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Application of Microbial Carbonate Biomineralization in Biotechnologies of Building Materials Creation and Restoration: Analysis of the State and Prospects of Development

Microbial carbonate biomineralization is intensively developing area of nature-like technologies. It expands the range of tools for the control over the processes of structure formation at various technological stages of the life cycle of composite building materials: from designing a raw material mixture to self-healing during service. Like any interdisciplinary field, the technology of carbonate biomineralization in building materials science passed to the stage of the accumulation of empirical results that require generalization and analysis, having gone through the stages of studying natural analogues of the processes proposed for borrowing and the theoretical justification of the prospects for their applied use. The paper presents a review of publications over a twenty-year period with respect to such criteria as the gender of the used bacterial cells; applicable precursors of biochemical reactions; the effect of biomineralization on the properties of composite materials; characteristic features of phase formation products. The existing methods for the introduction of bacterial cultures and precursors in the technologies for the production of composite building materials using carbonate biomineralization are generalized and classified.

Keywords: carbonate bio-mineralization, bio-concrete, self-healing concrete, bio-cementation, bacterial culture.

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Применение микробной карбонатной биоминерализации в биотехнологиях создания и восстановления строительных материалов: анализ состояния и перспективы развития

Микробная карбонатная биоминерализация – интенсивно развивающееся направление природоподобных технологий – расширяет спектр инструментов управления процессами структурообразования на различных технологических этапах жизненного цикла композиционных строительных материалов, от проектирования сырьевой смеси до самозалечивания при эксплуатации. Как любое междисциплинарное направление, технология карбонатной биоминерализации в строительном материаловедении, пройдя стадии изучения природных аналогов процессов, предполагаемых к заимствованию, теоретического обоснования перспектив их прикладного использования, перешла в стадию накопления эмпирических результатов, требующих обобщения и анализа. В работе представлен обзор публикаций за двадцатилетний период по таким критериям, как родовая принадлежность используемых бактериальных клеток; применяемые прекурсоры биохимических реакций; влияние биоминерализации на свойства композиционных материалов; характеристические особенности продуктов фазообразования. Обобщены и классифицированы существующие способы введения бактериальных культур и прекурсоров в технологиях получения композиционных строительных материалов с применением карбонатной биоминерализации.

Ключевые слова: карбонатная биоминерализация, биобетон, самовосстанавливающийся бетон, биоцементация, бактериальная культура.

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Introduction

The increased interest in nature-like technologies is reasoned by the need to reduce energy consumption in production, increase the durability of materials and structures, use renewable raw materials, etc. A classic example of borrowing natural processes and transferring them to the level of industrial use in the production of building materials are technologies with the application of microbial carbonate biomineralization.

Microorganisms played a significant role in the geological evolution of the Earth and they continue to make a significant contribution to the development of the inorganic component of the modern environment, participating in both phase-forming and destructive processes occurring in rocks, living organisms, as well as in various types of materials and structures. In this regard, the phenomenon of the formation of minerals with the participation of microorganisms inspired scientists to develop nature-like innovative materials used in various interdisciplinary areas, one of which is modern building materials science [1].

Despite the existing publications of both foreign and Russian authors on the topic of biomineralization [2–78], they do not cover the completeness of the existing problems, methods and approaches. In some cases the studies are summary in nature and they do not differ in the depth of study on the technological features of the use of biomineralization and the processes of structure formation, which limits the possibility of the spread of carbonate biomineralization technologies in building materials science. In order to generalize the research results of various scientific groups, identify the applied technological solutions and their influence on the properties of the obtained composite materials, the proposed analysis of scientific publications is relevant.

Calcium carbonate, being an integral phase of cement stone, which is formed, as a rule, in the post-technological (service) period, due to the natural carbonation of $Ca(OH)_2$ (a water-soluble product of hydration of clinker minerals), has a strengthening effect on the matrix of cement stone, clogging pores and increasing water resistance of the composite. One of the possible ways to obtain calcium carbonate is microbial carbonate biomineralization.

In modern literary sources, crystallization mechanisms occurring with the participation of microorganisms are described in detail [12, 14]. It is also known that one of the ways to eliminate defects in building materials is precise microbial carbonate biomineralization. However, in the development of technological processes for the production of building materials, the issues related to the effective selection of inorganic precursors and their concentrations for the initiation of carbonate crystallization, rational technological parameters of biocementation, as well as the influence of the raw materials of the concrete mixture, the conditions for the consolidation of binders and the operation of composite materials on the lysis of microorganisms, remain insufficiently studied.

Results of Published Data Analysis

Terminological issues.

The analysis of the scientific literature revealed some divergence in interpretation in understanding a number of definitions used to describe the above mentioned processes, and therefore, it is necessary to dwell on terminological issues. Microbial biomineralization covers the phase formation processes that occur with the participation of microorganisms (bacteria, algae, micromycetes). For example, blue-green algae (cyanobacteria) produce calcium carbonate, which is a by-product of the technology of biological CO_2 sequestration [2]. This process is not used in biotechnologies used to create and restore building materials. There were no facts of production of calcium carbonates by micromycetes (as a rule, calcium oxalates are formed [3, 4]). Accordint to the above mentioned aspects it follows that bacterial carbonate biomineralization is only one of the special cases of microbial biomineralization. However, when describing biotechnologies used in building materials science, these concepts are often considered identical.

From the point of view of technological biomineralogy, biotechnology of the production of building materials is a set of biochemical processes. These processes occur as a result of the influence of microbiological objects on inorganic matter, during which in the environment – matrix, i.e. building material, the formation of new phases takes place.

