

Article

Application of Granular Microbial Preparation and Silicon Dioxide Analcime for Bioremediation of Ecocide Areas

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Abstract: As a result of the Kakhovka dam explosion, a huge area of soil was contaminated with toxic organic waste of various origins. The sustainability of soil ecosystems affected by floods requires effective approaches to eliminate the consequences as quickly as possible. Therefore, the goal of this work was to study the efficiency of the application of granular microbial preparation (GMP) and silicon dioxide preparation Analcime for the degradation of toxic organic waste to restore the soil after floods as well as man-made and natural disasters using model ecosystems. It is based on the combination of microbial fermentation of organic waste via GMP, improvement in soil quality via silicon dioxide preparation Analcime ($\text{Na}[\text{AlSi}_2\text{O}_6] \cdot \text{H}_2\text{O}$), followed by the application of phytoremediation methods for affected soil bioremediation. Such parameters as time detention (Td) and degradation coefficient (Dc) served to estimate the efficiency of organic waste degradation. The detoxification efficiency was determined via growth inhibition coefficients of indicator plants. The coefficient of waste degradation (Dc) via GMP was four–eight-fold higher compared to untreated variants and ranged from 35.1 to 41.8. The presence of methane in the variants of the experiment with GMP indicated the complete degradation of solid waste to final non-toxic products. The addition of GMP and Analcime enhanced the viability and antioxidant protection systems of seedlings of test plants (*Cucumis sativus* «Konkurent» and *Amaranthus caudatus* L.). The proposed approach is promising to be applied in the polluted sites of Europe or Asia for soil treatment as well as alternative energy obtaining.

Keywords: Nova Kakhovka ecocide zone; microbial degradation of organic waste; methane production; hydrogen production; phytoremediation; environmental biotechnologies



Citation: Havryliuk, O.; Bida, I.; Hovorukha, V.; Bielaieva, Y.; Liubinska, A.; Gladka, G.; Kalinichenko, A.; Zaimenko, N.; Tashyrev, O.; Dziuba, O. Application of Granular Microbial Preparation and Silicon Dioxide Analcime for Bioremediation of Ecocide Areas. *Sustainability* **2024**, *16*, 1097. <https://doi.org/10.3390/su16031097>

Received: 16 November 2023

Revised: 16 January 2024

Accepted: 25 January 2024

Published: 27 January 2024



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1. Introduction

Soil resource supports terrestrial ecosystems and allows for the primary production of more than 90% of food, fodder, and fibers. Preservation of soil physical, chemical, and biological fertility is a key condition for social stability and economic development. Military actions destroy natural resources and entire ecosystems on which the economy, agriculture, life, and health in general depend [1]. Pollution of soil is a widespread problem in both Europe and Asia. The destruction of the Kakhovka dam on 6 June 2023 is considered the

largest artificial disaster in several decades [2]. This event has already been classified as an ecocide [3,4]. In total, the Kakhovka Reservoir contained 18 cubic km of water [5]. It provided irrigation for the southern regions of Ukraine and northern Crimea via the North Crimean Canal, the Kakhovka Canal, and the Dnipro–Kryvyi Rih Canal and was also used to cool the 5.7 GW Zaporizhzhya Nuclear Power Plant [6,7]. The explosion of the Kakhovka dam caused a powerful flood, as a result of which significant areas under the dam along both banks of the Dnipro River and nearby were flooded; in particular, four cities were partially flooded: Nova Kakhovka, Oleshki, and Gola Prystan on the left bank as well as Kherson and several dozen villages on the right bank [8]. Instead, the 2155-square-kilometer reservoir, which was a habitat for numerous species of fish and other animals, rapidly dried up [9]. All these events led to the following consequences: the destruction of the fauna and flora of a very large area; pollution of the flooded territories with toxic substances, in particular, heavy metals; a sharp change in the groundwater regime; the permanent coverage of rural and urban areas with a layer of sludge up to 2 m thickness; permanent changes in the microclimate of the territories along the Dnipro River [9,10]. However, the most hazardous consequence of these events was the accumulation of huge amounts of organic matter of various origins [11]. It includes plant biomass [12], cesspool waste, carcasses of fish and farm and wild animals [13], as well as human corpses [14]. In addition, as a result of the flood, old cemeteries were washed away, and huge volumes of sewage and fecal matter were released into water streams [15]. The catastrophic consequences are still threatening because the accumulation of untreated organic compounds contributes to the reproduction of pathogenic microorganisms and outbreaks of epidemiological diseases, such as cholera [16]. The lack of implementation of appropriate specially developed scientifically based methods to regulate the state of water ecosystems and soil will inevitably lead to the total degradation of the unique ecosystem of the southern regions of Ukraine [17], as well as the long-term unsuitability of these territories for habitation [18]. Problems related to natural or accidental floodings impact human settlements globally [19], and according to the Organization for Economic Co-operation and Development (OECD), damages from floods annually impact hundreds of square kilometers with imposing costs in the order of 40 billion dollars [20]. In these regards, strategies for the sustainable management of soils impacted by floods include technologies aiming to accelerate the biodegradation of organic pollutants for a fast recovery of soil fertility. Among technologies, while digging and dumping of surface deposited sediments are feasible for relatively small areas, sustainable remediation of large areas impacted areas such as that of the Kakhovka accident zone can only be based on cost-effective natural-based solutions. The aim of this work was to study the efficiency of the application of granular microbial preparation (GMP) and silicon dioxide preparation Analcime for the degradation of toxic organic waste to restore the soil after floods, assessed by the laboratory microcosms. We hypothesize that the combined use of microorganisms and phytoremediation can result in providing soil restoration in the Kakhovka ecocide zone. The obtained results will be useful for the purification of polluted areas in Europe and Asia.

