



Review

## Prospect Research on the Diversity of Extracellular Mineralization Process Induced by Mineralizing Microorganisms and Its Use as a Treatment for Soil Pollutants

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Abstract: Microbial-induced mineralization is a process in which metal ions in the environment are processed by microorganisms, forming deposits of crystals with cementing and void-filling functions. Cementing crystals can fix metal ions, reduce permeability, improve soil strength, and play a positive role in soil remediation and pollution control. This paper first introduces the principle of microbial-induced mineralization and analyzes its mechanism of action in the treatment of soil organic and inorganic pollutants. Then, the mineralization principle of different types of mineralized bacteria in soil (fungal metabolism involving organic acid complexation and metabolic urease catalysis, sulfur oxidation by sulfur-oxidizing bacteria, dissimilatory sulfate reduction by sulfate-reducing bacteria, ammonification by ammoniating bacteria, reverse digestion by denitrifying bacteria, urease catalysis by urease-producing bacteria, acetic acid fermentation by methanogenic bacteria, and  $\rm H_2/CO_2$  reduction) is elaborated, the influencing factors in the treatment of soil pollutants by mineralization technology in practical application are analyzed, and the current status of mineralization treatment for different types of pollutants is summarized. Finally, the future prospects of soil pollutant treatment are outlined to promote research into microbial-induced mineralization technology for the treatment of soil pollutants.

**Keywords:** microbial-induced mineralization; MICP; soil improvement; pollution prevention; mineralizing bacteria



Citation: Guo, B.; Liu, B.; Chen, J.; Jing, C.; Zhong, M.; Shan, Q. Prospect Research on the Diversity of Extracellular Mineralization Process Induced by Mineralizing Microorganisms and Its Use as a Treatment for Soil Pollutants. Sustainability 2023, 15, 4858. https://doi.org/10.3390/su15064858

Academic Editors: Qichun Zhang and Touqeer Abbas

Received: 8 November 2022 Revised: 24 February 2023 Accepted: 4 March 2023 Published: 9 March 2023



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## 1. Introduction

Soil pollutants mainly include inorganic substances (salt; alkali; acid; F and Cl; heavy metals such as Hg, Cd, Cr, As, Pb, Ni, Zn, and Cu; and radioactive elements such as Cs and Sr) and organic substances (organic pesticides, petroleum, phenols, cyanide, benzo(a)pyrene, organic deterrents, pathogenic microorganisms and parasitic eggs, etc.) [1]. From April 2005 to December 2013, the Ministry of Environmental Protection and the Ministry of Land and Resources jointly conducted a survey on soil pollution in China. The results showed that the total soil pollution in China was mainly non-organic pollution, followed by organic pollution, and the proportion of other pollution was relatively small. Soil pollution is more prominent in abandoned industrial land, and the main pollutants in different types of industrial land and surrounding areas are different, mainly zinc, mercury, lead, chromium, arsenic, and polycyclic aromatic hydrocarbons [2]. Most heavy metal pollutants in soil not only distribute and accumulate at the pollution sources but also diffuse to the surrounding areas to different degrees [3,4]; living in areas where heavy metals exceed the standard for a long time will cause gene mutation and even carcinogenesis. At the

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same time, metal pollutants, unlike ordinary pollutants, can be degraded and accumulate in soil, crops, and the human body through circulation, posing a great threat to human health [3,5]. In industrial and agricultural production, due to the use of non-standard treatments and accidents in oil exploitation and transportation, up to 36 million hm<sup>2</sup> of farmland is polluted by pesticides, petroleum hydrocarbons, and polycyclic aromatic hydrocarbons [6]. These organic pollutants quickly become difficult to remove from the soil after diffusion, migration, adsorption, and desorption, resulting in their distribution and continuous accumulation in soil at different depths [7–10]. As with inorganic heavy metal pollution, organic pollutants also have strong carcinogenicity, especially polycyclic aromatic hydrocarbons. Due to their lipid solubility, they can migrate, metabolize, and accumulate in plants and seriously endanger health through the food chain [11]. Microbial-induced mineralization can fix heavy metal ions by microbial action [12,13]. At the same time, microbial mineralization technology can also significantly reduce soil permeability, and some studies have pointed out that the permeability coefficient after mineralization can reach the order of  $1 \times 10^{-7}$  cm/s [14,15], which has a great effect on the control of the diffusion and migration of organic and inorganic pollutants and treatment of soil pollutants [14]. At present, the treatment of soil pollutants mainly includes immobilization/stabilization technology, ectopic elution technology, permeable reaction wall technology, etc. Although these technologies can achieve the purpose of soil pollutant control to a certain extent, they also have major drawbacks, requiring high resource consumption, destroying the original ecology, and easily producing secondary pollution [16–18]. Microbial-induced mineralization technology can effectively fix and store inorganic heavy metal pollutants; the resulting minerals have a strong control effect on the migration and diffusion of inorganic substances, do not easily produce secondary pollution, have strong stability, have great application prospects for the treatment of soil pollutant engineering, and are low cost. Therefore, this paper summarizes the mineralization of a variety of soil mineralization microorganisms and the influencing factors and research status, proposes the feasibility and effectiveness of mineralizing microorganisms in the treatment of soil organic and inorganic pollutants, and finally puts forward the prospect of its future development.