If we separate the processes that take place with the participation of various types of microorganisms, then bacterial (microbial) carbonate mineralization is the formation of calcium carbonates (usually calcite), induced by metabolites of bacteria (most often, urease-producing).

Another widely used concept in biotechnology is bio-concrete. However, the interpretations of this term are so diverse that they do not allow using it as a key one. Thus, for example, bio-concrete is understood as: a material that combines the functions of concrete and substrate for plants, used in phytodesign; a concrete with biopositive properties in the context of environmental friendliness of the material; as well as concrete, in the formation processes of which bacteria are involved.

In this regard, the subject of this article is the analysis of literary sources addressing the issues of bio-concrete creation, self-healing concrete, bio-hybrid composite building materials, microbiological concrete, as well as bio-cementing and microbial-induced sedimentation.

Analysis of publication activity dynamics.

During the analysis we selected the articles published in leading peer-reviewed Russian and international issues that were publicly available for the period from 1999 to 2019.

In 1999, one of the first articles on the possibilities of the use of biomineralization in the process of regeneration of architectural monuments was published [5]. Modern scientific research, reflecting the possibilities of the use of carbonate biomineralization in the technologies for the production of building materials, is on the path of revealing the problem at a fundamental level (review articles [6–29]) and conducting interdisciplinary research, which allows approaching the development of replicated technologies (results of empirical studies). In total we analyzed 76 scientific papers. This number did not include works on carbonate mineralization not related to building materials science, as well as theoretical reviews, which present popular description of foreign experience.

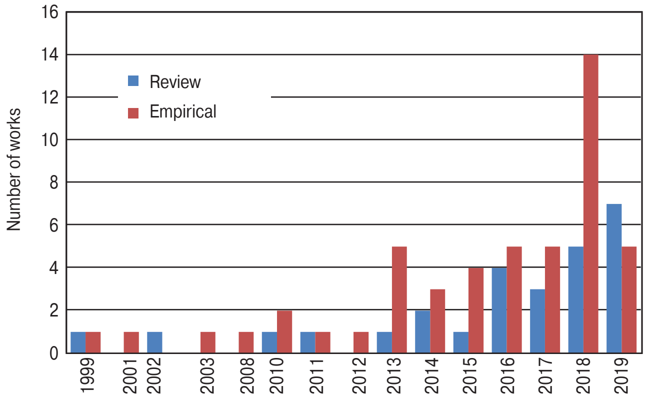

Fig. 1. Flow dynamics of review and empirical articles on carbonate biomineralization of building materials

Рис. 1. Динамика потока обзорных и эмпирических статей, посвященных карбонатной биоминерализации в строительных материалах

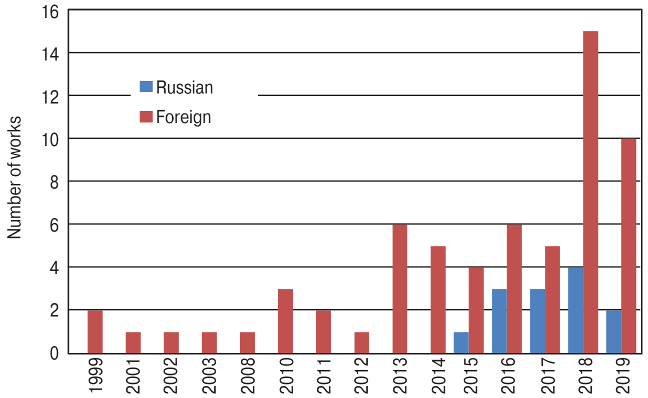

Fig. 2. Dynamics of publication activity of Russian and foreign authors

Рис. 2. Динамика публикационной активности российских и зарубежных авторов

The ranking of articles (Fig. 1) by type of publication in the context of time periods shows a steady increase in interest in this topic. The peak of publication activity was in 2018. However, the number of articles for the first half of 2019, which has already reached 63%, allows predicting the excess of indicators in 2018.

The interest of Russian scientists in this type of technology and, as a result, the appearance of publications with the results of relevant studies, dates back to 2015 (Fig. 2). The increase in the publication activity of Russian specialists in the field of building materials science lags behind foreign ones.

The publications of such foreign authors as W. De Muynck [14, 30, 31], N. De Belie [10, 14, 30–35], N.K. Dhami [11, 18, 19, 32], F. Hammes [20], as well as scientific groups led by Russian researchers V.T. Erofeeva [6, 9, 36] and S.P. Sivkova [37–43] deserve special attention, as they reflected modern fundamental ideas about this type of nature-like technologies and summarized the results of the experience of their application in building materials science.

The analysis of the results of experimental studies (Tables 1–3) [30–78] made it possible to structure publications on carbonate biomineralization in building materials according to the following criteria:

– type of the bacteria used;

– precursors – inorganic substances involved in biochemical reactions of carbonate biomineralogenesis in building materials;

– the availability of information on laboratory results testing on microbial carbonate in the matrix of building material;

– correlations between the strength characteristics of the cement matrix and the content of bacterial cells;

– characterization of the features of phase formation products (mainly the shape of crystals is described) formed under the action of microbial enzymatic activity.

Effectiveness of various bacteria.

The management of carbonate biomineralization processes in the cement system using bacterial cultures is possible through the determination of the most effective microorganisms (genus and type of bacteria) and nutrients for them, providing that they are compatible with the components of the building material involved in the process. The analysis showed that preference is given to bacterial cultures of *Bacillus* species (Fig. 3), *Sporosarcina* and *Myxococcus* are less used in biomineralization processes, the remaining bacteria (*Pseudomonas, Paenibacillus, Stenotrophomonas, Enterococcus, Proteus, Rhodobacter, Streptomyces* and *Staphylococcus*) did not receive much attention in analyzed publications, which may indicate their low efficiency for this type of technology or insufficient knowledge. Almost all authors use bacteria as a monoculture in their studies, and only one mention of a combination of two microorganisms of the Bacillus species was found [65].