2. Materials and Methods

2.1. Characteristics of the Granular Microbial Preparation

For waste fermentation, a modification of GMP based on a diversified methanogenic microbiome was used (Figure 1). Digested methane tank sludge was used as an inoculum source to produce the preparation. The sludge was collected at the Bortnytska Aeration Station of Kyiv (Ukraine). The preparation was made by employees of the Department of Biology of Extremophilic Microorganisms of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.



Figure 1. The appearance of the granular microbial preparation used in this study.

The GMP is dry cylindrical granules with a size of $2.0\text{--}3.0 \times 4.0\text{--}25.0$ mm.

The GMP contained a diversified microbiome, initial nutritive substrate, and regulators of microbial metabolism. The distinctive feature of the GMP was a diversified microbiome that provided the decomposition of polymer compounds of plant and animal origin to end products (CH_4 , CO_2 , and H_2O) through the functioning of syntrophic metabolism of chemoorganotrophic microorganisms. The GMP includes the following physiological groups: aerobic heterotrophic ($3.2 \times 10^6\text{--}7.8 \times 10^6$ CFU); facultative anaerobic heterotrophic ($2.2 \times 10^6\text{--}5.4 \times 10^6$ CFU); obligate anaerobic heterotrophic ($7.4 \times 10^5\text{--}2.1 \times 10^6$ CFU); denitrifying ($6.2 \times 10^4\text{--}3.1 \times 10^5$ CFU); dissimilatory sulfate-reducing microorganisms ($7.2 \times 10^3\text{--}3.8 \times 10^6$ CFU); and methanogenic bacteria ($6.3 \times 10^4\text{--}2.1 \times 10^5$ CFU). The GMP includes dry organic compounds (e.g., 1% polymer carbohydrates) that provide the rapid growth of the microorganisms, thereby facilitating the effective degradation of toxic compounds (solid and liquid organic waste) with the production of valuable energy carriers (hydrogen or methane). The regulators of microbial metabolism are non-toxic compounds that shift microbial metabolism toward the rapid hydrolysis (degradation) of solid and liquid organic compounds (environmentally hazardous waste).

2.2. The Characteristics of the Analcime Preparation

Analcime preparation is the highly dispersed silicon mineral with the formula $\text{Na}[\text{AlSi}_2\text{O}_6] \cdot \text{H}_2\text{O}$. According to the manufacturer's instructions (Crop Terravita GmbH, Solingen, Germany), the preparation has the following composition, %: 41.29–41.86 SiO_2 ; 0.34–1.23 Na_2O ; 0.005–0.006 Co; 0.0030–0.0040 Ti; 1.70–1.75 TiO_2 ; 3.12–3.14 K_2O ; 0.007–0.009 Ni; 0.0239–0.0246 Zr; 10.77–11.21 Al_2O_3 ; 0.06–0.12 P_2O_5 ; 0.0048–0.0049 Cu; 0.0240–0.0349 Ba; 0.09–0.16 MgO; 0.011–0.037 S; and 0.0079–0.0079 Zn. (Active) silicon is not less than 1589 mg/kg.

2.3. Model Soils

Model polluted and affected soils were used in this study. Pollution modeling of soil carried out after the consequences of the explosion of the Nova Kakhovka dam was characterized. In the Nova Kakhovka ecocide zone, the main soil types are represented mainly by chernozem and sand. Therefore, chernozem, sand, and their mixture were used to model the soil damaged by the flood. Soil and sand were provided by the employees of the M. M. Hryshko National Botanical Garden, National Academy of Sciences of Ukraine (Kyiv, Ukraine).

To optimize the structure of model soils and immobilize microorganisms and sorb soluble toxic compounds formed during the decay of waste, the highly dispersed silicon preparation Analcime ($\text{Na}[\text{AlSi}_2\text{O}_6] \cdot \text{H}_2\text{O}$) was used (Section 2.4).

2.4. Model Solid Waste

The most epidemiologically and ecologically hazardous waste in the Nova Kakhovka ecocide zone is solid organic remains of plants, animals, corpses from cemeteries, and sewage fecal masses, which generally refer to animal and plant polymers. Therefore, a mixture of rotten fish and potatoes was used during the examination of the degradation of toxic waste (Figure 2). Fish and potatoes were purchased at Aushan supermarket (Kyiv, Ukraine).