## 2. Mechanism of Microbial Mineralization to Control Pollutants

Microbial-induced mineralization involves a series of biological metabolic and chemical reactions. The basic principle of microbial-induced mineralization is that urea is decomposed by microorganisms capable of producing urease to produce CO<sub>3</sub><sup>2-</sup> and NH<sup>4+</sup>, resulting in an increase in local environmental pH [19,20]; it reacts with divalent metal ions in the surrounding environment to form carbonate precipitation [21]. Microorganisms play a very important role in the mineralization process; it not only secretes urease to decompose urea but the microbial cell itself is also a kind of colloidal substance with a negative charge (-COOH, -OH, C=O) [22,23], and divalent metal ions in the solution environment can be aggregated in the surrounding environment of cells through adsorption and electrostatic attraction (Figure 1a) [24], supersaturation of the local area, and the formation of carbonate precipitated crystal nuclei. With the continuous decomposition of urea by urease, CO<sub>3</sub><sup>2-</sup> and divalent metal ions around microorganisms constantly react to form calcite precipitation. During the reactions, bivalent and other-valence heavy metal ions are encapsulated, leading to consolidation and co-precipitation; this results in the formation of stable structures of heavy metals containing carbonate mineral precipitates, eliminating heavy metal pollutants in the soil, as shown in Figure 1b [22,25–29]. The generated mineral precipitates block pores in porous media, greatly reducing their permeability [14], reducing the diffusion and migration of organic pollutants in the porous media, and effectively controlling the levels of organic pollutants in the soil [30,31]. The main biochemical reactions in microbial mineralization technology can be expressed by simplifying the following equation (taking  $Ca^{2+}$  as an example) [32]:

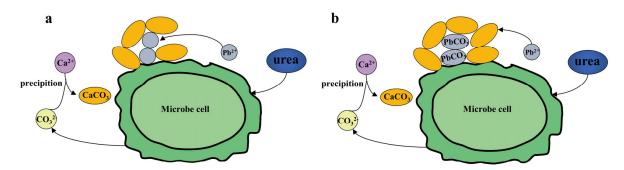
$$CO(NH_2)_2 + 3H_2O \rightarrow 2NH_4^+ + HCO_3^- + OH^-$$
 (1)

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$$HCO_3^- + H_2O + OH^- \rightarrow CO_3^{2-} + 2H_2O$$
 (2)

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+}$$
 (3)

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow Cell - CaCO_3$$
 (4)



**Figure 1.** Microbial mineralization [22]. (a) is the direct encapsulation of metal ions during microbial mineralization, (b) is the formation of other carbonates coprecipitated with calcium carbonate.

#### 3. Soil Mineralization Microorganisms

Microbial mineralization is everywhere. It is a common phenomenon in nature: fungi, algae, sulfur-oxidizing bacteria, sulfate-reducing bacteria, ammonifying bacteria, denitrifying bacteria, iron-oxidizing bacteria, and urease bacteria have the mineralization ability; from shell, bone, and teeth to limestone caves, the known forms of biological mineralization include more than 60 kinds of minerals. They are formed by microorganisms through direct or indirect mineralization through photosynthesis, sulfur oxidation, sulfate reduction, ammonification, nitrate reduction, iron oxidation, urea decomposition, etc. [33–35]. In addition to cyanobacteria and other algae and iron-oxidizing bacteria, the mineralization process mainly occurs in water environments; other microbial mineralization processes occur in the soil. The microbial decomposition of urea and calcium carbonate precipitation caused by urease bacteria are the most studied [12,36,37].

## 3.1. Fungi

Fungi have a wide variety of applications, and up to a million species of fungi have been found, which play an important role in agriculture, forestry, animal husbandry, medicine, and other aspects [38–40]. For microbial-induced mineralization techniques, although most of the current attention has been focused on bacteria-induced biomineralization, fungi are also involved in this process. Fungi produce organic acids, such as oxalic acid, and contribute to the formation of various metal complexes, such as metal-oxalate, while some urease-positive fungi also have the effect of producing urease to decompose urea and produce carbonate [41,42]; moreover, fungi have higher biomass and metal tolerance, which can be used as the best potential candidate for microbial mineralization of soil heavy metals [43].

