takes place.

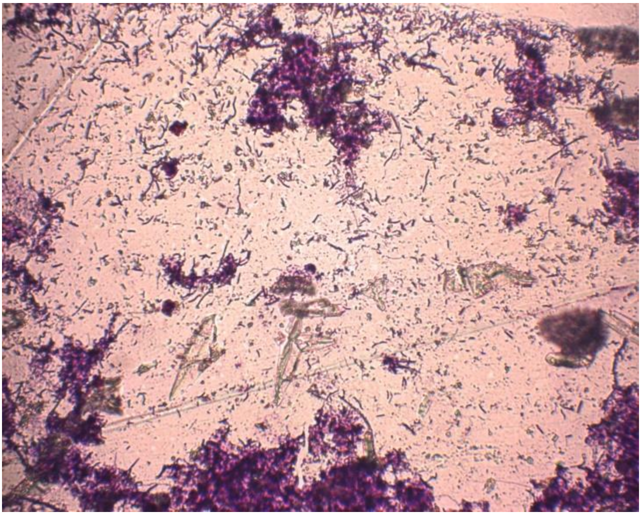


Figure 3.12 – Formation of crystal structures in the bacterial mass of anaerobic sludge. Light microscopy. Gram staining. Magnification: x100

Fig. 3.13 spatially presents the process of formation and disintegration of aggregates of anaerobic SS. A much larger number of small mineral particles in the sludge flakes from the pilot reactor in comparison with the control one has affected the sedimentation properties of the sludge, that is the bioflocculation properties. It should be noted that at the stage of disintegration the sludge index of the experimental SS has been 60–80 cm^3/g, while it has reached 250 cm^3/g on the control line.

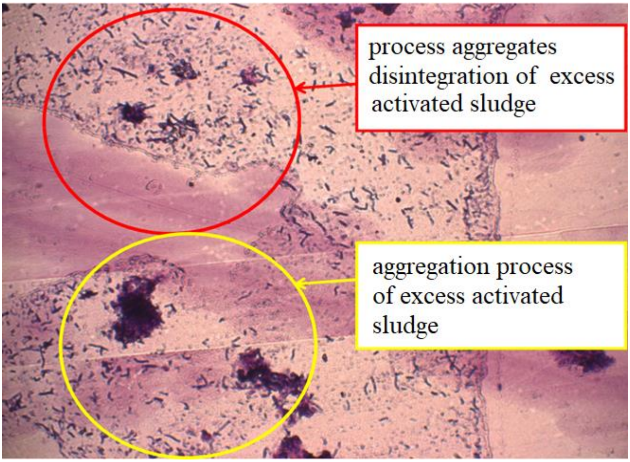


Figure 3.13 – Formation of bacterial aggregates in the process of anaerobic stabilization under conditions of sulfate reduction. Light microscopy. Gram staining. Magnification: x40

During the joint anaerobic stabilization of sewage sludge (SS) and phosphogypsum, an association of microorganisms is formed which contains a significant amount of sulfate reducers the number of which reaches $1.0 \cdot 10^{10}$ CFU/cm^3. Received micrographs of fermented sewage sludge have made it possible to observe various forms of cell aggregates with an increase in the microorganism growth rate (Fig. 3.14). Bacteria that acidify acetate dominate the association of microorganisms, which is connected with incomplete oxidation of organic matter to acetates. Therefore, the removal of additionally formed volatile fatty acids (VFA) is of first-rate importance for the system stability, primarily acetates. Otherwise, the acidification of the medium and decrease in pH to 5.0 takes place.

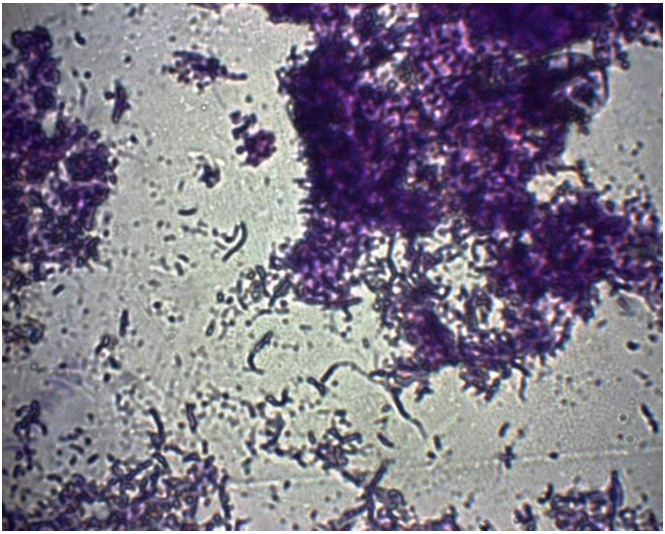


Figure 3.14 – Formation of bacterial aggregates under conditions of sulfate reduction. Light microscopy. Gram staining. Magnification: x100

In the field of view in Fig. 3.15. ruby-red acid-resistant spore-forming microorganisms related to *Desulfomicrobium sp.* can be seen.

Thus, anaerobic active sludge, obtained during anaerobic stabilization, is the association of microorganisms where bacteria that acidify acetate dominate.

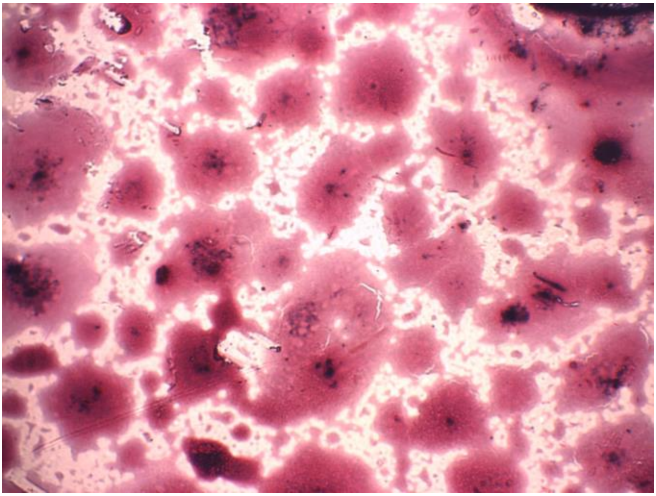


Figure 3.15 – Acid-resistant spore-forming bacteria of the association of microorganisms-destructors. Light microscopy. Ziehl-Neelson staining. Magnification: x40

In the process of anaerobic stabilization under conditions of sulfate reduction due to the active release of volatile fatty acids the diffusion of phosphate ions from the microbial cell occurs. Phosphorus in the cells is absorbed against the concentration gradient through an energy-dependent process with the help of specific protein transporters. The structural system performs absorption and release of phosphorus with a divalent metal cation, ion $MeHPO_4^+$ is transferred, where Me is Mg^{2+}, Ca^{2+}, CO^{2+}, Mn^{2+}, and the like. Also, the leasing of these microbial cells with the release of polyphosphates is possible.

Fig. 3.16 schematically represents the process of anaerobic conversion of SS along with dehydrating phosphogypsum with the disintegration and re-aggregation of the anaerobic sludge. This process is of first-rate importance for further development as syntrophic groups of microorganisms.

Near the equilibrium line of the solution CH_4 – gas CH_4 an active layer is formed in SS near the upper boundaries of which gas bubbles begin to form. The scheme depicts the process of phosphate ion release from the sludge combined with the release of volatile fatty acids and calcium and potassium ions with their inclusion into the SS organic structure.

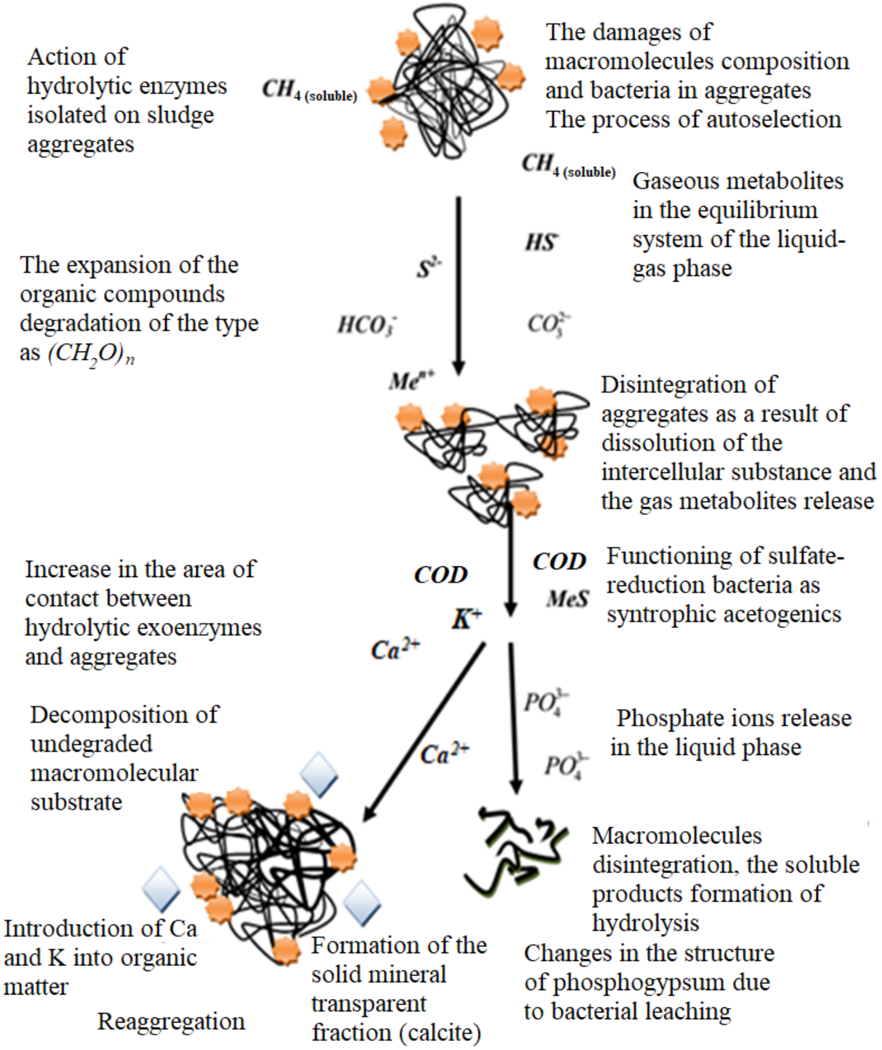


Figure 3.16 – The general scheme of factors involved in the process of anaerobic conversion of sewage sludge and phosphogypsum under sulfidogenic conditions (Chernysh et al., 2018)

One of the important processes is the aggregation of particles with the extension of the contact surface between the organic residue of the substrate, dead microbial biomass, and hydrolytic microorganisms. On the other hand, their maximum diameter has been limited by the disintegration of large aggregates. As a result, the relative position of individual bacteria in the aggregates has been changing continuously.