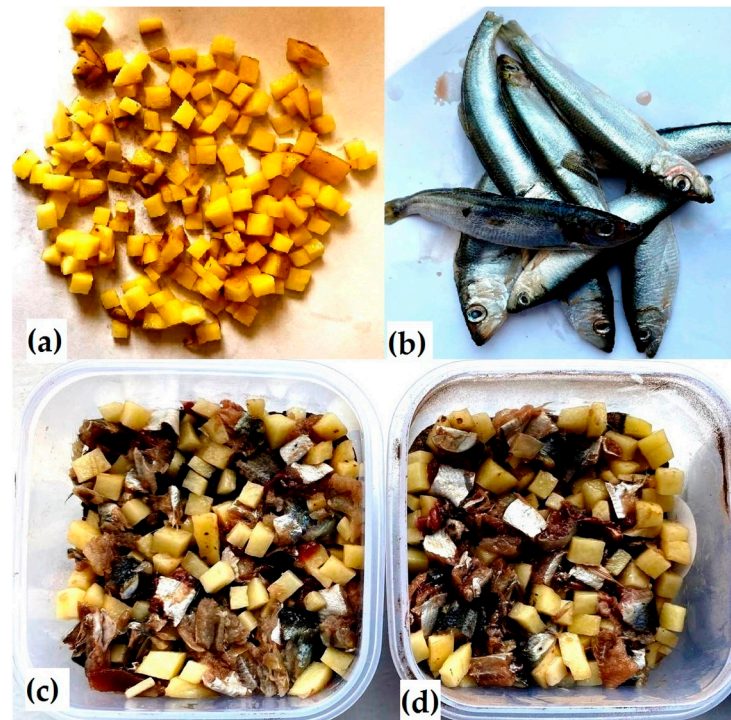


Figure 2. The experiment settings to model the degradation of environmentally hazardous organic waste in the Nova Kakhovka ecocide zone: (a) potatoes; (b) fish; (c) mixed waste (potatoes and fish), sand, GMP, and Analcime; (d) mixed waste and sand without GMP and Analcime.

2.5. Creation of Model Ecosystems

Plastic containers with a volume of 0.4 L (linear parameters: $12 \times 10 \times 7$ cm) were used to create model ecosystems polluted with organic waste on the surface. Each container was filled with 75 g of Analcime and separately with 175 g of chernozem, sand, and their mixture in a 1:1 weight ratio, forming a 2.5 cm layer (Table 1). To model the pollution, 60 g of waste with a particle size of $0.5 \times 0.5 \times 0.5$ cm (a mixture of fish and potatoes in a 1:1 weight ratio) was applied on the soil surface. The GMP (3 g) was added to the surface of the model soil to study the degradation of organics.

Table 1. The composition of model ecosystems.

№	Factors				
	Chernozem	Sand	Analcime	Fish and Potatoes	GMP
1	+ ¹	— ²	+	+	+
2	—	+	+	+	+
3	+	+	+	+	+
4	+	—	—	+	—
5	—	+	—	+	—
6	+	+	—	+	—

¹ Presence of factor; ² Absence of factor.

In the end, the containers were filled to the top with tap water to model the flood conditions. Resazurin sodium salt (Sigma-Aldrich, St. Louis, MO, USA) was used to prepare the 0.01% solution to control the redox potential during the fermentation as an indicator of anaerobic conditions because it created leucoform at $Eh < -100$ mV.

In the control variants, only 250 g of model soil (separately chernozem, sand, and their mixture in a 1:1 weight ratio) as well as 60 g of waste with a particle size of $0.5 \times 0.5 \times 0.5$ cm (a mixture of fish and potatoes in a weight ratio of 1:1) were used. Waste and the surface layer of soil were mixed to deepen the waste in the surface layer by 1 cm. Model ecosystems of soil pollution with solid organic waste are presented in Figure 3.



Figure 3. The appearance of model system of soil pollution with solid organic waste.

To measure the concentration of synthesized gas, the gas traps were installed into the containers. They consisted of a plastic container with a lid. A hole made in the lid was hermetically sealed with a rubber gasket. Gas samples were taken by piercing the rubber gasket with a syringe. To obtain statistically significant results, each model system was triplicated. The samples for the analysis were taken independently from each of them.

2.6. Measurement of the Main Metabolic Parameters of Anaerobic Degradation of Organic Waste

This study of the fermentation dynamics of the model mixture of organic waste was performed by the change in the following parameters: pH; Eh of the culture fluid; composition; and content of the synthesized gas. The values of pH and Eh were determined via the potentiometric method via the ionomer EZODO MP-103 (GOnDO Electronic, Ltd. Taiwan). The combined electrodes with BNC connectors (models PY41 and PO50, GOnDO Electronic, Ltd. Taiwan) were used to control pH and Eh, respectively [21].

The composition of the gas phase was determined using the gas chromatography method [22]. The chromatograph is equipped with two steel columns—one (I) for the analysis of H_2 , O_2 , N_2 , and CH_4 , and the second (II)—for the analysis of CO_2 . Column parameters were as follows: I – $l = 3$ m, and $d = 3$ mm, with molecular sieve 13X (NaX); II – $l = 2$ m, and $d = 3$ mm, with Porapak-Q carrier; column temperature $+60$ °C; evaporator temperature $+75$ °C; detector temperature (catharometer) $+60$ °C; detector current—50 mA; carrier gas—argon; gas flow rate— $30\text{ cm}^3/\text{min}$. The gas concentration was calculated from the peak square of the gas phase components.