Use of precursors.

The effectiveness of the management of structural changes in the cement system is reasoned by matrix compaction by reducing the pore space adding not only an inoculum (bacterial cell culture) or lyophilisate (bacterial cells in the form of dry powder), but also carbonate crystallization initiation precursors that stimulate the process of biomineralogenesis in the building material environment. In order to form optimal crystallization conditions, a number of precursors are used (a single component of

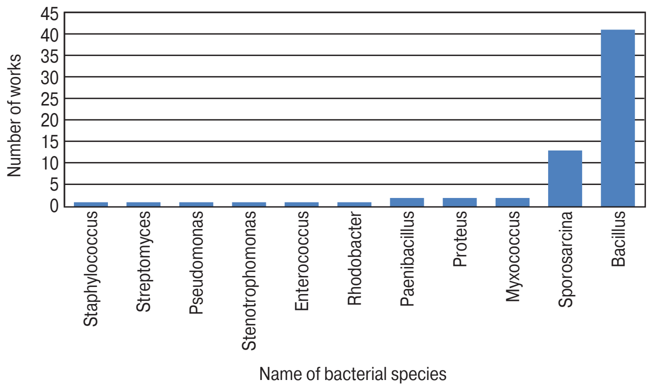

Fig. 3. Frequency of use of bacterial cultures

Рис. 3. Ранжирование бактериальных культур по частоте использования

Table 1

Introduction of carbonate mineralization precursors and a bacterial agent or groups of agents into a concrete mix

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	The composition of building material	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source Link
1. The introduction of a bacterial culture in mixing water with precursors						
Bacillus	30×10^5 c/ml	peptone 5 g/l+ yeast extract 3 g/l+ $2(C_3H_5O_3)\cdot Ca$ 80 g/l+ $(NH_2)_2CO$ 20 g/l	Cement-concrete mixture (Portland cement 429.31 kg/m^3 + water 193,19 kg/m^3 + filler 10 mm 965,95 kg/m^3 + sand 792,08 kg/m^3).	Compressive strength on the 90th day – 30MPa (12% increase)	–	[44]
			Mix for geopolymer concrete (water 33,33 kg/m^3 + filler 10 mm 1233,32 kg/m^3 + sand 530 kg/m^3 + boiler fly ash 290 kg/m^3 + POFA modificator 123,33 kg/m^3 + sodium hydroxide solution 47,62 kg/m^3 + sodium silicate solution 119,05 kg/m^3 + plasticizer 6,67 kg/m^3).	Compression strength on the 90th day – 32 MPa (22% increase). Less weight loss in 5% solution of H_2SO_4.		
Bacillus subtilis	50% by mass at OD_{600} 0,107; 0,20; 0,637; 1,221	–	Portland cement of ASTM Type-1 + sand 0,075–4,75 mm + crushed stone fraction 12,5 mm (1:2,57:2,71)	Concrete with OD_{600} 0,637 turned out to be the most effective at increasing density.	–	[45]
			Portland cement of ASTM Type-1 + sand 0,075–4, 75 mm + crushed stone fraction 12,5 mm (1:2,68:2,04).			
			Portland cement of ASTM Type-1 + sand 0,075–4,75 mm + crushed stone fraction 12,5 mm (1:1,28:1,73)			
Bacillus sphaericus	10 и 20 ml	NH_4Cl 0,2 g/l + KH_2PO_4 0,02 g/l + $CaCl_2$ 0,225 g/l + KCl 0,2 g/l + $MgCl_2\cdot6H_2O$ 0,2 g/l	Portland cement + sand M_{cm} 3,2 + coarse filler12,5 and 20 mm	The increase in compressive strength of 30.84% for 10 ml and 31.11% for 20 ml after 28 days. The increase in fracture strength by 5%.	–	[46]
Bacillus pumilus	$1,5\times10^8$ c/ml, 12×10^8 c/ml, 24×10^8 c/ml	$(NH_2)_2CO$ 20 g/l + $CaCl_2$ 2,8 g/l + nutrient broth 3 g/l + NH_4Cl 10 g/l + $NaHCO_3$ 2,12 g/l	Portland cement of 42,5 type Technology features: part of the samples was hardened in a solution with precursors; the other part contains precursors in the mixture. Hardening temperature 28°C	The maximum increase in compressive strength on the 7th day was 23.8% when precursors of crystallization initiation were introduced into concrete with a bacterial agent	–	[47]
Bacillus sp.	5×10^6 c/ml, 5×10^7 c/ml, 5×10^8 c/ml	Nutrient broth 8 g/l + $(NH_2)_2CO$ 2% + $CaCl_2$ 25 мМ	Portland cement + sand + coarse filler (1:1,32:3,29). Technology features: hardening of concrete samples with the initiators of biomineralization in their composition was carried out in a harvest medium	50% reduction in porosity, a decrease in water absorption and a maximum increase in compressive strength were at a concentration of 5×10^7 c/ml – by 40%.		[48]
Bacillus subtilis	$1,82\times10^{10}$ c/ml	10 g/l peptone + 5 g/l of yeast extract	Portland cement of 42,5 type 294 kg/m^3 + limestone filler (density 3,09 and 2,7 g/cm^3) 57 kg/m^3 + plasticizer MC Power-Flow 3140 2,1 kg/m^3	In bacterial samples, self-cementing phenomena of cracks 400 mcm of width were observed after 44 days	Needle-like crystals	[49]