Under anaerobic conditions, SS microorganisms (especially in the absence of rival denitrifying agents) phosphorous accumulating bacteria are capable of removing organics, using energy produced as the result of the intracellular polyphosphate decomposition. As a result of this process, the release of orthophosphates into the environment takes place.

The replacement of metal complex in microbial cells by Ca^{2+} of phosphogypsum occurs in the process of ion exchange, which stimulates, in its turn, the exopolyphosphates activity, whereby the released orthophosphate forms complexes with cations Ca^{2+} which are "released" from the cell into the external medium with the help of transport systems.

In the course of sulfate-reduction metabolism, the release of hydrogen sulfide takes place which influences the activity of cell enzyme systems of the active sludge resulting in the release of metal-phosphate complexes, and sulfide and hydrogen sulphide precipitate together with metal ions in the sulfide solid fraction. The iron phosphates decompose and iron sulfide is formed. The phosphate ions are emitted into the liquid phase.

Qualitative characteristics of the SS microbial system components vary depending on two processes: the adaptation of a microorganism species or adaptive dynamics with the change of species in the functional group. It is important that the association of various ecological and trophic groups of microorganisms functions as a whole with the cooperative trophic relations that determine the perspectives of chemical interactions. The self-regulation of the microbiological system takes place. The artificial determination of such self-regulation for the implementation of the required treatment stage is marked by the control issue which cannot be considered as regulatory concerning certain types of bacteria in the system.

The microbial community can be schematically represented as a dynamic system (Fig. 3.17) with input and output parameters. The change of this system is based on the influence of external factors (temperature, nutrients supply, etc.) and relevant system response to these factors – qualitative (species diversity) and quantitative (biomass) changes, the release of certain products of biosynthesis, and the like). Furthermore, such ecological and biochemical system has a number of subsystems at the level of microorganism groups or their species with the dynamics of matrix development and the metabolite production, which determines the direction of reactions in the purification systems and the mode of functioning of the technological system. Correspondingly, these subsystems determine the internal parameters of the system.

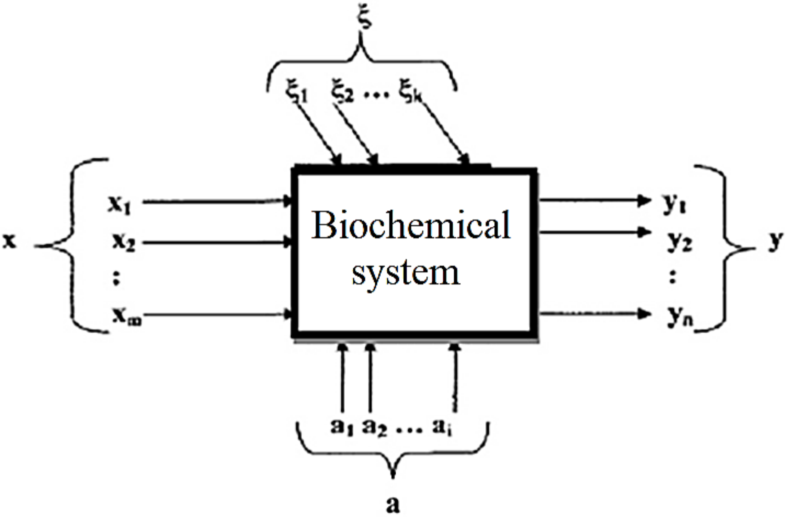


Figure 3.17 – Structural scheme of the microbiological system

In this case, the internal parameters of the system $a=(a_1, a_2,...a_i)$ are kinetic characteristics of the subsystems. There is a connection between the subsystems. Thus, the output parameters of one subsystem are a part of the input parameters of another subsystem. The change of the state of a subsystem-m at this moment at time t_2 depends on the change of the state of the subsystem-n at some time t_1. Microbiocenosis structurally changes in space and time, which is a process of succession. These dynamics are included in the “subordinate” structure of the trophic relations among populations of various microorganisms (subsystems) which can be represented as a system of biochemical reactions and corresponding “combined” kinetics. And parameters of uncertainties $\xi = (\xi_1, \xi_2, ... \xi_k)$, i.e. the part of input variables $x = (x_1, x_2, ..., x_m)$ and the internal parameters of the system play an important role in the bifurcation point when the association transits to another level of organization, for example, the change of the dominant species of microorganisms after the introduction of new electron acceptors into the system. In turn, the input vector changes $y = (y_1, y_2, ... y_n)$.

3.6 General regularities of synergistic changes in the microbial species composition in the system of anaerobic stabilization of wastewater and sewage sludge with phosphogypsum under sulfate reduction

In the process of phosphorus removal under sulfide genesis, the component characteristics of the biochemical silt deposit system change depending on the adaptive dynamics of species changes in a functional microbial ecological and trophic group. It is important that a microbial community function as a single entity with cooperative trophic links that determine the plan of chemical interactions by the thermodynamic characteristics. A microbial system regulates itself, so any artificial influence on this self-regulation should be reasonable and this kind of control cannot be considered regulatory in relation to certain strains of bacteria at their inoculation to the system.

Fig. 3.18 presents the pattern of Gibbs function change – free energy of formation of a biochemical system structure with a bioagent – by a dominant microbial species or an ecological and trophic group directly involved in the process of wastewater and silt deposit treatment. At that, the metabolism cycle of matter and energy is the driving force of non-self-generating processes.

From the point of view of “dark” non-self-generating processes, “thermodynamic forces” are the driving forces of self-organization and the development of biostructures of all hierarchical levels. It should be noted that Gibb's function of the formation of molecules and supramolecular structures as complex systems often coincides with Gibbs function of the formation of the corresponding natural systems under the condition of biotechnological systems.

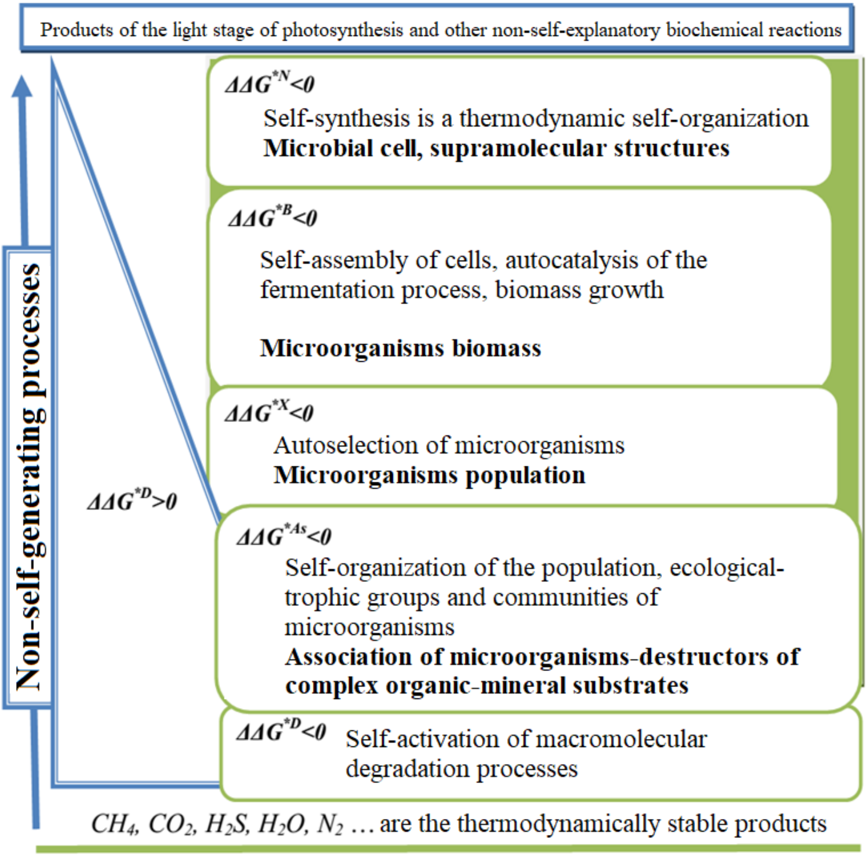


Figure 3.18 – Chart of the change of the Gibbs function for the formation of complex micro-ecosystems, ΔG^*_i, in the occurrence and biodegradation of chemical bonds in complex organic compounds (*D*), cells (*N*) and biomass (*B*), microorganism populations (*X*), associations (*As*)

Therefore, it should be considered not as a local closed system like a laboratory cultivator, but something of an industrial scale, for example, in the industrial process system of silt deposit treatment in waste treatment plants in large cities. Biogas production is enhanced by improving methanogenesis conditions - that is, by rationalizing pH value, the amount of macro and trace elements entering the system, etc. However, such changes indirectly affect the species composition of methanogens (hydrogenotrophic and acetoclastic) and trophically related microbial groups (homoacetogenic, sulphate-reducing, denitrifying bacteria, etc.). At that, the dominance of individual microbial species and genera in a community is determined by the thermodynamic benefits of using different electron acceptors (SO_4^{3-}, CO_2, CO, NO_3^- etc.).

Ecosystem stability cannot be maintained and secured if the law of internal dynamic equilibrium is violated. This law regulates environmental stress, provided that the “component balance” and “spatial balance” are not violated. It is exactly these “balances” that are the development standards of energy-efficient and resource-saving technologies and should be the basis of the development of environmental protection measures. The essence of this law lies in the fact that a biochemical system (artificial or natural) possesses internal energy, matter, information, and dynamic qualities that are mutually interconnected to such an extent that any change in one of these parameters results in other parameters, or the same one but in a different place or at a different time, undergoing associated functional and quantitative changes that retain the amount of matter, energy, information and dynamic parameters of the entire biochemical system. This provides the system with such properties as equilibrium maintenance.

3.7 Development of biotechnological solutions of phosphogypsum recycling in the wastewater treatment plant

The sequence of thermodynamic oxidation-reduction potentials as preferred acceptors is applied to inorganic compounds. The sequence of electron acceptor use includes:

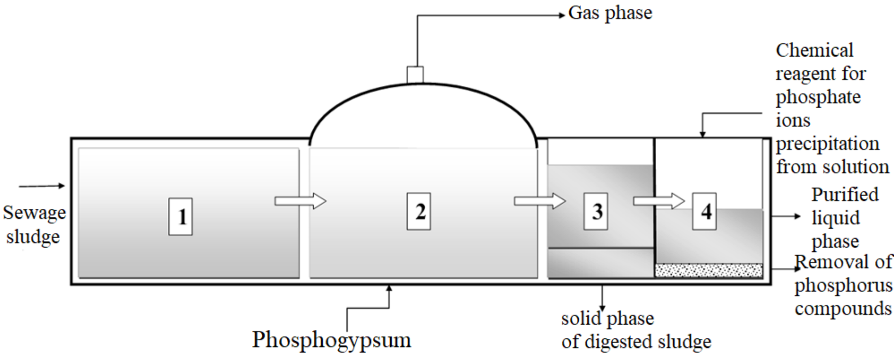
1. nitrate reduction or denitrification;
2. reduction of sulfur compounds or sulfate reduction associated with the process of phosphorus release from the cells of phosphorus-accumulating silt microorganisms;
3. reduction of carbon dioxide to methane or methanogenesis.