## 3.2. Sulfur-Oxidizing Bacteria

The current research on sulfur-oxidizing bacteria (SOB) mainly focuses on autotrophic bacteria. However, the heterotrophic type has a faster growth rate and stronger anti-interference ability than the autotrophic type and is widely distributed and abundant in nature. For example, the existence of heterotrophic sulfur-oxidizing bacteria has been found in mining areas, lakes, soils, oceans, and other environments [44,45]. Sulfur atoms have six electrons(e<sup>-</sup>) in their outermost shell, which can bond in many ways to form elemental or multiple valence values, such as -2, 0, +2, +4, and +6. SOB can completely oxidize the low-priced, reduced sulfide or elemental sulfur to sulfate (SO<sub>4</sub><sup>2-</sup>) and then form metal salts with divalent metal ions in the environment and realize metal recovery through the leaching process [45–48]. At present, the research on sulfur-oxidizing bacteria is mainly

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focused on the treatment of wastewater and waste gas, and the treatment of heavy metal pollutants in soil is less studied [49–51].

#### 3.3. Sulfate-Reducing Bacteria

Sulfate-reducing bacteria (SRB) are a kind of anaerobic heterotrophic bacteria with strong vitality, which are widely distributed worldwide. This is especially true in anoxic land and water environments, such as soil, seawater, underground pipelines of river water, and extreme anaerobic environments rich in organic matter and sulfate, such as oil and gas reservoirs, rivers and lakes, and marsh mud. At present, there are hundreds of known sulfate-reducing bacteria [52]. SRB in an anaerobic environment can take organic matter as an electron donor, absorb extracellular SO<sub>4</sub><sup>2</sup>-dissimilar sulfate, and reduce it to generate H<sub>2</sub>S and exclude extracellular mineralization [53]; at the same time, HCO<sub>3</sub><sup>-</sup> is produced, H<sub>2</sub>S is a weak acid, and degassing leads to an increase in pH, eventually leading to carbonate precipitation [54–56]. If  $H_2S$  is not released as a gas, it reacts with heavy metal ions to form an insoluble metal sulfide precipitate [57,58]. Meanwhile, chlorinated organic compounds, mono-aromatic compounds, polycyclic aromatic hydrocarbons, and alkanes can also be used as SRB electron donors [59]. The process is shown in Figure 2. It has a certain potential to treat organic pollutants as well as heavy metal pollutants. At present, its application is mainly in the treatment of heavy metal pollution, and the treatment effect is remarkable [60–64].

$$2(CH_2O) + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- + CO_2 + H_2O$$
 (5)

$$H_2S + M^{2+} \rightarrow 2H^+ + MS$$
 (6)

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \tag{7}$$

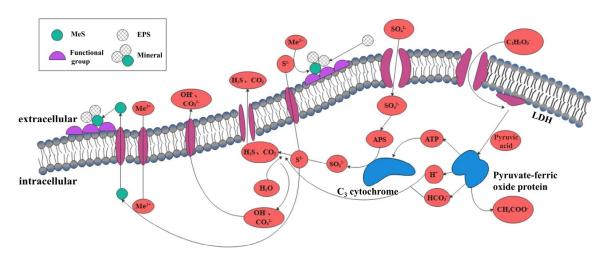


Figure 2. Metabolic process of sulfate-reducing bacteria [65].

## 3.4. Ammonifying Bacteria

Ammonifying bacteria, as facultative anaerobic bacteria, are widely distributed in soil and water systems [66]. As an important part of the nitrogen cycle, excreta produced by humans and animals on land and water, as well as decomposing animal and plant carcasses, are transformed into NH<sub>3</sub> by ammoniating bacteria, and  $CO_2$  is also produced in environments in which  $O_2$  is involved [67,68]. NH<sub>3</sub> hydrolysis increases the environmental pH and induces the formation of carbonate, which reacts with divalent cations in the environment to form carbonate precipitates [55]. At present, there are few studies on

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ammonifying bacteria, few studies focus on environmental nitrogen cycling [69,70], and research on microbial mineralization is limited.

$$RCHNH2COOH + O2 \rightarrow RCOOH + CO2 + NH3$$
 (8)

$$RCHNH_2COOH + H_2O \rightarrow RCHOHCOOH + NH_3$$
 (9)

## 3.5. Denitrifying Bacteria

Denitrifying bacteria are equally widespread in nature and are abundant in soil, manure, and sewage. When soil oxygen is insufficient, nitrate is used as an electron acceptor through reduction to reduce various products, such as nitrite, ammonia, nitrogen, etc., and produces  $CO_2$ , while improving the environmental pH [71]. In alkaline environments,  $CO_3^{2-}$  generated from  $CO_2$  reacts with divalent metal ions to form carbonate precipitation [72–74]. This mineralization process has the potential to degrade organic matter and has the advantages of high efficiency and economy. However, the current research on denitrification is mainly focused on denitrification, and the research on its mineralization is limited [75–78].