2.7. Calculation of Fermentation Parameters to Evaluate the Efficiency of Decomposition of Model Organic Waste by GMP

The main indicators that made it possible to evaluate the efficiency of the fermentation of organic waste were the destruction coefficient (Dc), the concentration of hydrogen and methane, and the duration of the process (T, days). The degree of waste fermentation was assessed by determining the waste destruction coefficient (Dc). It was calculated as the ratio of the initial and final mass of model waste in terms of absolute dry mass. To calculate Dc, the conversion factor of raw mass into dry mass (Dm) was determined for the initial mixture of waste and residues that remained unfermented. Before fermentation, part of the waste mixture was weighed, dried at 105 °C to constant weight, cooled to room temperature, and then weighed again. After fermentation, unfermented solid waste particles were washed with distilled water, dried to constant weight at 105 °C and weighed. The coefficient Dm was calculated according to the following formula:

$$Dm = m_1:m_2, \quad (1)$$

where m_1 is the mass of initial wet waste, and m_2 is the mass of dry waste. The coefficient of destruction of waste (Dc) was determined as follows:

$$Dc = m_2:m_3, \quad (2)$$

where m_2 is the initial mass of dry waste, and m_3 is the mass of dry unfermented residues.

The duration of fermentation (T, days) was calculated as the time from the introduction of waste to the model ecosystems until the moment of their maximum destruction and the termination of the fermentation process.

2.8. Assessment of the Direct Toxicity of the Obtained Soil Mixtures Using Biological Tests with Plants of *Cucumis sativus* «Konkurent» and *Amaranthus caudatus* L.

Direct toxicity of the soil was performed on seeds of *Cucumis sativus* “Konkurent” and *Amaranthus caudatus* L. The soil samples were placed in Petri dishes and moistened. On the surface of the soil in each dish, 50 seeds were placed. The seeds were covered with soil from the top and moistened. Over the next 10 days, Petri dishes with the tested substrate were watered with an equal amount of water. The seedlings were carefully freed from the soil after 10 days. Then, they were rinsed, dried with filter paper, and weighed. The length of the aboveground and root parts of the plants were measured separately. Measurements of the length of the aboveground and root parts of the test plants were carried out using a ruler with an accuracy of 1 mm. Control is provided by seedlings that have grown in the soil of the control point [23].

2.9. Data Analysis

To obtain statistically significant results, each model system was replicated 3 times. The samples for the analysis were taken independently from each of the replicated model systems. All other experiments were also conducted in triplicate. The statistical analysis of data was performed via Microsoft Excel 2016. It included the calculation of mean values and standard deviations (SDs) with a 95% confidence level.

3. Results

3.1. Dynamics of Metabolic Parameters during the Degradation of Solid Organic Waste by the GMP

The GMP was used for rapid and effective degradation of environmentally hazardous multicomponent solid waste of animal and plant origin. Fish and potatoes were used to model soil pollution with solid waste in the Nova Kakhovka ecocide zone. The degradation process was estimated by a change in the metabolic parameters pH and Eh and the synthesis of gases both in the variants with the GMP and Analcime as well as in the control conditions without them (Figure 4).