Thus, wastewater and sewage sludge should be denitrified in a separate biological treatment unit for the sulfate reduction process to progress better. Let us highlight several important provisions required to build a process flow diagram:

1. denitrification pretreatment of silt deposits with a portion of a wastewater stream improves anaerobiosis in the bioreactor (tank);
2. phosphorus removal is intensified with the introduction of phosphogypsum into the anaerobic tank, which contributes to the beginning of the sulfate reduction process on the principles of autoselection;
3. it is more reasonable to remove phosphate ions from the liquid phase in a separate treatment unit using a chemical deposition.

Fig. 3.19 shows the process flow diagram. Certain provisions of the technology are patented inventions; the design of the anaerobic unit is covered by a utility patent for “Unit for anaerobic treatment of organic waste”.

Denitrification is the first stage of the process that occurs in a biological unit that receives silt deposits with a portion of a wastewater stream. The second stage, anaerobic microbial degradation under sulfate reduction, occurs after silt deposits have been transferred from the biological unit into the anaerobic tank, where phosphogypsum introduction takes place and where gas is supposed to be withdrawn from the system to be later utilized.



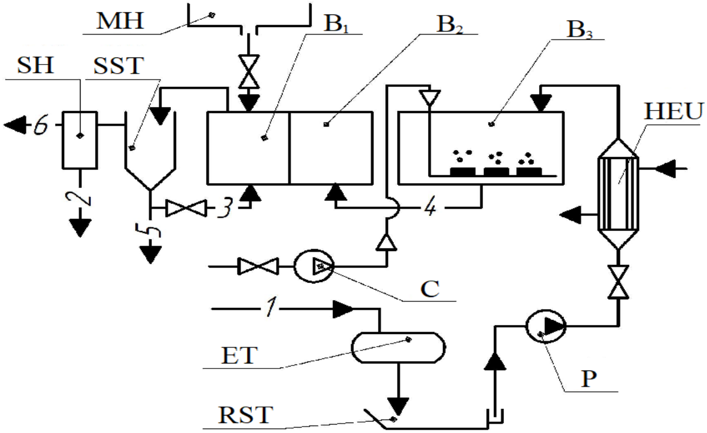
1 – denitrification unit; 2 – anaerobic reactor of dephosphotation treatment under the sulfate reduction conditions; 3 – sedimentation tank; 4 – reagent phosphorus removal unit

Figure 3.19 – Schematic of biological treatment of sewage sludge together with phosphogypsum

The third stage is phase separation during the settling process. The solid phase in the settling tank is removed from the system, while the liquid phase is directed into the phosphorus removal unit (fourth stage), where dissolved phosphorus compounds are precipitated with an agent, for example, like calcium hydroxide. Phosphate ions can be precipitated from the liquid phase of silt deposits with various reagents and later be used as agricultural fertilizers. It should be noted that the mechanism of phosphate sorption in silt deposits is complemented by the process of binding phosphate ions with metal hydroxides. Furthermore, phosphorus is included in the hydrated potassium complexes present in the sludge from silt maps.

The startup of the wastewater and sewage sludge treatment unit provides for the presence of a “starting” biomass amount of destructor microorganisms that act as an inoculum. The main stages of the startup period are as follows: cultivation of essential ecological and trophic groups with the help of fermenters under laboratory conditions; separation of viable biomass; introduction and immobilization of essential ecological and trophic microbial groups at anaerobic stages (B_2 та B_3) and the aerobic (B_1) stage that can serve as a separate aeration chamber tank, where inoculation of activated sludge from the existing urban treatment plants is possible; fillup with wastewater and sewage sludge, promotion of the development of a microbial community formed under autoselection by introducing dihydrate phosphogypsum; the transition of the unit to the flow-through mode.

Fig. 3.20 illustrates a process flow diagram of wastewater and sewage sludge treatment units with phosphogypsum recycling.



1 – input stream to be treated; 2 – sludge containing precipitated phosphorus compounds; 3 – recycling of a portion of digested sewage sludge; 4 – nitrate reverse cycle; 5 – digested sewage sludge to be recycled; 6 – treated liquid phase; ET – equalization tank; RST – radial-flow settling tank; P – pump; HEU – heat exchange unit; B_1 – aerobic bioreactor; B_2 – anaerobic bioreactor for the denitrification stage; B_3 – anaerobic bioreactor for the phosphorus removal stage under sulfate reduction with the release of phosphorus compounds; MH – measuring hopper for phosphogypsum; SST – secondary settling tank; SH – settling hopper for reactant removal of phosphorus from the liquid phase; C – compressor

Figure 3.20 – Process flow diagram of wastewater and sewage sludge treatment using phosphogypsum (Chernysh et al., 2017)

The developed process flow diagram includes a three-stage treatment process with a high-rate first stage of aerobic oxidation with the sludge age of 0.5 days and hydraulic retention time of 0.45 hours. The efficiency of COD removal can reach 32–50 % at this stage. With a short retention time of 0.45 hours, organic matter is only sorbed to active sludge and does not have enough time to be absorbed by the cell, which has a positive impact on further gas generation in anaerobic bioreactors (B_2 та B_3). The denitrification stage takes place in B_2 and is carried out with a maintained activated sludge age of 5–10 days depending on the temperature. The dissolved oxygen level is maintained within the 0.1–0.97 mg/dm^3 range. Hydraulic retention time runs at 1–2 days under psychrophilic conditions (20 °C), and 0.5–1 day under mesophilic conditions (35 °C). The longer the hydraulic retention time of the substrate (fresh sludge) is, the higher the potential COD output of the dissolved substance is. It has been determined that under a stationary mode the output of dissolved organic matter from B_2 with an extended sludge bed (hydrolysate and the residue of dissolved urban wastewater matter) can reach ~ 30 % of the biodegradable wastewater substance COD within half a day. Sewage sludge from previous stages of biological treatment is digested during the phosphorus removal stage under sulfatereduction which occurs in B_3. The formation rate of volatile fatty acids and acetates, as well as phosphorus removal efficiency, reach maximum values when the optimum sludge age lies in the range of 5–11 days. Thus, under mesophilic conditions, sludge retention time (including the initial induction period of not less than 5 days, which is only observed upon unit startup) required to achieve the indicators of organic matter decomposition up to 65 % and the degree of phosphate anion release not less than 70 %, equals 10 days. The specific level of gas generation equals over 650 dm^3/g of the benzene substance. The hydraulic retention time of wastewater equals 1.5–2 hours.

The immobilization efficiency criterion of microbial cells based on the repeated increase of the biomass yield of free cells indicates the saturation of the phosphogypsum medium with biomass of destructor microorganisms. It should be noted that most of the microorganisms are immobilized within the first 5 days, and then, with an increase in contact time, the degree of cell adsorption decreases dramatically. At that, there occurs phosphogypsum recycling of about 6,5 kg/ m^3 of sewage sludge sent for treatment.

The wastewater and sewage sludge treatment technology that includes an aerobic-anaerobic conversion process provides a spatial microbial succession and a trophic hydrobiota chain, involving technogenic mineral resources (phosphogypsum) to stimulate the development of essential ecological and trophic groups.

The following parameters are monitored to control the efficiency of the treatment process: wastewater temperature upon anaerobic bioreactor entry; pH value of the environment upon entering and leaving anaerobic and aerobic bioreactors; dissolved oxygen concentrations (pO_2) upon entering and leaving the aerobic bioreactor; oxidation-reduction potential value (Eh) in the anaerobic bioreactor; station pumping unit alarm; tank fluid level alarm; phosphate concentration (PO_4^{3-}) upon entering and leaving anaerobic and aerobic bioreactors. After phosphate precipitation, the treated water in the settling hopper can accumulate in accumulation tank sections and run by gravity into the pump area for treated wastewater from urban treatment plants (Chernysh et al, 2015–2018).

To improve microbial living conditions and, correspondingly, the effect of wastewater and sewage sludge treatment, the anaerobic bioreactor is introduced with air coming from air blowers, thus saturating (aerating) wastewater with atmospheric oxygen. Air is supplied into the airlift area of each section through perforated pipelines. During the aerobic stage, oxygenated wastewater circulates through the operating area B_1 and is purified by microbes immobilized on fibrous media. B_1 is aerated by compressor-induced air from the atmosphere and gases released from B_2.

Battery-type anaerobic bioreactors (B_2 and B_3). These consist of sections (at least 23 for each one) interconnected in series with a gravity flow mode. A bioreactor section is a vertical plastic pipe with a diameter of 350 mm and a height of 4.5 m. Each B_3 section is automatically supplied with phosphogypsum from the measuring hopper. Gas outlet manifolds connected with reactor sections are mounted in the upper part of units. The area between bioreactor walls and protective polystyrene sheet shields serves as an airlift area. Upon its circulation, activated sludge in B_3 coagulates with phosphogypsum particles, which is accompanied by matrix forming and allows to reduce the loss of active biomass from the system. At that, low-grade wastewater heat may be used, as described in (Chernysh et al., 2017).

The methodology based on the equalization of wastewater COD mass balance following is commonly used for designing the processes of biochemical wastewater treatment. This methodology, however, does not consider the physical and chemical properties of mineral media that can be used upon wastewater and sewage sludge treatment, and the indicators of joint development of ecological and trophic microbial groups in one community. Accordingly, we took these factors into account and created our approach for calculating the volume of the anaerobic bioreactor for the phosphorus removal stage in m^3:

$$V_{AnB} = \left(\frac{w_{SS} \cdot m_{SS}}{\rho_{SS} \cdot 100\%} + \frac{m_{PG}}{\rho_{PG}} \right) \cdot \left(\frac{Y_{X/(S+D)} \cdot (\Delta C_{COD} + \Delta C_P)}{\tau \cdot \left(1 - Y_{X/(S+D)}\right) \cdot \sum \mu_{iMC} \cdot X_{gen}} \right), \quad (3.15)$$

where m_{SS} is mass of sewage sludge that enters the phosphorus removal stage, kg; w_{SS} is sewage sludge moisture, %; m_{PG} is phosphogypsum mass, kg; ρ_{SS} and ρ_{PG} are respective sewage sludge and phosphogypsum density, kg/m^3; ΔC_{COD} is COD load, kg/m^3; ΔC_P is phosphate load, kg/m^3; τ is time spent at the phosphorus removal stage, days; μ_{iMC} is the specific growth rate of *i*-type of microbial communities, $days^{-1}$; X_{gen} is total productive biomass in the bioreactor, kg/m^3; $Y_{X/(S+D)}$ is the economic coefficient of biomass yield based on a complex substrate (COD/Phosphogypsum).