$$CH_3COO^- + 2.6H^+ + 1.6NO_3^- \rightarrow 2CO_2 + 0.8N_2 + 2.8H_2O$$
 (10)

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \tag{11}$$

$$Ca^{2+} + HCO_3^- + OH^- = CaCO_3 + H_2O$$
 (12)

#### 3.6. Urease Bacteria

The mineralization and removal of heavy metal contamination by urease-producing bacteria are the most widely studied and mature technologies. Urease-producing bacteria, including Bacillus, Sporobacteria, Enterobacteria, and Pseudomonas, are widely present in various soil environments [79–82]; in the process of metabolism, urease is secreted to accelerate the hydrolysis of urea, increase the environmental pH, and produce  ${\rm CO_3}^{2-}$  to form carbonate precipitation [29,83,84]. This process is shown in Figure 3 [85,86]. Ureahydrolyzation-induced mineralization by urease bacteria has many advantages compared with other mineralization pathways, such as having a simple mechanism, low cost, and the ability to produce a large amount of carbonate precipitation in a very short time as well as green environmental protection. Therefore, urease bacteria are widely used in the field of induced mineralization [32].

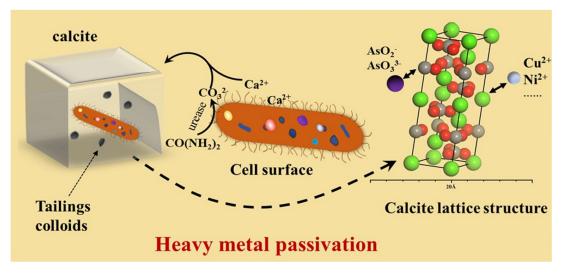


Figure 3. Urease-producing bacteria [86].

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The isolation, screening, and application of urease-producing bacteria have been studied systematically by many scholars. Most scholars use urea as a medium to coat a diluted soil suspension, separate it, and then determine its urease production ability. The 16S RNA gene is used to identify it and determine the species of bacteria.

N. Jalilvand et al. isolated four urease-producing bacteria from calcareous soils in Iranian mines and studied them by buying *Sporosarcina pasteurii* PTCC1645 (DSM33). It was found that, among these isolates, *Stenotrophomonas rhizophila* (A323) and *Variovorax boronicumulans* (C113) produced the highest amounts of carbonate minerals of heavy metals. *S. pasteurii* had the highest removal rate of Pb, Cd, and Zn, with a removal rate of about 95% [79]. Eshetu Mekonnen et al. screened three strains (*Bacillus paramycoides*, *Citrobacter sedlakii*, and *Enterobacter bugandensis*) with high urease production abilities from Ethiopian soil with a wide range of growth conditions (pH (4.0–10.0), NaCl (0.25–5%), and temperature (20–40 °C)) [87]. Fatemeh Elmi et al. screened a new multiextremophile, *Bhargavaea cecembensis*, from soil samples in desert areas of Iran. This strain can grow at a temperature of 50 °C, pH of 9–11, and NaCl of 20–25% *w/v* [88]. Ignatius Ren KaiPhang et al. isolated five strains of Bacillus urease from an acidic peat environment, which could produce urease at a low pH [89].

Many of the above studies have concluded that strains of different genera screened in different environments are quite different, so the use of selected bacteria in the bioremediation of contaminated sites may be more effective. Although there have been many studies on these bacteria, more systematic strain-application parameters have not been obtained in many studies, so there remains much room for further research.

#### 3.7. Methanogens

Most methane on Earth comes from methanogenesis by microorganisms, which are widely distributed in marine freshwater sediments, paddy soil, the animal gastrointestinal tract, and geological and geothermal environments [90,91]. There are three ways of methanogenesis, and methane in nature mainly comes from acetic acid fermentation and  $\rm H_2/CO_2$  reduction pathways, which simultaneously react with an increase in pH [92,93]. Carbonate precipitation can be formed in this environment [94,95]. At present, there are many studies on the mechanistic mineralization of methanogens, and the mineralization ability of methanogens is unquestionable [96,97], but its application in the treatment of heavy metals has not yet occurred, which is a huge development prospect.