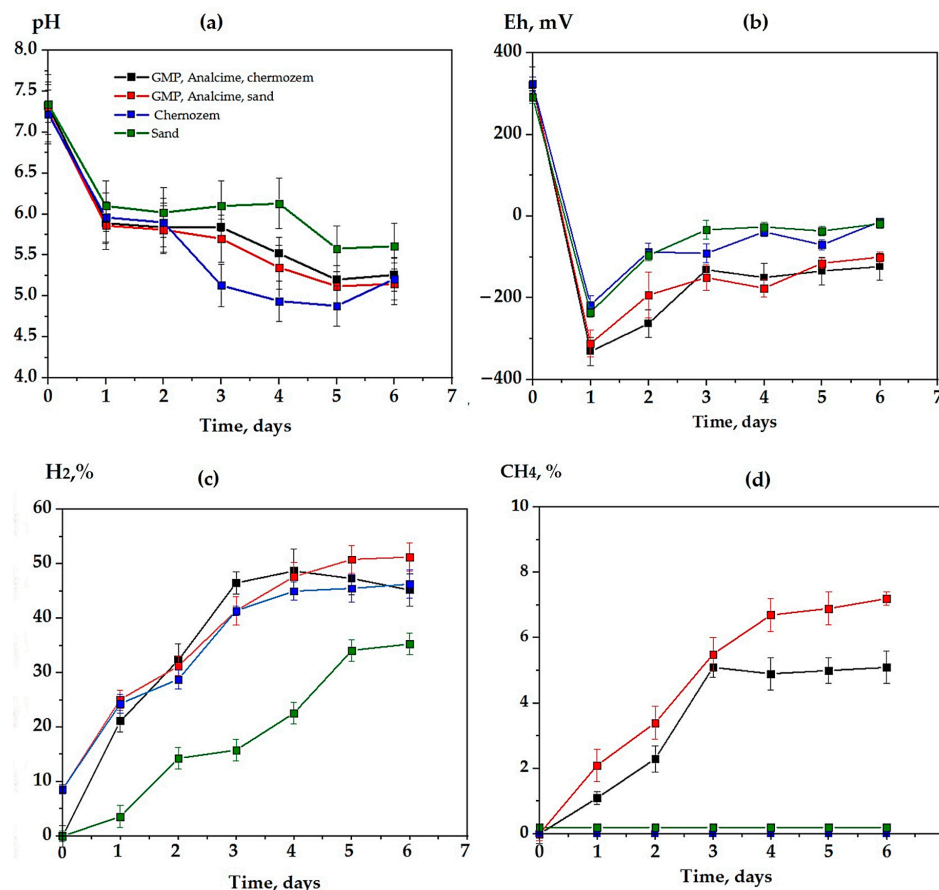


Figure 4. Dynamics of metabolic parameters pH (a), Eh (b), H₂ (c), CH₄ (d) during the degradation of solid organic waste: black line indicates waste degradation by the GMP in the presence of Analcime in chernozem; red line—waste degradation by the GMP in the presence of Analcime in sand; blue line—waste degradation without the GMP and Analcime in chernozem; green line—waste degradation without the GMP and Analcime in sand.

The synthesis of methane evidenced the high efficiency of GMP application. Synthesis of CH₄ means that the degradation of waste is complete and occurs with the formation of final non-toxic end products—CH₄, CO₂, and H₂O. In the absence of GMP, only the first stage of anaerobic degradation of waste took place. It is the hydrolysis with the formation of H₂ and toxic organic acids and alcohols (formate, acetate, methanol, ethanol, etc.) [24] in high concentration with acidification of model soil. Such compounds block the further degradation of waste to final products, are extremely toxic for all links of the food chains of natural ecosystems, and lead to their death.

Thus, the maximum concentration of methane in variants with GMP was 3.8–7.2% (Figure 4d). In all model ecosystems, pH and redox potential (Eh) decreased, which was a natural process for the growth of anaerobic microorganisms. The maximum decrease in the redox potential was observed in model ecosystems with GMP and Analcime and was −320...−310 mV (Figure 4b, 1, 2—red and black lines). In control variants (Figure 4, 4, 5—blue and green lines), the Eh also decreased but did not fall below −230...−220 mV.

Hydrogen was synthesized in all model ecosystems. However, in the models with GMP-Analcime (Figure 4c, 1, 2—red and black lines), hydrogen concentration was higher than in the control ones (Figure 4c, 4, 5—blue and green lines).

3.2. Efficiency of Model Waste Degradation via the GMP

The GMP was shown to significantly accelerate the efficiency of waste degradation compared to control model ecosystems (without GMP). Thus, the degradation coefficient (Dc) ranged from 35.1 to 41.8 in variants with the GMP. In control variants, Dc was four–

eight times lower and ranged from 5.4 to 9.1. The duration of degradation in experimental variants via the GMP and Analcime was only 6 days.

A significant advantage of the GMP is the suppression of putrefactive decomposition of solid organic waste and the growth of fly larvae and worms in the upper aerobic layer of polluted model ecosystems. The GMP contributed to the reduction in the redox potential to values of $-320 \dots -310$ mV, which was optimal for anaerobes and suppressed or completely inhibited the growth of aerobic microorganisms. In model ecosystems with GMP-Analcime, anaerobic bacteria provided complete degradation of solid organic waste, as evidenced by the presence of methane in the gas phase. In model ecosystems, the degradation of waste without GMP was accompanied by the reproduction of insect larvae and rot worms (Figure 5). This indicates another indisputable advantage of the application of the GMP for waste degradation in the Nova Kakhovka ecocide zone.

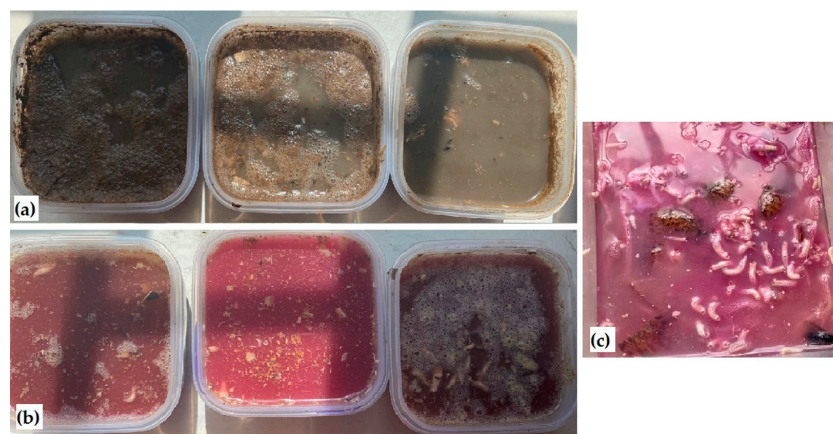


Figure 5. Degradation of solid organic waste and inhibition of its putrefaction by the GMP: (a) model ecosystems after waste degradation using GMP-Analcime; (b) control model ecosystems after waste degradation; (c) decay of waste with the reproduction of insect larvae and rotting worms.

3.3. Evaluation of the Toxicity of Obtained Soil Mixtures Using Biological Tests with Plants

The final stage of this research was to evaluate the toxicity of the obtained soil mixtures using biological tests with plants of *Cucumis sativus* “Konkurent” and *Amaranthus caudatus* L. (Figure 6). Both direct soil toxicity from all substrate variants mentioned in Table 1 were examined at water extracts from the soil in concentration ratios of 1:10 and 1:100.