Indicators of treatment efficiency in the B_1–B_2–B_3 system can reach 95.5 % for COD and 99.5 % for phosphates. At that, not less than 60 % of COD and 70 % of phosphates are destroyed during anaerobic stages (denitrification and phosphate removal under sulfate reduction). If the composition of wastewater and sewage sludge are dominated by nitrates, it is advisable to initially provide anaerobic stages (B2-B3), where the organic substrate is oxidized by nitrates with release of free nitrogen, and for the final oxidation of organic matter aeration stage should be provided.

CHAPTER 4

Biochemical processes of contaminated soil remediation by biogenic composites based on sewage sludge and phosphogypsum

4.1 The problem of contaminated soil remediation and ways of decision

The growing anthropogenic load on the ecosystems is accompanied by the change of the natural soil-forming process. The intensive use of black earth in Ukraine in recent years has resulted in a significant loss of humus, which is accompanied by negative changes in agrophysical, physico-chemical, and biological properties of soil. Correspondingly, the efficiency of agricultural methods and the productivity of crops are decreasing (Dadenko et al., 2013). At the same time, various organic-mineral and mineral fertilizers are used to stimulate the growth and development of crops. The use of phosphate, nitrogen, and potassium fertilizers in dosage established by the standards is a very effective means to increase crop yields. But nowadays it is still difficult to solve the problem of heavy metals reduction in plant systems. The processes of migration and accumulation of heavy metals take place due to root exudate that moistens soil aggregates and binds cations of two- and polyvalent metals with the help of carboxyl and hydroxyl groups of polysaccharides, amino acids, and carboxylic acids in complex compounds and concentrates these cations resulting intoxication land ecosystems.

The investigation by Carmona et al. (2011) was established in two tailing ponds, in which experimental plots were designed, using marble wastes, pig manure, and sewage sludge as amendments to reclaim the mine soils. Bioavailable metals decreased in the amended plots, although increments in some metals after 5 years of monitoring were observed, owing likely to the erosion of tailings surface and decreases in organic matter, which initially immobilized metals.

Many research studies (JDerome et al., 1999; Derome, 2000; Anderson et al., 2013; Goulding, 2016) the effects of liming, correction fertilizer, and site-specific fertilizer treatments on heavy metal (Cu, Ni) and macronutrient (Ca, Mg, K) availability in the organic layer. Liming had only a relatively small reducing effect on free and exchangeable Cu and Ni concentrations at 0.5 km. The lack of pH increase following liming may be due to the precipitation of Fe, present in very high concentrations close to the smelter, as $Fe(OH)_3$, resulting in the loss of neutralizing bicarbonate and hydroxyl ions, but the release of Ca and Mg. Liming strongly increased Ca and Mg availability (Derome, 2000).

The lime and phosphogypsum are applied as soil amendments to alleviate Al^{3+} toxicity and improve the root environment, increasing the efficiency of applied nutrients and benefiting crop growth and yield (Blum et al., 2013). Phosphogypsum is a solid waste produced by the phosphate ore industry, which has been used as soil fertilizer in many parts of the world for several decades. The positive effects of phosphogypsum in ameliorating some soil properties and increasing crop yields are well documented. However, few studies have focused on the impact of phosphogypsum applications on soil biota, as well as the contribution to soils with elements in mobile fractions of phosphogypsum which may affect freshwater species as well (Hentati et al., 2015).

Thus, the study of laws and mechanisms of the biochemical transformation of heavy metals in natural and artificial ecosystems for optimization and balancing of nutrition of crops remains relevant. The applied nature of this problem can also be considered in the aspect of biotechnological systems development and biotechnical means of natural mechanisms stimulation of the soil complex protection while rehabilitating natural and anthropogenic landscapes contaminated by heavy metals. And the problem solving of phosphogypsum treatment is very important today.

The change of the activity of the soil biotic component is influenced not only by seasonal changes but also by several other factors, such as the degree of anthropogenic loading and cultivation. The bacteria with carbonate and sulphate respiration as well as microorganisms involved in the synthesis and decomposition of humus substance play an important role in the biochemical transformations of toxicants. The natural aerobic-anaerobic transformations result in the formation of a stable solid fraction with fluctuating domination of sulfides and/or carbonates and organometallic complexes. These groups of slightly soluble and insoluble compounds are often considered separately. But while interacting with soil microbiota they are formed together and are a natural barrier against the influence of toxicants, isolating them from the root system of plants. At the same time, the natural biochemical mechanisms underlying environmental protective function are poorly studied. Their main feature is the system and synergetic nature of transformations. It can cause dysfunction of these mechanisms at an increased level of anthropogenic loading on the ecosystem. The uncertainties, prolonged toxic effects on natural and artificial phytocenosis arise when the soil ecosystem is in a critical state when the system becomes unstable as to fluctuations.

Thus, the most important task of environmental research is to find ways to stimulate the natural protective properties of soils under conditions of intensive anthropogenic load.

The research aim is to model the stimulating process of the natural protective properties of the soil complex due to the influence of biogenic composite material based on sewage sludge and phosphogypsum. To achieve the aim, the following tasks were set:

– microscopic examination of biocomposite samples obtained as a result of anaerobic fermentation of sewage sludge together with phosphogypsum under the condition of biosulphate reduction;

– research of the fractional composition changes of heavy metals on the example of lead in the soil before and after biocomposite processing based on sewage sludge and phosphogypsum;

– model development of biogenic composite influence based on sewage sludge and phosphogypsum on protective properties of soils.

4.2 Directions of natural regulation of the soil buffer properties

In the biochemical transformations of toxicants, an important role is played by bacteria with processes of carbonate and sulfate respiration, as well as microorganisms involved in the synthesis and decomposition of humus substances. In the course of natural aerobic-anaerobic transformations, a stable solid fraction is formed with fluctuation domination of sulfides and/or carbonates and organometallic complexes in it. These groups of insoluble and insoluble compounds are often considered separately. However, they interact with soil microbiota formed together and are a natural barrier against the action of toxicants, isolating them from the root system of plants. At the same time, the natural biochemical mechanisms that underlie the implementation of the ecological protector function are not fully understood. Their main feature is the system-synergistic nature of transformations. It can cause dysfunction of these mechanisms when increasing the level of anthropogenic loading on the ecosystem. When a soil ecosystem is in critical condition, in which the system becomes unstable about fluctuations and uncertainty arise, prolonged toxic effects on natural and artificial phytocoenuses arise.

From the point of view of biomineralization, bacterial metabolism affects the oxidation-reduction reaction in the soil medium and the rate of release of nutrients and deposition of heavy metals (Konhauser et al., 2012), respectively, in the process of their fixation in a complex biogenic organomineral fraction of soil. Each bacterium has a biogeochemical metabolism, which is best suited for adaptation in specific environmental conditions. The basic scheme of the bacterial surface, which shows the process of adsorption of cations of metals, is depicted in Fig. 4.1. In this case, metal cations can react with dissolved anions in the external environment, such as silicon dioxide, sulfate, phosphate, sulfide, and bicarbonate. Depending on the available anions, different minerals can be formed on the bacterial surface.

In the process of metabolic induced bio-mineralization, secondary minerals are formed as by-products of microbial metabolism containing metals in a chemically bound form, respectively, the extraction of the latter from the cycle of substances in the ecosystem (Fig. 4.2) (Chernysh and Plyatsuk, 2018)

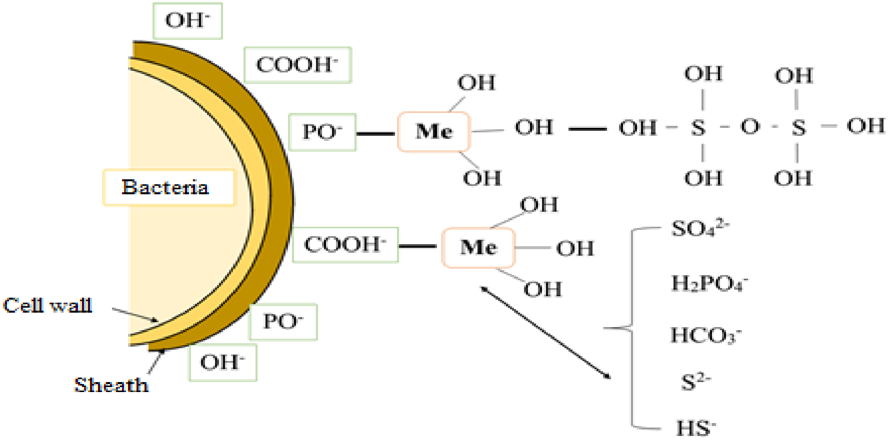


Figure 4.1 – Schematic representation of the process of bacterial mineralization with binding and deposition of metals in the minerals

Composition

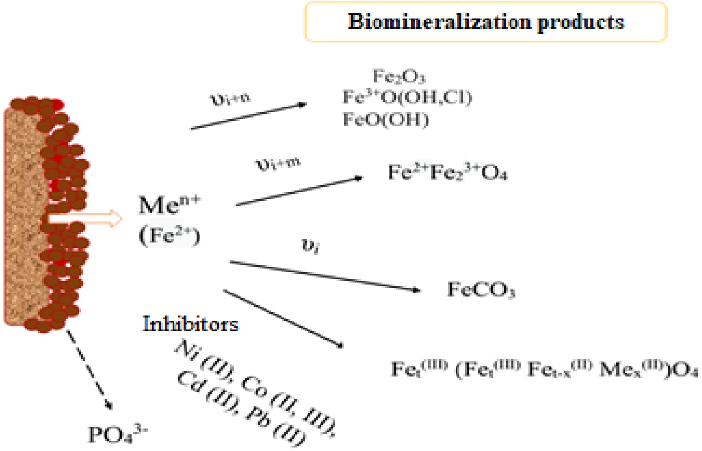


Figure 4.2 – Schematic representation of metal bio-mineralization

The conceptual model of ferrihydrite bio-mineralization shows the process of aggregation of ferritic acid nanoparticles ($2{,}5Fe_2O_3 \cdot 4{,}5H_2O$) around the bacterial cell in the direction of Fe^{2+} (II) transformation into various minerals with a certain velocity (υ_i, υ_{i+m},

υ_{i+n}). In this case, the release of biogenic compounds (in particular, phosphates), followed by their transition to the space of the rhizosphere. Also, in the process of suppressing the action of heavy metals, the tread function for microorganisms creates a ferritic oxide film (Fig. 4.2).