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	The composition of building material	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source Link
Bacillus megaterium	5×10^7 CFU / ml	Nutrient broth 8 g/l + NaCl 5 g/l + 2% $(NH_2)_2CO$ + 25 мМ $CaCl_2$	Portland cement + sand with M_{cm} 2,89 + crashed limestone 29,5 mm (1: 1,54: 2,86) + boiler fly ash	At a concentration of fly ash in solutions of 10%, 20%, and 40%, the bacterial cells increased the compressive strength of the solution by 19%, 14%, and 10%, respectively, as compared to control samples. Water absorption was reduced by more than 3 times.	–	[50]
Sporosarcina pasteurii	10^8 c/ml 10^9 c/ml	Medium NH_4 – YE: 20 p of yeast extract + 10 g $(NH_4)_2SO_4$ + 0,13 M tris-buffer (pH 9,0) + 20 g of agar. NB medium: 3 f of nutrient broth + 20 g $(NH_2)_2CO$ + 10 g NH_4Cl + 25,2 mm $NaHCO_3$	Cement: sand (1:3).	The compressive strength increases with increasing cell concentration from 108 to 109 c / ml by 4.9% for bacteria grown in NB medium and by 4% for bacteria grown in NH4 – YE medium, which indicates that the medium does not significantly affect the efficiency of calcifications.	–	[51]
2. Mixing a bacterial culture with precursors with subsequent adding to the mixing water						
Bacillus sp. CT5	–	$(NH_2)_2CO$ 2% + $CaCl_2$ 25 мМ	Portland cement + sand + gravel 10–20 mm (1: 1,82: 3,24)	The increase in compressive strength of 35%, damage to samples aged in a sulfate solution after 365 days was not visualized.	–	[52]
Bacillus megaterium	10% of weight	Peptone 5 g/l+ nutrient broth1,5 g/l+ yeast extract 1,5 g/l+ NaCl 5 g/l+ 2% $(NH_2)_2CO$ + 25 мМ $CaCl_2$	Soil: sand (1:1) + cement 7%. Technology features: during the hardening of samples (28 days), the surface was treated with a 30 ml solution with precursors by spraying daily.	Strength increase by 22%, porosity decrease by 44%.	Calcite crystals	[32]
Bacillus megaterium	10, 20, 30, 40, 50×10^5 c/ml	Glucose 10 g/l+ K_2HPO_4 2,5 g/l+ KH_2PO_4 2,5 g/l+ $(NH_4)_2HPO_4$ 1 g/l+ MgSO4·7H_2O 0,2 g/l+ $FeSO_4 \cdot 7H_2O$ 0,01 g/l+ $MnSO_4 \cdot 7H_2O$ 0,007 g/l	Cement + water + fine filler+ coarse filler	At a bacterial concentration of 30×10^5 c/ml, the compressive strength on the 60th day was 40 MPa (the increase by 50%). bending – 4.3 MPa (an increase of 53%). With increasing concentration of bacteria, strength decreases.	–	[53]
Bacillus coagulans	1,5×10^8 c/ml 12×10^8 c/ml 24×10^8 c/ml	$(NH_2)_2CO$ 20 g/l + $CaCl_2$ 2,8 g/l + nutrient broth 3 g/l + NH_4Cl 10 g/l + $NaHCO_3$ 2,12 g/l	Portland cement of 42, 5 type + sand M_{cm} 2,6 + granite gravel 20 mm + water. Technology features: hardening temperature was 30^{o}C.	6.25% increase in compressive strength at a concentration of 24×10^8 c /ml	–	[54]

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	The composition of building material	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source Link
Sporosarcina pasteurii	5,5×10^6 c/ml	Tryptic soy broth 30 г + $(NH_2)_2CO$ 20 г + $CaCl_2$ 25 мМ	Concrete mix. Technology features: concrete samples with a bacterial culture were placed in a solution with precursors for 28 days.	Water absorption of samples with bacteria (21.38%) was 6.03% higher than the control (21.24%). The compressive strength was 42.13 MPa, which was 30.27% higher than the control samples (32.34 MPa).	–	[55]
Bacillus mycoides *Bacillus circulans*	2,4×10^{10} c/ml	$2(C_3H_5O_3)\cdot Ca$15 g/l	Portland cement CEM I 52.5 N 145 g + silica 25 g + sand 460 g + water 125 ml + $2(C_3H_5O_3)\cdot Ca$ 15 g	The compressive strength increases by 21 days from 24.88 to 28.72 MPa (by 15.4%).	–	[56]
			Portland cement CEM I 52.5 N 145 г + silica 25g + sand 460 g + water 125 ml + $2(C_3H_5O_3)\cdot Ca$ 15 g + diatomite powder	The compressive strength increased by 21 days from 44.36 to 46.3 MPa (by 4.4%).		
Sporosarcina pasteurii *Pseudomonas aeruginosa*	Bacterial culture / cement (0,47)	Nutrient broth 8,0 g/l + NaCl 5,0 g/l + 2% $(NH_2)_2CO$ + 25 мМ $CaCl_2$	Portland cement + sand with M_{cm} 2,89	Compressive strength increased by 28.15% compared to the control. Water absorption decreased by 6 times.	–	[57]
Bacillus subtilis *Bacillus sphaericus*	2–5×10^7 c/ml	Dick nutrient broth (peptone 3 g/l + $(NH_2)_2CO$ 10 g/l + $NaHCO_3$ 2 g/l)	Cement mixture	By the 28th day of hardening, the bending strength was maximized when *B. sphaericus* was added to 11.5 MPa, which was 36% higher compared to the control, and the compressive strength increases to 108.3 MPa (by 37%).	–	[37, 39]
Bacillus subtilis	100×10^8 c/ml	Peptone 5 g/l+NaCl + 5 g/l+ yeast extract 2 g/l+ plain broth 1 g/l	Portland cement + sand M_{cm} 2,7+ coarse filler of 20 mm + harvest broth 13 g/l	The compressive strength on the 28th day increases to 43.55 MPa (by 14%), bending strength to 4.11 MPa (by 25%).	Rhombohedron crystals	[58]
Bacillus megaterium				Compressive strength on the 28th day increases to 46.68 MPa (by 20%), bending strength to 3.88 MPa (by 17%).		
Enterococcus faecalis	1% (2,1 l/m^3) 3% (6,3 l/m^3) 5% (10,5 l/m^3)	Nutrient broth 25 ml + $(NH_2)_2CO$ 10 ml	Portland cement 420 kg/m^3 + coarse filler 1,115 kg/m^3 + water 207,9 l/m^3 + fine filler 685 kg/m^3	The maximum values of physical and mechanical properties were demonstrated by samples with the addition of 3% *E. Faecalis:* compressive strength increased by 23%, bending strength increases by 14%.	–	[59]
Bacillus cereus		Nutrient broth 25 ml + $MgSO_4\cdot 7H_2O$		The samples with the addition of 5% *B. cereus*: compressive strength increased by 13%, bending strength increased by 11%.		