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$$
 (13)

$$CH_3COOH \rightarrow CH_4 + CO_2$$
 (14)

$$Ca^{2+} + 4H_2 + 2HCO_3^- \rightarrow CaCO_3 + CH_4 + 3H_2O$$
 (15)

#### 4. Influencing Factors of Mineralization in Soil

The process of microbial-induced mineralization is affected by a variety of factors, and the effects of microbial mineralization under different factors vary greatly, thus greatly affecting the efficiency of soil pollutant control. Therefore, considering the relatively controllable factors such as the concentration and composition of the reaction solution, temperature, properties of cementing medium, and injection method in practical engineering application, it is of great help to realize effective treatment in its application.

## 4.1. Reaction Solution Concentration and Composition

In the microbe-induced mineralization, the reaction solutions are mainly bacterial liquid and cementation liquid, and microorganisms play two roles in the formation of crystals. First, the microbial body acts as a nucleation site during crystal formation and simultaneously decomposes urea in solution, releasing a large amount of  $HCO_3^-$ ,  $CO_3^{2-}$ , and  $OH^-$  [98]. Second, the EPS matrix and other organic matter secreted by bacteria and some negative ion groups can attract  $Ca^{2+}$  to act as the nucleation site of crystals [99];

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they also regulate the type and morphology of calcium carbonate crystals [100–103]. The concentration and composition of the cement are mainly reflected in the microscopic crystal type, appearance, size, and crystal distribution in the cement [102,104–106]; macroscopically, it is mainly reflected in the amount of calcium carbonate, compressive shear strength, and permeability [107,108].

Rowshanbakht studied the effect of bacterial concentration on the size of calcium carbonate crystals. He observed that the average crystal size increased with the increase in bacterial concentration, and the strength of mineralized soil also increased [109]. When Okwadha was diluted with deionized water with bacterial concentrations of  $10^6$ ,  $10^7$ , and 108 CFU/mL to induce calcium carbonate deposition, it was found that the urea decomposition rate and calcium carbonate precipitation amount were positively correlated with the concentration of the bacterial solution, and when the urea and calcium ion concentration reached a certain level, the concentration of the bacterial solution was the main factor determining the amount of urea decomposition and calcium carbonate production. These two different test results indicate that the activity and calcium carbonate production rate of the bacterial solution will be affected by the culture batch, but the activity of the bacterial solution obtained by the dilution of the same batch has a great correlation with the concentration [110]. LM Lee used 0.25 M, 0.5 M, and 1 M bacterial concentrations of cementing fluid to solidify silty residual soil. The experimental results showed that the experimental shear strength was stronger with the increase in bacterial concentration and began to decrease when the concentration was higher than 1 M [111]. Ng used Bacillus giant to cement sand soil and found that calcium carbonate was mostly deposited on the intergranular contact points of sand particles at concentrations of 0.50 mol/L and 0.25 mol/L cementing fluid, and the deposition of calcium carbonate at a 0.50 mol/L concentration was more and more compact than that at a 0.25 mol/L concentration [112].

Han-Jiang used Sporosarcina pasteurii to cement Ottawa sand with different concentrations (0.25-3 M) of cementing fluid. The experimental results showed that with the increase in concentration, the structural strength of the sample increased first and then decreased, and the cement effect of 1 M cementing fluid concentration was the best [113]. Zhang used Sporosarcina pasteurii (American Type Culture Collection, ATCC 11859) to cement 200-380 µm industrial sand in calcium chloride, calcium acetate, and calcium nitrate cementation solution with three different calcium source concentrations of 0.5 mol/L and found that the unconfined compressive strength of the sample cemented by calcium acetate cementation solution was 1.4 times that of the sample cemented by calcium chloride and calcium nitrate cementation solution, and the void scale distribution of the sample was more uniform [114]. Harun AKO G UZ et al. treated sand with 0.75 M calcium chloride, calcium nitrate, and calcium acetate as a carbon source for Viridibacillus arenosi K64 (GenBank ID:KR873397). It was found that the permeability of sand soil decreased by 80.8%, 23%, and 90.4% after the treatment of calcium chloride, calcium nitrate, and calcium acetate, respectively. The treatment effect of calcium acetate was the best to reduce the permeability [115].

## 4.2. Temperature

Temperature change has a great influence on the growth and reproduction of microorganisms and the activity of urease in functional metabolism, which changes the yield of calcium carbonate, deposition rate, crystal type, crystal morphology, particle size, and cementation mode of calcium carbonate between soil particles and has a direct influence on the treatment of pollutants induced by microbial mineralization.