On the smallest scale, many organic substances in various types of soils and deposits are associated with clay minerals, forming organo-mineral complexes (V. G. Dobrovol'skij, 2004). If chemical sorption is the driving mechanism for this association, then it will be optimized for smaller minerals size by the ratio of surface area to pore volume. Such primary organomineral complexes are often observed with clay minerals and oxyhydroxides of metals of various sizes from a submicrometer to a micrometer. Mechanisms that are most likely to create a primary complex, are (J. M. Hayes, 2006; S. A. Thorarinn, etc., 2007): 1) sorption of dissolved organic molecules on the surface of the mineral; 2) aggregation of macromolecular (colloidal) organic matter with minerals. Most often, it is quite complicated to determine the difference between these two mechanisms in the study of fine-grained fractions, in which most of the organic matter is located.

In a thin section of chernozem can be particles that are joined together with a porous space. It contains an accumulation of water and nutrients and occurred in the metabolic activity of the soil biota. The highly dispersed fraction of the soil complex consists of minerals that have a layered crystalline structure in which the layers are interconnected not as tightly as the ions in the fragile minerals structures. This feature of these minerals promotes the sorption of scattered heavy metals, and their concentration in this fraction is an order of magnitude higher than that of fine-grained minerals (quartz, mica, silicates, etc.) and in the soil as a whole.

In general, in the transformation of heavy metals in the soil complex, a humus substance plays a significant role, which increases the destruction of pesticides, fixes pollutants, including heavy metals, reducing their inflow into terrestrial ecosystems. In acidic and neutral media humic acid molecules can coagulate and stand out in the form of structured sediment consisting of large globular aggregates. Probably, the density of organic matter does not exceed 1.25–

1.80 g/cm^3, and its weight capacity is approximately two times higher than that of the clays.

It should be noted that an important mechanism for regulating the buffering properties of soils is the preservation of a certain level of the stock of microbial metabolites (sugars, organic acids, and alcohols, amino acids, etc.). There is contributed to the conservation of soil microbiotics in periods when the soil or the part of a specific microzone receives fresh nutrients. Accordingly, even in unfavorable external conditions, at an elevated level of anthropogenic loading, there is a minimum reserve of nutrients necessary for the preservation of the biotic component of the soil complex. This is important, as when introducing organo-mineral composites to achieve high efficiency of the restoration of contaminated soils, it is necessary to stimulate the development of natural ecological trophic groups of microorganisms that become depressed under the influence of external destructive factors.

If diffuse access to substrates in hierarchical aggregate structures restricts the biological use of their microorganisms, then physical access can be enhanced by various forms of physical impairment. In soils, after the plowing, there is a decrease in the macro aggregate structures, while the micro aggregates and the primary organ-silicon structures remain. The activity of peroxidase on arable land is 51–52 % higher than on virgin soil, the activity of polyphenol oxidase is higher in arable land by 30 and 50 %. The ratio of the activity of polyphenol oxidase to peroxidase is a conditional coefficient of humification (K_{gum}), which indicates the orientation of the synthesis processes of humus substances. In general, the activity of these enzymes depends on many factors: the flow of nitrogen and phosphorus, temperature, aeration, and microbial biomass. The content of humus most strongly changes in the first 15 years after the plowing of virgin soil due to the rapid mineralization of labile forms of humus. It should be noted that the methods of soil treatment in different ways impact on microorganism’s growth on the profile of the soil horizon. Thus, in the case of flat-cut soil treatment, the number of eutrophic microflora in the soil layer is increased from 0 to 10 cm in comparison with the variants where the plow was applied for 25–30 cm. However, in the deeper layers of the soil (20–30 cm), the opposite pattern is observed: the number of microorganisms at Flat-cut processing is twice less than on plowing options.

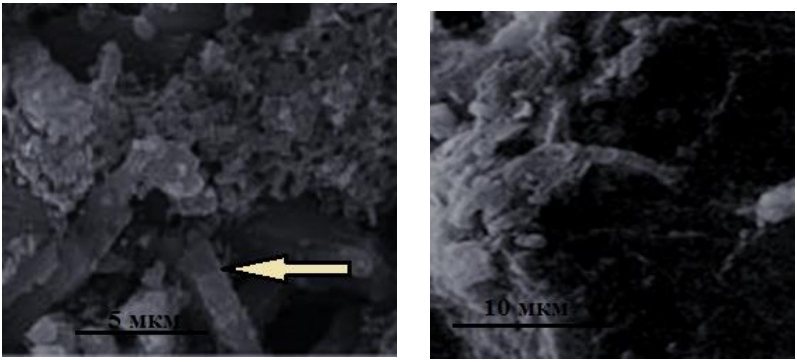
The change in the activity of the soil biotic component is influenced not only by seasonal changes but also by several other factors, such as the degree of anthropogenic loading and agricultural processing. In the biochemical transformations of toxicants, an important role is played by bacteria with processes of carbonate and sulfate respiration, as well as microorganisms involved in the synthesis and decomposition of humus substances. In the course of natural aerobic-anaerobic transformations, the formation of a stable solid fraction with fluctuating domination of sulfides and/or carbonates and organometallic complexes takes place. These groups of insoluble and insoluble compounds are often considered separately. However, they interact with soil microbiota formed together. There is a natural barrier against the action of toxicants, isolating them from the root system of plants. At the same time, the natural biochemical mechanisms that underlie the implementation of the ecological protector function are not fully understood. The system-synergistic nature of transformations is their main feature. It can cause dysfunction of these mechanisms when increasing the level of anthropogenic loading on the ecosystem. When a soil ecosystem is in critical condition, in which the system becomes unstable about fluctuations and uncertainty arises, prolonging toxic effects on natural and artificial phytocoenoses arise (Chernysh and Plyatsuk, 2017–2018).

Thus, in conditions of intensive anthropogenic loading, the most important task of environmental research is to find ways to stimulate natural soil's protective properties (Chernysh et al., 2017).

4.3 The research of changes in the fraction composition of heavy metals on the example of lead in the soil before and after processing by biocomposite based on sludge deposits and phosphogypsum

The previous research, carried out in the laboratories of Sumy State University, proved the expediency of using phosphogypsum as a mineral substrate for the development of microorganisms association of organic substance decomposers of sewage sludge and wastewater under anaerobic conditions during bio-sulphate reduction and using of processing product as an environmentally safe composite material in environmental protection technologies. The previous research of the anaerobic microbiological destruction of sewage sludge together with phosphogypsum revealed the regeneration process and the release of phosphate ions and formation of complex sulfide fraction (Chernysh, 2013; Plyatsuk and Chernysh, 2014; Chernysh and Plyatsuk, 2017).

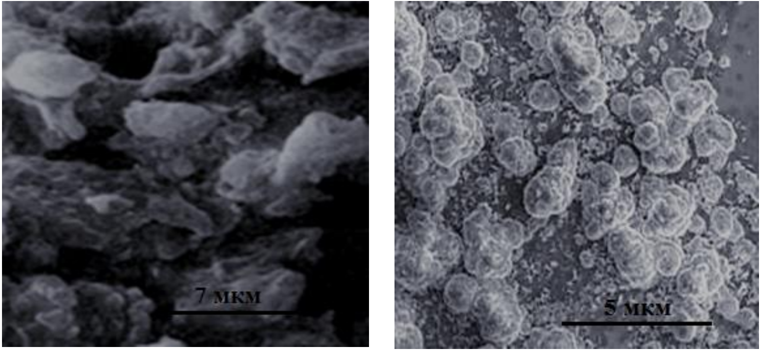
As a result of X-ray diffractometric analysis of the components spectrum of the processing product, we obtained information on the metals speciation and their distribution according to mineral phases. At the same time, a complex sulfide fraction that chemically binds heavy metals in its structure and neutralizes the possibility of their involvement in the natural cycle of the matter is formed at the stage of anaerobic destruction under the condition of sulphide genesis. Thus, such main components of biocomposite mineral constituent were identified: quartz – SiO_2; potassium hydroxide – KOH and potassium hydrogen phosphate hydrate – $K_2H_2P_2O_7 \cdot H_2O$; brushite – $CaPO_3(OH)_2 \cdot H_2O$, these compounds have active metal sorption sites in ion-exchange reactions; calcite – $CaCO_3$; phosphorus oxide – P_2O_5; ammonium sulfate (Mascagnit); complex sulfide fraction. It should be noted that the process of aggregation of sewage sludge fraction with components of phosphogypsum transformed is present. Fig. 4.3 presents the results of structure research of the biogenic composite, carried out using SEM analyzer PEMMA-102 (publicly held company “SELMI”, Sumy, Ukraine).



a) selective fixation magnification:5 microns

b) aggregation, magnification:10 microns

(arrow shows pyrite crystals)



c) sedimentation/deposition, magnification: 7 microns

d) biosorption, magnification:5 microns

Figure 4.3 – Scanning electronic microphotographs of biogenic composite material structure

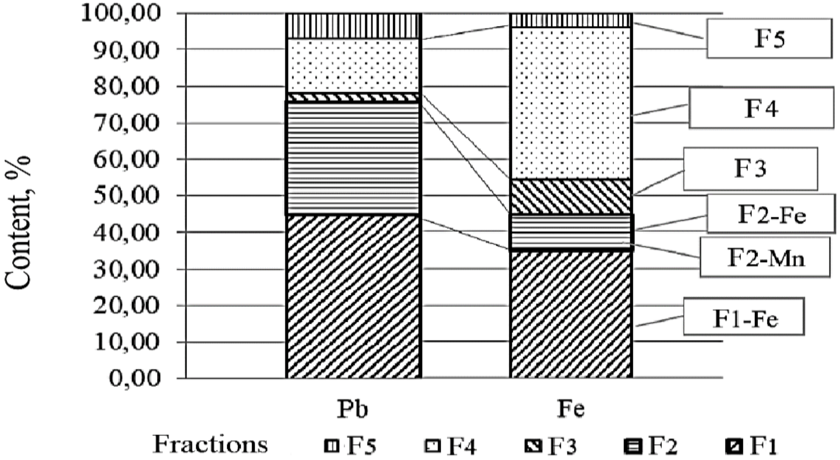
The transformations of components of organic and mineral waste mixture characteristic for the structural changes of soil complex aggregates during autocatalytic regulation of buffer properties take place due to anaerobic mineralization.

The microscopic examination of biogenic composite aggregates showed that the organic substance in these associations is nonuniformly distributed film with small organic molecules, often consisting of “bubbles” of the size of a colloidal particle of the organic components (Fig. 4.3, g).

The biocomposite based on sewage sludge and phosphogypsum was mixed with the soil layer of 0–20 cm. The repeatability of the experiment is three times.

The results of successive soil extractions before processing are shown in Fig. 4.4. The largest portion of Pb (about 67 %) was extracted as a portion of F1-Fe and F2-Fe, bound to amorphous oxides and hydroxide Fe: FeOMe, $(FeO)_nMe$, FeOMeOH, for example, $PbFe_2O_4$ (Chernysh, 2018).

As Fig. 4.4 shows, metal oxyhydroxide (76 %) can be referred to as the main fraction, and only 22 % belong to the residuum closely bound to the soil matrix (with silicates and sulphides).