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	The composition of building material	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source Link
Bacillus subtilis *Paenibacillus brevibacillus* *Bacillus methylotrophicus* *Paenibacillus dendritiformis* *Stenotrophomonas maltophilia* *Bacillus licheniformis*	1×10^4 c/ml 1×10^6 c/ml	$2(C_3H_5O_3)\cdot Ca$	Portland cement + sand + crushed stone 20 mm (1:1,54:2,86)	*B. subtilis* showed the highest compressive strength (increase of 28.61%) of cement mortar at 10^4 c/ml compared to other (*P. Brevibacillus* – 22,1%; *P. Dendritiformis* – 19,9%; *B. Methylotrophicus* – 16%; *B. Licheniformis* – 12,7%, *S. Maltophilia* – 9,6%)	–	[60]
3. Mixing a binder with precursors						
Bacillus megaterium *Sporosarcina pasteurii* *Bacillus cohnii*	3×10^7 c/ml	($(NH_2)_2CO$ + $2(C_3H_5O_3)\cdot Ca$) 1% fo 100 g of cement	Cement of OPC 43 grade, river sand M_{cm} 2.72 + crushed stone M_{cm} 12.5 mm (1: 1.71: 2.92). Technology features: precursors added to the binder, followed by mixing with water with an inoculum.	In the samples with *B. Megaterium* with the addition of $C_6H_{10}CaO_6$ the increase in compressive strength by 40% and tension by 46% was recorded	–	[61]
4. Addition of immobilized bacterial cultures						
a) Absorption						
Bacillus subtilis	7,6 l/m^3	$2(C_3H_5O_3)\cdot Ca$ – 18,7 kg/m^3 of cement mortar	Portland cement 837 kg/m^3 + sand 1400 kg/m^3 Technology features: bacterial cultures were absorbed in crushed limestone	Increase in compressive strength from 40.8 MPa (for control samples) to 41.6 MPa	–	[62]
Bacillus sphaericus	1% of mass	$(NH_2)_2CO$ 1 or 3% of mass + 12% solution (3 g/l peptone + 2 g/l $NaHCO_3$ + additionally $(NH_2)_2CO$ 10 g/l)	Portland cement 3 or 5 % of mass of sand + sand M_{cm} 2,8 Technology features: bacteria sedimentation on a mineral carrier – $Al(OH)_3$, followed by filtration and drying. A bacterial additive was introduced into the composition of carbonate sand in an amount of 1 mass %.	The maximum increase in compressive strength on the 14th day (14.6 MPa) at a concentration of 5% of Portland cement and 1% of $Ca(OH)_2$.	–	[42]
Bacillus sphaericus	0,2 mass. %	–	Gypsum Technology features: bacteria sedimentation on microcellulose, followed by filtration and drying. The bacterial additive was mixed with gypsum in an amount of 0.2 mass % and was mixed with water W / T = 0.56.	The maximum increase in bending strength on the 28th day was by 23%, the increase in compressive strength was by 38%.	Crystal new growths	[38]