Keykha injected the same amount of A. baburai fluid at pH 9 and temperatures of 30 °C, 40 °C, and 50 °C to cement the silty sand column and measure the unconfined compressive strength. It was found that the strength of the consolidated sand column was the highest at 40 °C [116]. Wang and Yuze conducted microscopic studies on carbonate precipitation induced by microorganisms at temperatures ranging from 4 °C to 50 °C and found that different types of calcium carbonate precipitate produced different sizes and

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quantities of precipitates by changing the temperature. A low temperature (4 °C) did not reduce the bacterial activity but reduced the bacterial growth and attachment rates, limiting the final amount of cementation. High temperature (50 °C) conditions significantly reduced the bacterial activity for a short period of time, while repeated injections of bacteria before every second injection of cement increased the final amount of cement [117]. Liang Cheng found in his investigation of the influence of physics and the environment on the MICP effect that the amount of CaCO<sub>3</sub> precipitated at 50 °C is three times that of 25 °C, and the crystal size produced at higher temperature is relatively small (2–5 μm), while the crystal size at lower temperatures is relatively large (15–20 μm) [118]. However, the results of Jie Peng et al.'s study on MICP soil improvement by temperature showed that the final precipitation amount of CaCO3 in aqueous solution and sand column at 10 °C was 92% and 37% higher than that at 30 °C [119]; this directly contrasts what the previous authors concluded. The reason may be that the relationship between the microbial metabolic efficiency and enzyme activity and temperature is diametrically opposite, and the control of experimental parameters is also different. The specific reason and the treatment efficiency of temperature in soil pollution need to be verified.

#### 4.3. Properties of Cementing Medium

Using different mineralization media for microbial mineralization has important effects on the treatment of soil pollutants; different mineralization media possess distinct properties, differing in composition, porosity, and organic matter. The distribution of pollutants in media is not the same, and they thus differ in mineralization efficiency and permeability; the form of media has a strong influence on the ability to control pollutants in the soil.

Yufeng Gao used MICP to study the seepage of irrigation channels and reservoirs built on a sandy soil surface; it was found that the seepage rate of treated soil samples was reduced by up to 379 times and the permeability resistance was significantly higher than that of untreated soil [120]. Shima Atashgahi used *B. pasteurii* and *B. megaterium* megacanthus to improve the properties of loess and found that the soil permeability coefficient could reach the order of  $1 \times 10^{-7}$  after continuous MICP treatment for 7 days [120]. Hideaki Yasuhara, in a carbonate cemented sand permeability test on 300 g Toyoura sand, found that the modified sample permeability was reduced by more than an order of magnitude [121]. Nader Hataf and Alireza Baharifard treated the soil of Shiraz landfill by *B. sphaericus* mineralization, and the experimental results showed that the soil permeability of the mineralization treatment was reduced from  $3.9 \times 10^{-5}$  cm/s to  $6.81 \times 10^{-7}$  cm/s at the maximum, forming an impermeable barrier that provided effective prevention of waste leachate infiltration [122].

#### 4.4. Injection Pattern

The effect of the injection pattern on the ability of soil microbial mineralization to process pollutants is notable. In field injection, due to the nature of the soil in different locations, bacteria liquid may spread unevenly, with different liquid diffusion ranges; different injection methods can also affect the formation of mineral compounds, so the injection pattern has a direct impact on the mineralization processing effect.

At present, there are three mainstream injection methods: the first is the infiltration method, which is slow drip irrigation to the sample; the second is the grouting method, in which a grouting pipe is inserted into the sample; the third is the stirring method, in which the sample and bacteria liquid and cement liquid stirring thoroughly contact. The penetration mode is uneven, and the dominant seepage channel is easily generated, which means that some areas are not affected [123]. The grouting mode is distributed from the center to the periphery, and it is easy to form biological blockage around the injection point [124]. The stirring method has better uniformity than other methods [125], but mechanical mixing disturbs the soil and may be unusable in some cases. T. Hamed Khodadadi treated the soil using both soaking and injection methods and found that the

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injection method was more effective in obtaining rhomboids. These results also suggest that mineralogical factors should be considered when determining the appropriate method for MICP treatment of soil in the laboratory so that the obtained samples can represent the in situ mechanical behavior of MICP-treated soil [126]. Jisheng Zhang et al. used a low-pH, single-phase method to analyze and discuss the effects of two different bacterial grouting strategies: multiple injection of low-concentration bacteria and single injection of bacteria. The results showed that the amount of CaCO<sub>3</sub> produced by multiple injection of low-concentration bacteria was three times that of a single high-concentration bacteria, and extending the grouting period would make the distribution of CaCO<sub>3</sub> more uniform [127]. Liang Cheng carried out the cementation curing experiment by single-phase injection of a low-pH mixed solution (bacteria and cementing solution). In this method, the lag stage of the biological cementation is controlled by the pH value, and the distribution of CaCO<sub>3</sub> is more uniform [128]. Kuan Zhang et al. proposed a new single-phase cementing method by adjusting the pH of bacterial liquid mixed with cementing fluid. When the pH of the bacterial liquid was adjusted to 5.0 and the cementing fluid was 1 M, the "window period" for precipitation generation was delayed by 1.5 h, which greatly improved the influence depth and uniformity of MICP [120].