F1 is oxides and oxyhydroxides of iron and manganese; F2 is exchange forms; F3 is carbonated, hydroxy carbonates; F4 is with organic substances; F5 is residuum (silicates and sulphides).

Figure 4.4 – Fractional composition of metals in the soil at successive chemical extraction on <2 mm of soil fraction before processing (concentration, %) (Chernysh, 2018)

The low portion of Pb and Fe (i.e. <2 % and <9.2 % respectively) is extracted as bound to carbonate fraction. These results proved that Pb had main bonds with amorphous particles of iron oxide and ferrous hydroxide of soil, which is confirmed by diffractometry research.

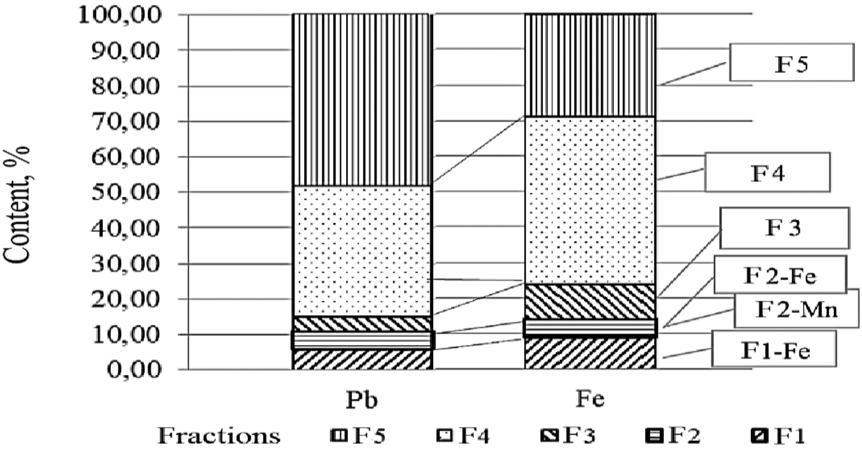
The diffractometric analysis showed the traces of hematite, jarosite, and scorodite, which are usual pyrite sedimentary products, which HM can generally bind to. The absence of residual mixed sulphide phases in soil may be connected with weathering of pyrite minerals.

At extraction of $NH_2OH \cdot HCl$, under these acidic conditions of pH (about 4), about 76% of the total lead was extracted. The mechanism of lead desorption from the surface of oxyhydroxide Fe due to competition with OH-ions can explain the rapid increase in lead extraction. The remediation of Fe remains very low (about 0.2% of total Fe). Fe remediation may be controlled by Fe hydroxide precipitation.

These results suggest that the most portion of Pb present in the soil is sorbed onto Fe oxyhydroxides. The remaining portion (about 22 % out of total Pb) can be considered as a portion coprecipitated with Fe and/or bound to resistant compounds (predominantly silicates).

Accordingly, this suggests that lead is bound to unstable oxyhydroxides in the soil complex.

After treatment five major fractions of metals in the "soil complex – biocomposite" system were generally compatible (86 %): 37 % were found in stable organic compounds and 49 % were in residual fractions, which have firm bonds with the matrix of mineralized sediments (silicates and sulphides) (Fig. 4.5). There were only 12 % of metals oxyhydroxides. The low portion of Pb and Fe are extracted as exchangeable and bound with carbonate fraction. Thus, it has been determined that at least 90 % of lead is bound in the inaccessible form to plants: primary and secondary silicate minerals, slightly soluble metal compounds (sulphides), and organo-mineral complexes. It should be noted that carbonate fraction increased by 2 % and, correspondently, it made 4 %, which is related to the substitution of calcium by lead in carbonate compounds contained in the composite, as lead ions have a similar size as calcium ions.



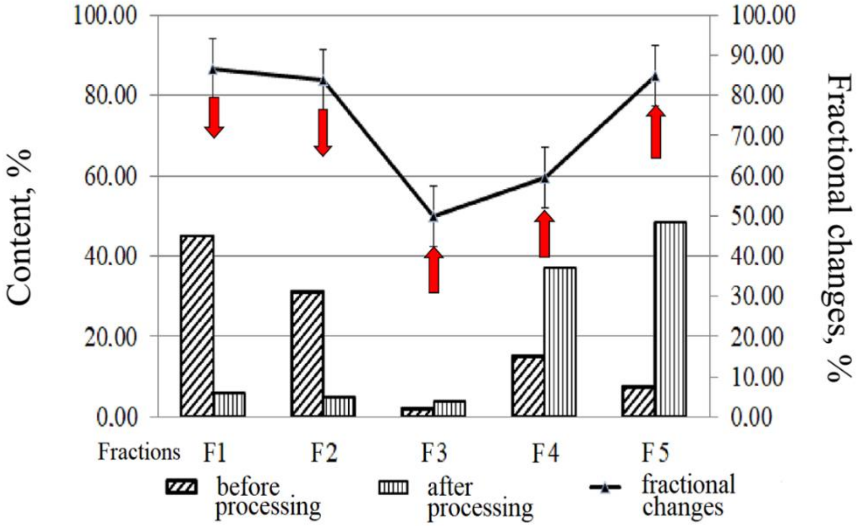
F1 is oxides and oxyhydroxides of iron and manganese; F2 is exchange forms; F3 is carbonates, hydroxy carbonates; F4 is with organic substances; F5 is residuum (silicates and sulphides).

Figure 4.5 – Fractional composition of metals in “soil-biocomposite” system at successive chemical extraction on < 2 mm fraction (concentration, %)
(Chernysh, 2018)

The results of Pb extraction out of contaminated soils under the biocomposite influence due to $NaCH_3COO$ solution are also shown in Fig. 4.5. It has been found that extraction with the help of this solution has no significant effect on the release of Fe and Pb. However, it indicates an increase in calcium solubility (approximately 500 times at pH about 5), and at the same time there observed phosphorus adsorption on Fe oxyhydroxides according to the results of diffractometric studies.

Due to sulphides formation, the concentration of metals in interstitial water of soil is significantly reduced and they become not available to organisms and plants. It should be noted that iron sulphide (II) and zinc sulphide in relation to copper and lead sulphides have more significant solubility product (for example, KS (FeS) ~ 10^{-19}, and KS (PbS) ~ 10^{-29}). Thus, lead sulphides can be referred to as a stable residual fraction in soil, the increase of which is due to the presence of exchange processes in complex sulfide fraction of biocomposite due to mineralization and humification.

Having analyzed the obtained results, the diagram demonstrating the change of metals fractional composition was created (Fig. 5.6)



F1 is oxides and oxyhydroxides of iron and manganese;
F2 is exchange forms; F3 is carbonates, hydroxycarbonates;
F4 is with organic substances; F5 is residuum
(silicates and sulphides).

Figure 4.6 – Combined analysis diagram of the changes of metals fractional composition in soil before and after biocomposite introduction (Chernysh, 2018)

The relative portion of the residuum during soil processing with biocomposite rapidly increases (Fig. 4.6). The accumulation of HM in soils and their distribution in fractions are also influenced by acid-base conditions. Thus, the restored soil had an acidity of 6.5 and the exchange fraction of lead decreased by 84 %. The number of elements bound with hydroxides and oxides of Fe and Mn also decreased by 87 %.

Let us consider the effect of biocomposite on the duration of protective soil properties, based on the dynamics of processes occurring in the soil complex, and extrapolate them on the biocomposite properties. So, if we take the change of the initial fractional composition of organic matter of soil as a time function of oxygen influence, then we can consider the processes of organic matter aggregation of soil together with the biocomposite taking into account microbial kinetics of soil biome. When degradation time is insignificant (from several days to several years), most of the organic matter remains in its original state with the help of some form of selective preservation (for example, oxygen sensitivity and natural slow decomposition constants), after which the aggregation takes place realizing the principal protective mechanism and, also, here biocomposite is "included" to stimulate protective function due to its physicochemical and biochemical transformations to bind toxic compounds into an insoluble form (Fig. 5.6). A longer period of oxidation of organic matter increases the relative importance of sorption and the biosorption of toxic components, in particular. The research results in (Löhr et al., 2014) suggest that the biominerals occlusion and co-precipitation with minerals of iron oxide may be an important protective mechanism in a long-term period, and at this stage, the formation of stable complex compounds takes place, which can take in heavy metals and remove them from the biochemical migration cycle in the environment (Chernysh, 2017, 2018).

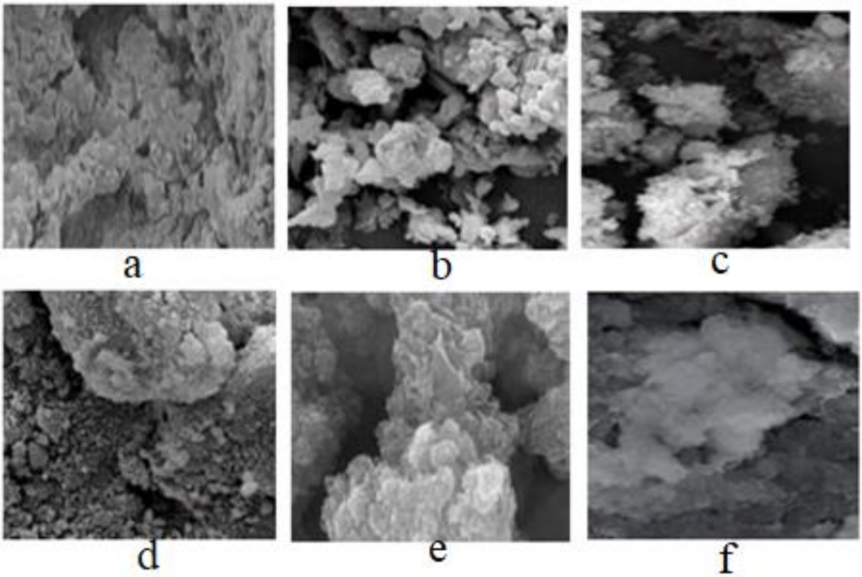
A microscopic analysis performed BSE images using a raster electron microscopy in combination with microanalysis of soil after introducing biocomposite based on SS and phosphogypsum showed the presence of quartz, gypsum, and compounds containing Fe and HM, in particular Pb, with a composition in the mineral fractions, which corresponds to the results of the diffractometric analysis. It should be noted that the strong binding of metals provides stable organo-mineral compounds.

Fig. 4.7 shows the general view of the humus layer on the soil profile after the introduction of the biocomposite.

Figure 4.7 – Photograph of the humus layer together with the beveled vegetation when the biocomposite is applied superficially

Fig. 4.8 shows mapping the SEM-EDX raster microanalysis of the superficial introduction of biocomposite on the based on sewage sludge and phosphogypsum was obtained by 2–3 mm fraction.