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	The composition of building material	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source Link
Sporosarcina pasteurii	10^6 c/ml	$(NH_2)_2CO$ 20 g/l + $CaCl_2 \cdot 2H_2O$ 49 g/l	Portland cement 405 kg/m^3 + sand 527 kg/m^3 + coarse filler LWAC 456 kg/m^3 + fine filler 152 kg/m^3 + water 160 kg/m^3 + plasticizer 0,05 kg/m^3. Technology features: coarse filler was impregnated with a bacterial inoculum with precursors for 6 days and then it was added to the concrete mix.	Water absorption of samples impregnated for 90 days with a solution of urea with calcium chloride decreased by 23% compared to the control. The strength of samples impregnated with a solution of urea with calcium chloride during 90 days increased by 21.5%, and by 26.4% on the 150th day	–	[63]
Rhodobacter capsulatus	4,6×10^6 c/ml	Yeast extract 1 g/l + disodium succinate hexahydrate 1 g/l + ethanol 1 ml/l + K_2HPO_4 0,5 g/l+ $MgSO_4 \cdot 7H_2O$ 0,4 g/l+ $CaCl_2 \cdot 2H_2O$ 0,005 g/l+ NaCl 0,4 g/l	Portland cement 35% + sand 2%+ boiler fly ash 20% + granulated blast furnace slag 45%. Technology features: immobilization of bacterial cultures with precursors in expanded vermiculite for 72 hours, replacement of 30% of the aggregate volume in concrete mix.	The maximum increase in compressive strength on the 28th day was 40.7 MPa compared to the control (38.9 MPa), the increase in compressive strength was by 38%. The compressive strength coefficient on the 28th day after exposure of the samples in a 5% solution of sulfuric acid was 1.02 compared to the control (0.97).	–	[64]
Bacillus subtilis *Bacillus sphaericus*	Absorbent Al_2O_3 with bacterial spores 3 mass. %	Dick nutrient broth (peptone 3 g/l + $(NH_2)_2CO$ 10 g/l + $NaHCO_3$ 2 g/l)	Cement mixture Technology features: sedimentation of bacteria on a mineral carrier – $Al(OH)_3$.	By the 28th day of hardening, the bending strength increases to 14 MPa, which was 28% higher compared to the control, compressive strength increased to 93.2 MPa (33.5%); porosity was reduced by 30.14%; the coefficient of capillary water absorption decreased by 66%.	–	[37, 39, 40]
Sporosarcina pasteurii *Bacillus subtilis* *Sporosarcina ureae*	10^4 c/ml 10^6 c/ml 10^8 c/ml	–	Cement + Zeolite; cement + pumice	The greatest increase in strength (by 20.1%) was recorded in the samples with immobilized bacteria *S. Pasteurii* in pumice at a cell concentration of 10^6 c/ml on the 270th day	–	[36]
Bacillus sphaericus + *Bacillus licheniformis* (1 : 1) (совместное введение биокультур)	–	$CaCl_2$ 40 g/l + yeast extract 2 g/l + $(NH_2)_2CO$ 65 g/l	Portland cements 16% + sand 4% + filler 40% + water. Technology features: immobilization of bacterial cultures with nanoparticles obtained in the process of cosedimentation 0,74 g Fe^{2+} ($FeSO_4 \cdot 7H_2O$) and 1,17 g Fe^{3+} ($FeCl_3 \cdot 6H_2O$)	Reduced water absorption with immobilized bacteria	Calcite crystals	[65]

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	The composition of building material	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source Link
			b) Encapsulation			
Bacillus sphaericus	1×10^9 c/ml	$(NH_2)_2CO$ 20 g/l+ yeast extract 20 g/l + $Ca(NO_3)_2 \cdot 4H_2O$ 12 g/l	Portland cement CEM I 52.5N + sand (1:3) Technology features: bacterial cultures with precursors were encapsulated in a hydrogel	Crack closure on the 28th day with a width of 0.2–0.3 mm, water absorption reduced by 68%	–	[33]
Bacillus sphaericus	1, 2, 3, 4, 5% of mass	$(NH_2)_2CO$ 20 g/l + yeast extract 20 g/l	Portland cement + sand Technology features: bacterial cultures were introduced into melamine microcapsules added to mixing water. Addition of capsules with bacteria in the amount of 0%, 1%, 2%, 3%, 4% and 5% in cement (by weight).	With addition of 1–5% of encapsulated cultures, the decrease in compressive strength was recorded by 15–34% on the 28th day and by 22–47% on the 90th day and a decrease in water absorption in the range of 20–30%. Crack closure on the 90th of day at a concentration of 5% bacterial microcapsules by weight of the solution.	–	[34]
Bacillus sphaericus	0,5 or 1% of mass	$(NH_2)_2CO$ 20 g/l + $Ca(NO_3)_2 \cdot 4H_2O$ 11,8 g/l + yeast extract 20 g/l	Portland cement CEM I 52.5 N + sand DIN EN 196-1 Norm Sand. The ratio of cement and sand was 1: 3, Water – cement ration was 0.5. Technology features: bacterial cultures were encapsulated in an alginate-based hydrogel.	With the addition of 1% encapsulated cultures, the decrease in compressive strength was by 23.4%, tensile strength was by 30% due to the formation of macropores in capsules and absorption of mixing water. The ability of bacteria to exhibit urease activity, biocompatibility of capsules and bacteria was proven.	–	[35]
Bacillus pseudofirmus	1,4×10^9 c/ml 3,2×10^9 c/ml 5,5×10^9 c/ml 8,6×10^9 c/ml 13×10^8 c/ml	$Ca(CH_3COO)_2$ in concentrations 7,1; 5; 100 g/l	Cement 450 g + sand 1080 g + water 225 g Technology features: bacterial cultures with precursors were encapsulated in expanded perlite.	Crack closure after 165 days with bacteria concentration 5,5×10^9 and 8,6×10^9 c/ml.	–	[66]

Table 2

Local processing of finished or exploited concrete products with solutions, containing carbonate mineralization precursors and bacterial agent or agent associations