# 5. Status of Research on the Microbial Mineralization Control of Inorganic and Organic Pollutants in Soil

Inorganic pollutants and organic pollutants pose a serious threat to soil environments, and microbial mineralization remediation is a very important method for solving this problem [129]. The microbial mineralization of inorganic heavy metal pollutants can fix them in mineralized products [86]; this is green and safe, does not produce secondary pollutants, and has been studied by many scholars, showing remarkable effects [130,131]. Few studies have been conducted on the microbial mineralization of organic pollutants, but the minerals produced by microorganisms can effectively plug pores and reduce permeability [132], and the diffusion of organic pollutants can be effectively controlled through microbial mineralization [133]. It also provides sufficient time and safety for other methods (such as biodegradation [134–136]) to be used to remove organic pollutants [137]. To date, there has been little research on this, but many scholars' research results for reducing permeability show that it has good application prospects. The process is shown in Figure 4.

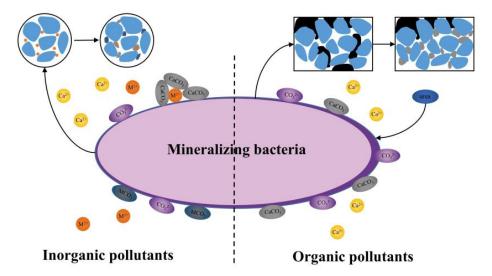


Figure 4. Microbial mineralization for soil pollutants.

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#### 5.1. Inorganic Contaminant

Marwa Eltarahony repaired Pb<sup>2+</sup> and Hg<sup>2+</sup> by CaCO<sub>3</sub> precipitation induced by *Proteus* mirabilis 10 B under aerobic and anaerobic nitrate utilization. The results showed that the removal rates of Pb<sup>2+</sup> and Hg<sup>2+</sup> under aerobic and anaerobic conditions reached 95.2% and 91.1% and 92% and 88.3%, respectively, after treatment in aerobic (144 and 168 h) and anaerobic (168 and 186 h) conditions [130]. Nasrin Ghorbanzadeh used Bacillus Pasteurelli to hydrolyze urea to remove Cd from sand and clay. The results showed that the initial concentration of Cd at 10, 20, 40, and 50 mg/kg<sup>-1</sup> decreased by 85.9%, 61.1%, 74.3%, 80.3%, and 89.3%, respectively, after 7 days of treatment [138]. XinyiQian used the fungus *Penicillium chrysogenum CS1* to mineralize soils contaminated with Cr(VI) and Pb. After the treatment, the percentage of exchangeable Cr(VI) decreased from 41.60% to 1.95%, while the exchangeable Pb decreased from 41.27% to 2.19% [42]. Varenyam Achal used Sporosarcina ginsengisoli CR5 to repair the soil contaminated with As(III). The results showed that the exchangeable As(III) in the soil decreased from 25.85 mg/kg to only 0.88 mg/kg after treatment [139]. Wilson Mwandira used a microbial-induced calcium carbonate precipitation technique in combination with the bacterium *Paracarulobacter sp* for lead bioremediation. The results showed that 1036 mg/L Pb<sup>2+</sup> was removed by co-precipitation of calcium carbonate and lead [140]. XuejiaoZhu used Bacillus cereus NS4 to repair soil highly contaminated by nickel. After microbial carbonate precipitation, the soluble exchangeable nickel concentration decreased from 898 mg/kg to 38 mg/kg, and the removal rate reached 95.8% [131]. Nasrin Jalilvand isolated four strains of bacteria with urease-producing metal tolerance from contaminated soil and carried out mineralization-removal experiments of the heavy metals zinc (Zn), lead (Pb), and cadmium (Cd). The results showed that after 72 h of treatment, S. pesteurii had the best removal effect, eliminating 98.71% of Pb, 97.15% of Cd, and 94.83% of Zn [79]. Varenyam Achal isolated a local calcification strain from the soil of the Urumqi mining area and repaired the copper-contaminated soil by MICP. In total, 95% of the copper in the contaminated soil was removed, and FTIR analysis showed that it produced two different forms of calcium carbonate: calcite and artifact [141]. The potential of MICP to repair strontium in aquifer quartz sand was evaluated in experiments, and the results showed that the strain removed 80% of the Sr in the soluble exchange fraction of aquifer quartz sand. At the same time, X-ray diffraction detected calcite, spherical aragonite, aragonite, and strontium carbonite (SrCO3) in the precipitation [142].