Thus, the features of the fractional distribution of the HM are related to the specificity of their interaction with the soil components. With the increase in the level of soil contamination by heavy metals, most of them are associated with oxides and hydroxides of Fe and Mn, resulting in a fraction of this fraction increases. In the course of implementation of the soil restoration during the introduction of biocomposites, an increase in the proportion of the metal in the residual fraction is observed, which indicates their strong binding in the mineral-organic structure and inaccessibility to plants.



a – general view; elements: b – Fe; c – Pb; d - Ca; e – S; f – Si
Figure 4.8 – SEM-EDX raster microanalysis map to 2 mm fraction (12X) (Chernysh, 2018)

Generally formed compounds of the HM in the system “soil complex - biogenic composite” are stable over a wide range of pH from 2.0 to 7.5 and belong to fractions that are resistant to chemical and microbiological impacts (Chernysh, 2018).

4.4 Description of the processes of stimulating the natural protective properties of soil using a biocomposite based on sewage sludge and phosphogypsum

As indicated in (Milanovskiy et al., 2003) fresh humus possesses obvious hydrophobic properties and is formed inside organo-mineral aggregates involving anaerobic bacteria. Thus, the microorganisms inside the soil aggregates differ from those on the surface – they are mainly anaerobic.

Therefore, the biogenic composite based on sewage sludge and waste phosphogypsum in the process of anaerobic fermentation under the conditions of bio-sulphate reduction can stimulate the development of necessary anaerobic groups of microorganisms in the intra-aggregate space. In this case, sulphatisation contributes to the formation of macromolecules of biogenic composites.

Fig. 4.9 presents the model of formation of a stable soil aggregate with fixation of metallic ions in the process of biosorption by a microbial cell.

Figure 4.9 – Model of the soil organo-mineral aggregate (Chernysh, 2017)

The origin, the formation process, the stability of aggregates, and, on the contrary, the processes of loss of aggregate structure, its degradation are the processes that directly affect complex fundamental physicochemical and biological soil-forming processes and at the same time, have practical relevance. But till now, the generally accepted and comprehensive theory of aggregates formation and management of structuring does not exist. Upon that, several researchers (Torn et al., 1997; Shein et al., 2014) state that to form a solid aggregate structure that does not come apart in water, the following conditions are obligatory: 1. organic substances serving as the starting material for humus formation should penetrate the soil; 2. local conditions are needed for metabolic activity of anaerobic microorganisms that transform organically into amphiphilic soil humus with dominant hydrophobic properties, which binds minerals forming a water-stable aggregate. These natural conditions can be implemented based on structural transformations in the system "soil complex – biocomposite".

The conceptual model of the influence of the proposed organo-mineral biocomposite on the protective function of the soil complex is formed and represented in Fig. 4.10.

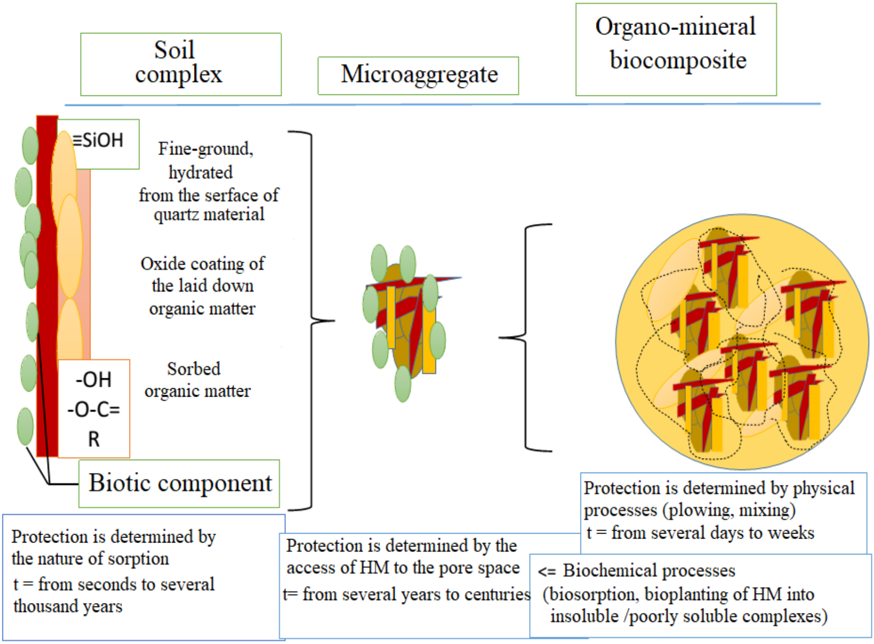


Figure 4.10 – Schematic representation of the process of step-by-step stimulation of soils protective properties after application of the organo-mineral biocomposite (Chernysh, 2017)

Contact of the organo-mineral biocomposite with the soil complex with the help of surface-sorption phenomena can have protective properties and lead to the formation of microaggregates consisting mainly of organic-mineral fine particles with the development of soil microorganisms on their surface. Subsequently, during the adaptation period, a natural microbial association is formed in the aggregates, which developed into the system "biocomposite –

soil” through the processes of microdiffusion and biosorption. Such a biotic component can play an important role in the conditioning and structuring of the environment, in particular, the mineral component (depositing of heavy metals), and influence the gradual release of biogenic matters from the biocomposite.

The formed microaggregates are depleted in readily available organic matters, because during the soil mineralization such compounds were used by microorganisms-destructors and, accordingly, are more resistant to degradation than the primary biocomposite applied into the soil.

In the process of interaction of the soil complex and the biogenic composite, a number of common products of interaction are formed. There are formed when the biotic component of the soil is transformed together with components of biocomposite into the processes of the joint aggregation of organo-mineral particles and biocomposite, selective fixation of metal ions, biosorption, and bioprecipitation. It should be noted that in a number of our studies, the possibility of using a biocomposite for biochemical binding of radionuclides was substantiated (Chernysh and Lego, 2016).

Based on the classical kinetics of the microorganism’s growth (Varfolomeev et al., 1990) we have

$$M = N + N \cdot P + X_i + X_i \cdot P + X_{i+1} + X_{i+1} \cdot P, \tag{4.1}$$

where N is the number of cells; M is total cell concentration; P is a metabolic product.

It should be noted that in the process of the organic component oxidation there is a dynamic change in the concentration of biomass that develops in the space of compatible aggregation of the soil complex and biogenic composite. Thus, the product of compatible aggregation (Ra) is essentially a biogenic product, which is created under the metabolic action of the soil biotic component at the stimulating effect of the proposed biocomposite. Accordingly, the use of biocomposites based on phosphogypsum will increase the agro-ecological indicators of the land rehabilitation area (Chernysh et al., 2014; 2017).

Thus, assumed that micro aggregates can form non-reactive complexes with each cell state X_0 (the primary state of the biotic component of the soil), X_1 (the state of biocomposite interaction and biomass increase in the active phase), X_2 (the state of stationary biomass growth). In this case, the actual metabolic activity of soil microorganisms and the effectiveness of biochemical binding or fixation of toxic compounds in the process of organic matter oxidation cause the restoration of disturbed soils and increase the size of microbial biome soil.

The process of sorption and precipitation of heavy metals is considered as secondary processes of selective fixation. There is the process of biochemical binding of heavy metal ions and taking into account the linear relationship between the concentrations of the product and the biotic component, we obtained (Chernysh and Plyatsuk, 2018):

$$\left(1+\frac{k_f}{k_d}+\frac{k_f}{k_a \cdot S_{\mathrm{org}} \cdot Y_{bioc}}\right)\frac{dM_{gr}}{d\tau}+\frac{M_{gr}}{Y_{\mathrm{p}} \cdot K_{sum\_ox}}=k_f d\tau \qquad (4.2)$$

where S_{org} is the total organic substrate, including the organic component of the biocomposite, g/cm^3; M_{gr} is the total value of the soil microbial biome at the depth of the biocomposite introduction, g/cm^3; Y_p is the economic coefficient of biogenic product yield on an additional substrate (biocomposite); Y_{bioc} is the economic coefficient of biomass growth on an additional substrate (biocomposite); k_f is the constant of biochemical binding or fixation of metals in organo-mineral structures; k_d is constant of dissociation; k_a is aggregation constant; K_{sum_ox} is the coefficient of total oxidation, which determines based on the assumption that the dynamics of organic matter oxidation linearly depends on the humification process, taking into account biocomposite stimulating relative to the biotic components of soil (Chernysh, Plyatsuk, 2018):

$$K_{sum\_ox}=K_{gum} \cdot \alpha_{bioc} \qquad (4.3)$$

where K_{gum} is a conditional coefficient of humification; α_{bioc} is a coefficient reflecting the effect of a biocomposite on the activity of polyphenol oxidase and peroxidase.

When determining the K_{sum_ox}, the type of soil, its acidity, which is primarily reflected in the value of K_{gum}, is taken into account.

In this case, the change in the total organic matter in time, taking into account the indicator of total oxidation, is found from the expression (Chernysh and Plyatsuk, 2018):

$$\frac{dS_{org}}{d\tau} = -\left(\frac{\mu \cdot X_0}{Y_{X/S}}\right) \cdot K_{sum\_ox} \tag{4.4}$$

where $Y_{X/S}$ is the economic coefficient of biomass output under the organic matter of the soil; S_{org} is the total organic substrate, including the organic component of the biocomposite, g/cm^3; μ is the rate of biomass growth, day^{-1}, which can be found by converting the Mono equation with allowance for the stimulating effect of the biocomposite:

$$\mu = \mu_m \cdot \frac{S_{\text{org}}^1}{K_S + S_{\text{org}}^1} \cdot \frac{C_{bioc}}{k_{\text{н}} + C_{bioc}} \tag{4.5}$$

where μ_m is the specific growth rate of soil microorganisms, day^{-1}; K_S is the half-saturation constant for the organic substrate, g / cm^3; S^1_{org} is the initial concentration of organic substrate, g / cm^3; C_{bioc} is a dose of biocomposite, g/cm^3; k_{hs} is the half-saturation constant for the mineral component of the biocomposite, g/cm^3.

Under initial condition $\tau = 0$, $M_{gr} = X_0$ integrating equation (4.2) we obtained (Chernysh and Plyatsuk, 2018):

$$ln\frac{M_{gr}}{X_0} + \frac{M_{gr} - X_0}{Y_{\text{p}} \cdot K_{sum\_ox} \cdot \left(1 + \frac{k_f}{k_d} + \frac{k_f}{k_a \cdot S_{org} \cdot Y_{bioc}}\right)} = \frac{k_f}{1 + \frac{k_f}{k_d} + \frac{k_f}{k_a \cdot S_{org} \cdot Y_{bioc}}} \cdot \tau \tag{4.6}$$

Here the description of the biochemical interaction of the biogenic composite with the soil complex is carried out.