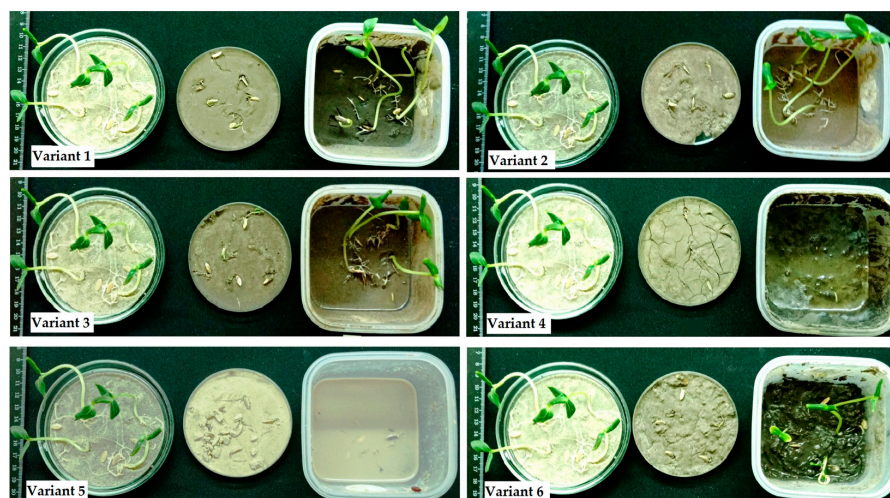


Figure 6. Direct toxicity of substrates with simulated drought and without simulated drought. Substrate options (variants 1–6) correspond to Table 1: (1–3)—model soils with GMP-Analcime preparation; (4–6)—model soils without GMP-Analcime preparation.

The influence of different concentrations and the presence of certain substances (the GMP and Analcime) on the growth of the studied plants was investigated. It was determined that water extracts from soil mixtures had varying effects on test reactions, depending on the concentration used. Without Analcime, water extracts from soil mixtures at concentrations of 1:10 and 1:100 resulted in only 25–50% of sprouted cucumber seeds. However, the addition of Analcime and GMP in variants 1, 2, and 3 (Table 1) led to active root system development in test objects, particularly at a concentration of 1:100. For amaranth seedlings, root length ranged from 60 to 90% at a concentration of 1:10, and 120–140% at a concentration of 1:100 compared to the control. Cucumber seedlings exhibited root lengths of 70–90% at a concentration of 1:10, with lateral roots varying within 60–70% compared to the control. The highest values were observed at a concentration of 1:100, with root lengths of 110–120% and lateral roots at 80–117% compared to the control.

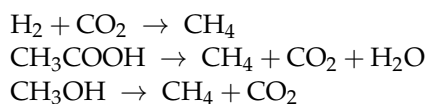
Using direct biotesting methods, samples 4–6 (Table 1, without Analcime and GMP) indicated the presence of allelopathically active substances with phytotoxic properties. Phytotoxic effects led to suppressed growth processes in test objects, affecting root length and lateral root formation. Samples 1–3 demonstrated active growth and development of both the aboveground (96–107%) and root systems (70–100%) of amaranth, respectively. There was also an increase in the numerical indicators of the mass of aboveground and root systems (95–130% and 80–110%, respectively) compared to the control. Cucumber seedlings (samples 1–3, Table 1) also showed active development of aboveground portions (110–140%), as well as growth of main roots (60–70%) and lateral roots (70–110%). The numerical indicators also increased, with aboveground mass at 60–70% and root system mass at 70–100% compared to the control.

Therefore, the addition of the GMP and Analcime promoted the growth of seedling sprouts under stressful conditions. The least direct phytotoxicity was observed in variants 1–3 (Table 1), where both GMP and Analcime were added. The protective effect of Analcime and GMP is primarily due to the activation of antioxidant defense and stimulation of root system development, indicating increased systemic resilience to adverse biotic and abiotic environmental factors. Additionally, Analcime improves the water regime of the substrate by creating an additional reservoir for moisture retention. The introduction of Analcime into the substrate significantly improved seedling growth and stimulated biomass accumulation in aboveground portions and roots, especially under optimal moisture conditions (60% field capacity). This enhancement of vitality and antioxidant defense systems in the seedlings by the studied substances demonstrates increased systemic resilience against toxic and hazardous biotic and abiotic environmental factors.