#### 5.2. Organic Contaminant

Mallavarapu Megharaj et al. noted various problems in the practical application of various methods to treat organic pollutants and showed the importance of reducing diffusion [143]. At the same time, although biodegradation is safe and effective, it takes a long time and a single strain cannot degrade all organic pollutants [144], so it is very important to treat organic matter and control the diffusion of pollutants. FengPan, in a study of the transport and transformation model of petroleum pollutants in the soil of the Loess Plateau, pointed out that a decrease in void space was an important factor leading to a decrease in saturated water conductivity of soil, and concluded that when  $K_s = 10.54$  cm/day was reduced to  $K_s = 3.03$  cm/day of uncompacted soil, the soil pollution was significantly reduced [133]. According to the national standard, the hydraulic conductivity of landfill liner compacted clay should be less than  $1 \times 10^{-7}$  cm/s, and the thickness should be more than 2 m [145]; using the equation to calculate the national standard generation, it is concluded that the time for the contaminant to pass through the anti-permeability system is at least 55 years [137]. J.CHU mineralized the sand surface to form a calcium carbonate cement layer, and it was found that the permeability of sand decreased from  $10^{-4}$  m/s to  $10^{-7}$  m/s when 2.1 kg of calcium carbonate was precipitated on the surface of sand per square meter [132], and the permeability drastically decreased. Viktor Stabnikov used dead bacteria with urease activity to decompose urea and produce calcium carbonate precipitation to seal the soil. The results showed that the hydraulic conductivity of the treated sand decreased from  $5.2 \times 10^{-4}$  to  $7.7 \times 10^{-9}$  m/s [15]. Jian Chu et al. conducted

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MICP studies on Bacillus isolated from tropical beach sand, and the permeability of sand was reduced to  $1.6 \times 10^{-10}$  m/s after six consecutive treatments [146]. KimVan Tittelboom often treats cracks in concrete through MICP and has found that the highest permeability coefficient is close to  $1 \times 10^{-12}$  m/s after biological treatment and coupling with other methods [147].

#### 6. Conclusion Outlook

Soil pollution is closely related to human health, so it is necessary to adopt scientific, safe environmental protection and the long-term and stable remediation of soil pollution. As a safe, environmentally friendly, long-term, and stable technology, microbial mineralization technology has been applied in various fields, and many studies have been conducted on the treatment of soil pollutants. Based on this paper, it can be concluded that microbial mineralization can mineralize and seal the vast majority of heavy metal pollutants in the soil, and the removal rate can reach more than 90% or even higher. For organic matter in the soil, from much research data, we can conclude that the hydraulic conductivity coefficient of the treated sand can reach up to  $1.6 \times 10^{-10}$  m/s, which can effectively control the diffusion and migration of organic matter. Although it can be concluded from the research results that microbial mineralization can effectively control organic and inorganic pollutants in soil, there are various factors affecting its treatment effect in practice. Based on the research in this paper, the following research prospects are proposed for the treatment of soil pollution by microbial mineralization:

- (1) Further research should be carried out on the mineralization mechanisms of various types of mineralized bacteria and the screening of new bacteria (such as salt-resistant bacteria, high-temperature-resistant bacteria, pH-resistant bacteria, etc.) to identify the mineralization processes of different types of bacteria and treat targeted pollutants.
- (2) Currently, more research has been carried out on soil inorganic contaminant treatment than organic pollutants and treatments to control them. Organic pollutants are difficult to degrade and have long treatment periods. Microbial mineralization can greatly reduce soil permeability and prevent the diffusion of pollutants; coupled with other soil treatment methods, it can eliminate organic pollutants, implying great application prospects.
- (3) At present, research on controllable mineralization is mostly confined to the laboratory, and its engineering applications are few. Research on the influencing factors of controllable mineralization under engineering application, such as the addition of chitosan and silk fibroin protein, should be expanded, and practical engineering application research should be carried out to transform controllable mineralization into a green and efficient technology.

**Author Contributions:** Conceptualization, B.G. and B.L.; methodology, B.G. and B.L.; software, B.G.; validation, B.G., J.C. and C.J.; formal analysis, B.G., B.L. and M.Z.; investigation, B.G.; resources, B.G.; data curation, B.G.; writing—original draft preparation, B.G.; writing—review and editing, B.L., B.G. and J.C.; visualization, Q.S.; supervision, B.L.; project administration, B.G. and B.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** Supported by Open Fund of Key Laboratory of Exploration Technologies for Oil and Gas Resources (Yangtze University), Ministry of Education, NO PI2021-06; Educational Commission of Hubei Province of China, D20201302; Supported by the National Natural Science Foundation of China (Grant No.52174019).

Institutional Review Board Statement: Not applicable.

**Informed Consent Statement:** Not applicable.

Data Availability Statement: Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

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