At the same time, it is possible to determine the relation $\frac{k_f}{1+\frac{k_f}{k_d}+\frac{k_f}{k_a \cdot S_{org} \cdot Y_{bioc}}} \cdot \tau, \frac{g}{cm^3} \cdot$ day as the oxidative ability of biomass in the process of interaction in the system “biotic component – biogenic product”, which determines the protective mechanisms of the soil complex in a consistent transformation with the biocomposite. Thus, this indicator includes the relationship of constants that reflect changes in the state of the soil complex during interaction with the biocomposite under the microbial biome action.

4.5 The development of influence model of the biogenic composite based on sewage sludge and phosphogypsum on protective properties of soils

Fig. 4.11 shows the transformation of an organo-mineral mixture of sewage sludge and phosphogypsum (biocomposite).

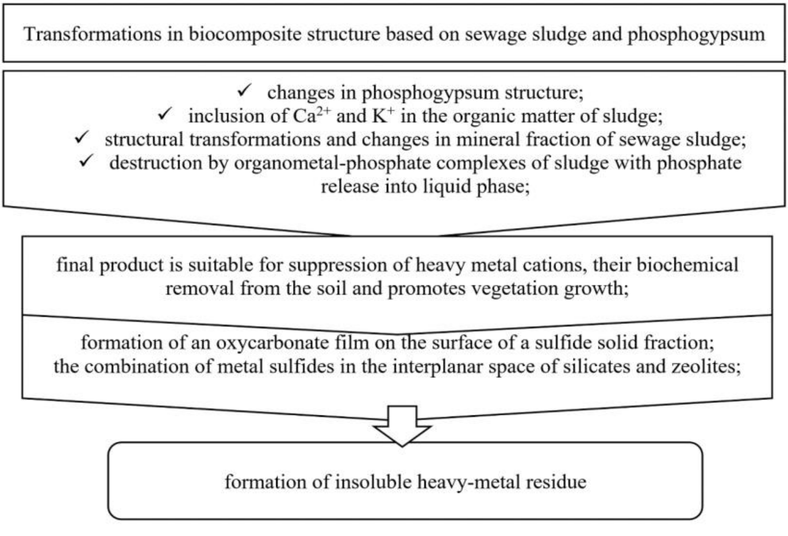


Figure 4.11 – Block diagram of transformations in a biocomposite based on sewage sludge and phosphogypsum formed in the process of anaerobic conversion under bio-sulfidogenic conditions (Chernysh, 2018)

Fig. 4.12 shows the combination of the most important soil protective mechanisms.

The components transformation of an organo-mineral mixture of sewage sludge and phosphogypsum has occurred under anaerobic conversion in the bio-sulfidogenic condition. These biochemical changes are characteristic for structural changes in the aggregates of the soil complex during autocatalytic regulation of buffer properties and which can be used for biochemical fixation of pollutants in the soil

This diagram (Fig. 4.12) does not attempt to quantify all protective mechanisms and ways of preserving the organic matter of the soil complex, but rather indicates that most of the organic matter stored at different time scales. There is likely to pass through successive overlapping of various protection mechanisms and can be stimulated by the introduction of the biogenic composite.

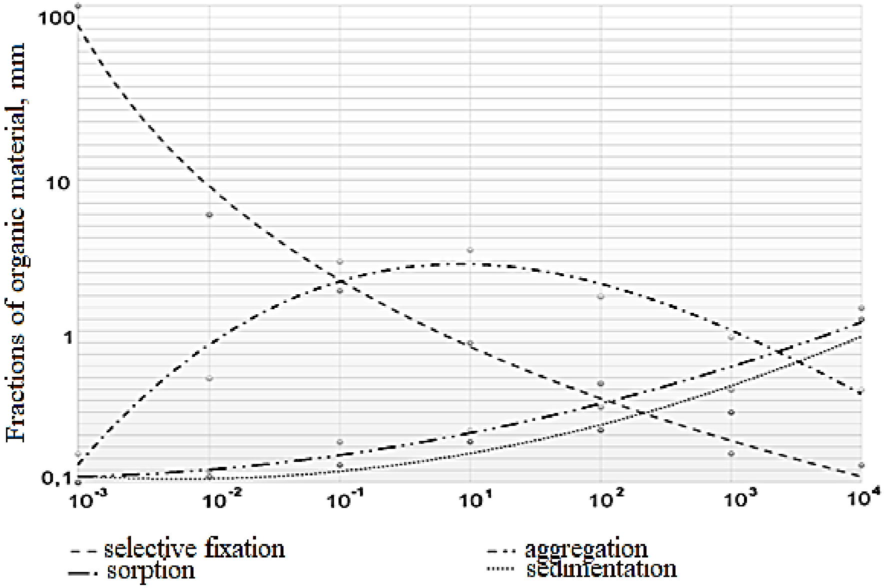


Figure 4.12 – Dependence between the sequence of imposing various protective mechanisms and fractions of organic matter of the soil complex (Chernysh, 2018)

Assume that the oxygen saturation of the porous space minimizes the time of stay at the earlier stages of the aggregation of organic matter, while the removal of oxygen from the system slows the increase in the process of association of mineral and organic substances, resulting in higher concentrations of organic matter in aggregates. Evidence of this oxygen effect can be seen in the flows of burials and types of organic matter in different deposition regimes, as illustrated in a number of geological works and the laws of distribution in (Torn et al., 1997; Thorarinn et al., 2007; Zonneveld et al., 2010; Löhr et al., 2014; Chernysh et al., 2017-2019) are investigated. Accordingly, a diagram with a graphical indication of the type of functions in the Microsoft Excel software package was constructed.

Consider the effect on the duration of the soil protective properties of the biocomposite based on the dynamics of processes occurring in the soil complex.

Fig. 4.12 shows extrapolating them to the properties of the biocomposite. So, if we take the change of the initial fractional composition of the soil organic matter as a function of the time of the oxygen influence, then we can consider the processes of aggregation of the soil organic matter together with the biocomposite with the binding to the microbial kinetics of the soil bacteria growth. When the time of degradation is insignificant (from several days to several years), most of the organic matter remains in its original state by some form of selective preservation (for example, oxygen sensitivity and natural slow decomposition constants). After these processes, aggregation takes place with stimulation of the soil protection function by bio-composite, which is associated with a number of its physicochemical and biochemical transformations from the binding of toxic compounds to an insoluble form (Fig. 4.11). With a longer period of organic matter oxidation, the relative importance of sorption, in particular the biosorption of toxic components, increases. It should be noted that occlusion in bio-minerals and heavy metals co-precipitation with minerals of iron oxide may be an important protective mechanism in the long haul on a large scale. At this stage, there is the formation of stable complex compounds that can include heavy metals with their excretion from the biochemical migration cycle in the environment (Chernysh et al., 2017–2018).

CONCLUSIONS

To reduce the man-made impact on the environment caused by phosphogypsum waste, the scientific bases of the ecological-synergistic approach to the system of environmentally safe waste treatment have been grounded, and biotechnologies of complex phosphogypsum recycling in environment component purification systems have been developed. The monograph presents scientific ideas, conclusions, and recommendations that, when combined, represent new scientifically valid results in the field of environmental security.

The directions of phosphogypsum (as a secondary raw material) impact on the environment, both in traditional areas of its application and in alternative technological solutions within the framework of the sustainable waste treatment concept, have been ecologically formalized. In particular, models of the impact that phosphogypsum (as a secondary raw material) has in traditional technologies and environmental protection biotechnologies, have been designed. Such models allow us to determine the directions of actions aimed at reducing the negative impact that phosphogypsum has on ecosystem components.

The comprehensive research methodology was formed for phosphogypsum recycling in environmental protection biotechnologies, that includes an integrated scheme of the conduction sequence of experimental and theoretical studies, where one should note the implementation of individual technological solutions aimed at protecting aquatic ecosystems, air protection, as well as soil remediation technologies. This interrelation manifests itself in the related areas of application of phosphogypsum mineral media. A unifying model of ecological and biochemical research has been developed to allow to assess the efficiency at which phosphogypsum as a mineral substrate is utilized by various microbial groups within the framework of environmental protection biotechnologies.

Scientific substantiation has been given to the environmentally safe utilization of phosphogypsum components as the bases of mineral media – biocomposites in biotechnological environmental protection systems – that will contribute to the stable operation of technological systems, eliminate the need for additional nutrients, and will allow to locally increase the level of environmental safety. At that, substantiation is also given to the environmentally safe synergistic concept of waste management upon its combined recycling and treatment. Several groups of biogenic composites that consist of a mineral base – dihydrate phosphogypsum with the addition of other technogenic organic and mineral waste, have been developed. We have identified the mechanisms of ecological and biochemical transformations of phosphogypsum components, making it possible to demonstrate the efficiency of using modified phosphogypsum granules as sources of macro and trace elements that are essential for stimulating the development of the required ecological and trophic microbial groups. The basic principles of the method for obtaining such phosphogypsum-based media are protected by a Ukrainian invention patent.

Scientific substantiation is given to the possibility of reducing the man-made environmental impact associated with the use of phosphogypsum for intensifying the conversion process of sulfur compounds into biosulfur in gas biopurification systems, which ensures the formation of a new direction of its use in air protection biotechnologies with the creation of an environmentally safe technology of phosphogypsum treatment and the generation of valuable products. We have conducted ecological and biochemical research of the process of microbial matrix development on modified phosphogypsum granules and given scientific justification to several biochemical transformations that, upon using phosphogypsum granules with a modified surface, allow increasing the performance of biodesulfurization systems to broaden the scale of their application in technological systems of environmental protection.

Scientific and theoretical substantiation is given to the possibility of reducing the man-made environmental impact associated with the recycling of phosphogypsum in the systems of anaerobic wastewater and sewage sludge stabilization aimed at intensifying the detoxification and phosphorus removal processes. Theoretical and experimental justification is given to the mechanisms of stimulating the process of phosphate release under anaerobic conditions by introducing the system with dehydrate phosphogypsum and, correspondingly, stimulating the development of sulfate-reducing bacteria that excrete their metabolic products into the environment, thus indirectly stimulating the activity of phosphate-mobilizing bacteria and intensifying the release of phosphate-ions into the solution, which occurs on the bases of system self-organization and autocatalysis within the framework of the development of the synergistic research concept.

The formalized ecological and biochemical model was developed for the hydrolysis of polymeric compounds of sewage sludge under the effect of a phosphogypsum supplement within the framework of aquatic ecosystem protection technologies, as well as the corresponding technological solutions for phosphorus removal, including the aerobic-anaerobic conversion process aimed at providing a microbial spatial succession and trophic chain of a hydrobionta with the involvement of technogenic mineral resources (phosphogypsum) aimed at stimulating the development of essential ecological and trophic groups.

The ecological and biochemical mechanisms were determined of heavy metal fixation in the “soil-modified biocomposite” system and identified a number of protective mechanisms that determine long-term fixation of heavy metals in insoluble or low-solubility organic-mineral aggregate structures. The role of the biotic component of the soil complex in the transformation of organic matter and the biochemical binding of toxic substances has been identified.

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