4. Discussion

These approaches intended to maintain the sustainability of soil affected by man-made or natural disasters that are of great importance now. We offered a comprehensive approach to the accelerated degradation of environmentally hazardous multicomponent decaying solid and liquid waste of animal and plant origin, as well as improvement in soil quality after the floods. It provides effective purification of contaminated soil as well as contributes to the improvement in its fertility, which is an important component of the sustainability of valuable natural resources. The basis of this microbiological method is the use of a granular microbial preparation. It is designed for the fast and effective degradation of multicomponent solid and liquid organic waste. Microorganisms of the GMP rapidly and effectively decompose a wide range of toxic organic waste, including solid and liquid polymer compounds of plant and animal origin (decaying animal and plant residues), as well as products of their hydrolysis (organic acids, amino acids, alcohols, aldehydes, ketones, etc.). Due to the diversified microbiome of the GMP, they are decomposed into final non-toxic products (H_2 , CH_4 , CO_2 , and H_2O). Rapid and effective decomposition of polymer compounds of plant and animal origin is achieved through the syntrophic (linked-sequential) metabolism of the physiological groups of microorganisms. The fermentation of organic compounds occurs in the following sequence:

- (1) Aerobic microorganisms consume organic compounds and create anaerobic (oxygen-free) conditions: $\text{CH}_3\text{COOH} + \text{O}_2 \rightarrow 2\text{CO}_2 + \text{H}_2\text{O}$ ($E_h = -50 \dots 0$ mV) [24];
- (2) Facultative anaerobic microorganisms under anaerobic conditions ferment organics and create low-potential ($E_h = -300 \dots -200$ mV) obligate anaerobic conditions through the formation of reducing agents, for example, $\text{NH}_2\text{-CH-SH}_2\text{-COOH} \rightarrow \text{H}_2 + \text{NH}_3 + \text{CO}_2 + \text{H}_2\text{O} + \text{S}^{2-}$ ($E_h = -250$ mV) [25];
- (3) Obligate anaerobic hydrolytic bacteria (*Clostridium* and others) perform the fermentation of animal and plant polymers with a significant reduction in their volume and weight [26]:
 Plant polymers: $[\text{C}_6\text{H}_{12}\text{O}_6]_n \rightarrow \text{N} \cdot \text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{CO}_2 + 4\text{H}_2$ ($E_h = -280$ mV);
 Animal polymers: $[\text{COOH-R-NH}_2] \cdot n \rightarrow \text{N} \cdot \text{COOH-R-NH}_2 \rightarrow \text{H}_2 + \text{CO}_2 + \text{NH}_4^+ + \text{NH}_3$ ($E_h = -250$ mV);
- (4) Methanogenic bacteria perform the decomposition of liquid and gaseous intermediates into final non-toxic compounds (methane, carbon dioxide, and water) [27,28]:



The ability to produce hydrogen or methane via the fermentation of organic pollutants is an additional promising feature in the development of profitable biotechnologies. Alternative energy obtained from waste is of interest to both Europe and Asia to reduce the share of fossil fuel and contribute to environmental protection.

Phytoremediation is another area that is rapidly developing in European and Asian countries. On the one hand, phytoremediation is an economically beneficial and environmentally safe technology in which plant biomass or living plants are used to remediate polluted areas [29]. On the other hand, plants are extremely sensitive to soil pollutants without a sufficient amount of plant-promoting microorganisms. Plant–microbe interactions increase the resistance of plants to extreme factors and contribute to the effectiveness of phytoremediation. Plant-promoting bacteria have already been used to purify the soil from organic pollutants [30]. Phytoremediation occurs by mechanisms including degradation (rhizodegradation, phytodegradation), accumulation (phytoextraction, rhizofiltration), dispersion (phytoevaporation), and immobilization (hydraulic control and phytostabilization) to degrade, remove, or immobilize pollutants. Depending on the nature of the pollutant, plants may use one or more of these mechanisms to reduce their concentration in soil and water [31]. Plants used for phytoremediation must possess some characteristic features, such as exhibiting stronger growth traits with high biomass, unpalatable nature, exuberant root system, and hyperaccumulation of target pollutants, accompanied by signs of stress resistance [32]. Maize (*Zea mays* L.), sunflower (*Helianthus annuus*), switchgrass (*Panicum virgatum*), hybrid poplar trees (*Populus tremula*, *Populus alba*), tobacco (*Nicotiana tabacum*), and rice (*Oryza sativa*) can absorb and accumulate organic pollutants [30].

The effectiveness of phytoremediation can be increased by the use of chelators, acidifiers, organic chemicals, fertilizers, transgenic plants, plant growth regulators, and microorganisms [33]. One of the ecologically safe and cost-effective methods to enhance crop productivity and protection is the application of silicon-containing minerals. Silicon positively influences plant growth and development as a bioelement, primarily acting as an adaptogen against abiotic and biotic stresses. Additionally, it has been established that the effectiveness of mineral fertilizers and pesticides increases when silicon-containing minerals are applied. Silicon-containing fertilizers are widely used in rice and sugarcane cultivation to improve yield and quality. In Dutch greenhouse farming, silicon was used to protect cucumbers and roses from powdery mildew and other diseases. Despite the promising prospects of silicon-containing minerals in ecological agriculture, the physiological mechanisms of their impact have not yet been fully understood, hindering their widespread

use. Herein, a natural silicon-containing mineral preparation, Analcime ($\text{Na}[\text{AlSi}_2\text{O}_6] \cdot \text{H}_2\text{O}$), was used to optimize the structure of model soils, immobilize microorganisms, and adsorb soluble toxic compounds generated during waste decomposition.