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	The composition of building material	Treatment method	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source Link
1. Surface treatment							
a) Spraying a solution with a bacterial inoculum and precursors							
Not indicated	–	–	Limestone	Local treatment by spraying with a bacterial inoculum and culture medium for 5–10 days	5 times reduction in water absorption	Spheroidal crystals	[67]
Bacillus sp. CT5	–	$(NH_2)_2CO$ 2% + $CaCl_2$ 25 мМ	Portland cement + sand + gravel 10–20 mm (1:1,82:3,24)	Local treatment by spraying with a bacterial inoculum and precursors for 28 days	The increase in compressive strength up to 40 MPa (11%) and resistance to sulfate corrosion for 365 days	Needle crystals of gypsum and ettringite	[52]
M. xanthus	1×10^7 c/cm^2	1% Bacto Casitone + 1% $Ca(CH_3COO)_2\cdot4H_2O$ + 0,2% $K_2CO_3\cdot0{,}5H_2O$	Calcarite	Local treatment by spraying the solution 2 times a day for 6 days	Consolidation Depth was 3 cm	–	[68]
b) Complete application of a solution with a bacterial inoculum and precursors							
Bacillus sphaericus	0,3 and 0,6 l of daily culture	$(NH_2)_2CO$ 10 g/l + nutrient broth 10 g/l + $CaCl_2$ 36 g/l or $Ca(CH_3COO)_2$ 51,25 g/l	300 kg of cement + 670 kg of sand + 1280 kg of gravel + 150 kg of water	Surface treatment with a bacterial inoculum with precursors using a brush	Reduced water absorption, reduced gas permeability	Rhombohedral crystals, granular grains	[31]
2. Product immersion in a solution of bacterial inoculum and precursors							
a) Partial immersion							
Bacillus sphaericus	1% of inoculum	Solution 1: yeast extract 20 g/l+ $(NH_2)_2CO$ 20 g/l. Solution 2: $(NH_2)_2CO$ 20 g/l + $CaCl_2\cdot2H_2O$.	Limestone	Partial immersion of samples in a crystallization solution for 7 days.	Reduced water absorption	Rhombohedral crystals	[30]
b) Full immersion							
Bacillus sphaericus	1% of inoculum	Solution 1: east extract 20 g/l + $(NH_2)_2CO$ 20 g/l. Solution 2: $(NH_2)_2CO$ 20 g/l + $CaCl_2\cdot2H_2O$	Limestone	Immersion of samples in solution with inoculum and precursors.	Decrease in water absorption, decrease in porosity.	Rhombohedral crystals	[30]
Bacillus sphaericus	0,3 and 0,6 l of daily culture	$(NH_2)_2CO$ 10 g/l + nutrient broth 10 g/l + $CaCl_2$ 36 g/l or $Ca(CH_3COO)_2$ 51,25 g/l	300 kg of cement + 670 kg of sand + 1280 kg of gravel + 150 kg of water	Immersion in solution with inoculum and precursors.	Reduced water absorption, reduced gas permeability	Rhombohedral crystals, granular grains	[31]

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	The composition of building material	Treatment method	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source Link
Bacillus sphaericus	–	$(NH_2)_2CO$ / $CaCl_2$ $(NH_2)_2CO + CaCl_2$	Building gypsum	Immersion of samples in the inoculum solution	After 24 hours of treatment with solutions, the performance was maximized in combination with $(NH_2)_2CO$ and $CaCl_2$ with inoculum: bending strength increased by 17%, compressive strength increased by 10%, water resistance coefficient increased by 82%; porosity decreased by 56%.	Fine Calcite Crystals	[43]
Bacillus sp. CT5	–	$(NH_2)_2CO$ 2% + $CaCl_2$ 25 мМ	Portland cement + sand + gravel 10–20 mm (1:1,82:3,24)	Immersion of samples in a bacterial solution with precursors for 28 days	Increase in compressive strength up to 42 MPa (by 40%) and resistance to sulfate corrosion for 365 days.	Needle crystals of gypsum and ettringite	[52]
Bacillus pumilus	1,5×10^8 c/ml 12×10^8 c/ml 24×10^8 c/ml	$CO(NH_2)_2$ 20 g/l + $CaCl_2$ 2,8 g/l + nutrient broth 3 g/l + NH_4Cl 10 g/l + $NaHCO_3$ 2,12 g/l	Portland cement of 42,5 type + coarse filler of 20 mm	Immersion in solution with inoculum and precursors	Compressive strength increased by 6.3% at 1,5×10^8 c/ml.	–	[47]
M. xanthus	0,1×10^9 c/ml (for limestone) 2×10^9 c/ml (for calcarenite)	Medium M-3: 1% Bacto Casitone + 1% $Ca(CH_3COO)_2 \cdot 4H_2O$ + 0,2% $K_2CO_3 \cdot 0{,}5H_2O$	Limestone, calcarenite (calcareous sandstone)	Immersion in a bacterial solution with precursors for 24 hours	Slight increase in the weight of the samples, decrease in porosity of 6%	Vaterite needle crystals, calcite rhombohedra	[69]
Bacillus subtilis, Bacillus sphaericus	2–5×10^7 c/ml	Nutrient solution: peptone 3 g/l + $(NH_2)_2CO$ 20 g/l + $CaCl_2$ 5 g/l	Portland cement	Immersion in harvest solution for 7 days	Strength increased by 12–18%; capillary porosity was reduced by 20-24%; coefficient of capillary water absorption decreased by 45–57%	–	[37]
				Samples with formed cracks were placed for 7, 14, 28, 35 days in nutrient solution	The filling of cracks with microcrystals was visualized. The value of the strength index of the samples was not restored		
Bacillus subtilis, Bacillus sphaericus	–	Nutrient solution + $(NH_2)_2CO + Ca(OH)_2$	Concrete mixture	Immersion in solution with inoculum and precursors for 28 days.	When using *B. subtilis* we observed the decrease: in bending strength by 33%; in open porosity by 25%, in capillary water absorption coefficient by 41%. Compressive strength increased by 11.7%.	–	[41]
		Nutrient medium + $(NH_2)_2CO + CaCl_2$			When using *B. sphaericus* we observed decrease: in bending strengthby 28%; in open porosity by 25%, in capillary water absorption coefficient by 41%. Compressive strength decreased by 12%.		