The approach presented in this research has several advantages. The granular microbial preparation was developed to accelerate fermentation of organic waste of animal and plant origin. Moreover, the GMP is convenient to use and stored for a long period because the granules do not contain moisture and have a large number of different physiological and taxonomic groups of microorganisms, in particular, spore-forming. The presented GMP has already been tested in some studies. In particular, for methane fermentation of biomass of invasive plants, in particular, *Pistia stratiotes* [34], *Ambrosia artemisiifolia* L. [35], and *Solidago canadensis* L. [36], with simultaneous detoxification of toxic heavy metals such as copper, chromium, and iron. The potential of the GMP in hydrogen production by dark fermentation has also been demonstrated. At the same time, the output of hydrogen reached 102 L/kg of solid waste and 2.3 L/L of liquid waste, and the fermentation resulted in a 91-fold reduction in the weight of the solid waste, while the concentration of organics in the liquid waste 3-fold decreased [37]. Using the GMP model, organic waste was fermented in columns for 14 days. At the same time, the weight of waste decreased by 20 times; the output of hydrogen was 27 L/kg, and the output of methane was 12 L/kg of waste [38]. Thus, the possibility of obtaining energy carriers by fermentation of invasive or phytoremediant plants is a separate interesting direction that contributes to the further development of renewable energy and environmental protection in Europe and Asia.

The application of highly dispersed silicon preparation Analcime in this research provided optimization of the structure of model soils, immobilization of microorganisms, and sorption of soluble toxic compounds. The combination of granular microbial preparation and silicon preparation Analcime ensured the effective fermentation of model waste and stimulated the growth of the investigated phytoremediation plants. The technology for the treatment of contaminated soils via the application of silicon-containing fertilizers has been developed. A patent for the invention to optimize the agrophysical, agrochemical, and biological components of the soil ecosystem was registered. This development is acceptable for the treatment of soils affected by hostilities [39].

In the literature, the process of phytoremediation is found mostly for soil purification from toxic metals. Thus, inoculation of *Helianthus annuus* seeds with *Pseudomonas citronellolis* SLP6 significantly enhanced plant growth, chlorophyll content, antioxidant enzyme production, and Cu accumulation potential under Cu contamination with and without salt stress [40]. Due to inoculation with soil rhizobacteria, the rate of dispersion of pyrene and the rate of removal of Ni in soils contaminated by the *Scirpus triquetra* plant increased. The removal rate of Ni in one of the Ni-contaminated soils was increased from 0.895‰ to 8.8‰ [41]. Phosphate-solubilizing bacterium significantly promoted the growth of *Wedelia trilobata* and improved its phytoremediation efficiency in Cu-contaminated soil through a positive effect on soil microflora, improving soil quality [42]. The multi-metal-tolerant *Bacillus cereus* significantly has a positive effect on the physiology, growth, and phytoremediation ability of *Chrysopogon zizanioides* on metal-contaminated soil due to the fabrication of hydrogen cyanide, siderophore, Indole Acetic Acid, N_2 fixation, as well as P solubilization [43].

The GMP manufacturing is cost-effective. The basis of this preparation is the biomass of digested methane tank sludge. This is municipal sludge, which is organic waste that often requires disposal [44,45]. However, for industrial and bioremediation technologies, digested sludge is a macronutrient source in sustainable plant biomass production [46], a suitable source for microbial preparations and fertilizers manufacturing [47,48], as well as building material for architecture applications [49,50]. The effectiveness of the preparation during its application may likely depend on weather conditions, in particular temperature and rainfall. In addition, the effectiveness could be decreased in dry weather or during application in arid regions. However, this preparation and biotechnological approach

should be applied immediately after floods to accelerate the effective degradation of organic waste in wet soils.

The proposed approach showed high efficiency of the bioremediation of model soil affected by flood, contributing to the accelerated restoration of polluted ecosystems in the Nova Kakhovka ecocide zone.

5. Conclusions

The application of the GMP with Analcime for the degradation of waste in the zone of Nova Kakhovka ecocide is a promising approach for the accelerated purification of soil from toxic waste contributing to its fertility and sustainability. The addition of the GMP and Analcime enhanced the viability and antioxidant protection systems of seedlings of test plants, which provides increased systemic resistance to toxic and hazardous biotic and abiotic factors of the environment. The combination of the microbiological method of fermentation of organic waste via GMP and Analcime is promising for the development of new bioremediation approaches to the restoration of a large area of soil in the Nova Kakhovka ecocide zone. The proposed combined GMP-Analcime preparation can be applied in the polluted sites of Europe or Asia for soil treatment as well as obtaining renewable energy from organic waste.

Author Contributions: Conceptualization, O.T., O.D. and N.Z.; methodology, O.T. and O.D.; software, O.H., V.H., I.B. and A.K.; validation, O.H., I.B., G.G., Y.B. and A.L.; formal analysis, O.H., I.B., V.H. and A.K.; investigation, O.H., I.B., G.G., Y.B. and A.L.; data curation, O.H., I.B. and V.H.; writing—original draft preparation, O.D., O.T., I.B., O.H. and V.H.; writing—review and editing, O.D., O.T., N.Z., I.B., O.H., V.H., A.K., G.G., Y.B. and A.L.; supervision, O.T., O.D. and N.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: The authors are grateful to the Presidium of the National Academy of Sciences of Ukraine for the encouragement of the bioremediation research of the Department of Extremophilic Microorganisms Biology of the D.K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine.

Conflicts of Interest: The authors declare no conflicts of interest.

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