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	The composition of building material	Treatment method	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source Link
Proteus mirabilis *Proteus vulgaris*	–	$(NH_2)_2CO$ 20 g/l + $CaCO_3$ 2,12 g/l + NH_4Cl 25 g/l + nutrient broth 3 g/l	Concrete samples	Immersion in nutrient solution for 28 days	Filling of cracks	–	[70]
3. Selective cementation							
a) Injection							
Sporosarcina pasteurii	60 ml for 400 ml of solution	$(NH_2)_2CO$ 0,4 M + $CaCl_2$ 0,4 M	Marble aggregate 0,5–1,4 mm 1904,3 g	Injection flow of 14 ml/min	Cementation was visualized 30 mm from the base. Porosity reduced	–	[71]
b) Introduction of prepolymer films with bacteria incorporated into the cracks							
Sporosarcina pasteurii	5×10^7 c/ml 5×10^8 c/ml 5×10^9 c/ml	$(NH_2)_2CO + CaCl_2$	Concrete mixture	Technology features: introduction of a polymer tape with bacterial cultures into simulated cracks in concrete samples with subsequent placement in solution of $(NH_2)_2CO$ and $CaCl_2$	At any cell concentration, a slight increase in compressive strength was recorded after 28 days	–	[72]

Table 3

Creation of materials using biocarbonate synthesis (biocarbonate cementation)

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	Filler type	Treatment method	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source link
Bacillus sphaericus	1% of mass	$Ca(OH)_2$ 3 or 5 % of mass + $(NH_2)_2CO$ 1 or 3 % of mass + 12% solution (3 g/l peptone + 2 g/l $NaHCO_3$ + additionally $(NH_2)_2CO$ 10 g/l)	Sand M_{cm} 2,8.	Technology features: the sedimentation of bacteria on a mineral carrier – $Al(OH)_3$, followed by filtration and drying. A bacterial additive was introduced into the composition of carbonate sand in an amount of 1 mass %.	The increase in compressive strength on day 14 (6.2 MPa) at a concentration 1% $(NH_2)_2CO$ and 5% $Ca(OH)_2$	–	[42]
Bacillu sp.	25 ml	$CaCl_2$ 82,5 g/l + $(NH_2)_2CO$ 20 g/l	Quartz sand with a grain size of 0.42 mm	The introduction of 25 ml inoculum per 50 ml of sand with the addition of a solution with precursors, followed by a fivefold repetition	The strength of the obtained material varied in the range of 0.765–0.845 MPa	–	[73]

The name of bacterial agent	Bacterial Additive Concentration	Crystallization Initiation Precursors	Filler type	Treatment method	Physico-mechanical properties of building material	Morphology of bioinduced new growths	Source link
Sporosarcina pasteurii	60 ml for 400 ml of solution	$(NH_2)_2CO$ 0,4 M + $CaCl_2$ 0,4 M	Marble aggregate 0,5–1,4 mm 1904,3 g	Injection flow of 14 ml/min	Cementation was visualized for 30 mm from the base. Porosity reduced.	–	[71]
Sporosarcina pasteurii	24 ml	$CaCl_2$ 24 ml	Sand compacted by:	Addition of inoculum with subsequent stirring	The maximum amount of carbonate (in mass.%):	–	[74]
			40% (298,8 g)		2,5		
			60% (307,6 g)		4,8		
			80% (316,9 g)		1,3		
Sporosarcina pasteurii	–	Urea + $CaCl_2$	Fine and medium grained sand	Sand impregnation	Strength and hardness improved from 3 to 12 MPa on medium-grained sand	Cubic crystals	[75]
Sporosarcina pasteurii	80 ml	Solution 1: $CaCl_2$ 1 m/l + $(NH_2)_2CO$ 1 m/l Solution 2: $CaCl_2$ 1 m/l + CH_3COOH + $(NH_2)_2CO$ 1 m/l	Limestone sand compacted by 40%	Injection of the inoculum with $CaCl_2$ $(NH_2)_2CO$ and soluble calcium obtained by mixing $CaCl_2$ and CH_3COOH, adding $(NH_2)_2CO$ to the samples with sand, followed by drying of the sample at 60°C for 48 hours.	The density of sand treated with soluble calcium was greater than that of samples treated with calcium chloride. The strength and stiffness of samples treated with soluble calcium were higher than that of control samples treated with calcium chloride.	The crystals obtained in samples with soluble calcium were needle crystals of aragonite; During the treatment with calcium chloride, calcite crystals of rhombohedral form were formed.	[76]
Sporosarcina pasteurii	OD_{600}=2,3	Main solution: $CaCl_2$ 147,02 g/l + $(NH_2)_2CO$ 60,06 g/l. Fixative solution: $CaCl_2$ 0,05 M	Quartz sand	Injection of samples with fixation (extracted cells from a sterile medium) and without fixation (injection without centrifugation by mixing with a solution). The ratio of bacterial cultures and sand 1:10 when injected with fixation and 1:25 – without fixation.	The maximum compressive strength (1,773 MPa) was achieved using the injection method with fixation solution, while without fixation it was 0.51 MPa.	Spherical crystals were formed without fixation, while jellylike crystals were formed in samples with fixation.	[77]
Sporosarcina pasteurii, Staphylococcus saprophyticus, Streptomyces globispora, Bacillus lentus	5×10^8 – 10^9 CFU/ml	$(NH_2)_2CO$ 20 $_{g/l}$ + $CaCl_2\cdot2H_2O$ 3,7 g/l + nutrient broth 3 g/l + NH_4Cl 10 g/l + $NaHCO_3$ 25,2 мМ	Sand with a filling density of 60% and 80%	Sand surface impregnation	The greatest amount of calcium carbonate was synthesized using bacteria *S. pasteurii*, which was 50% higher with a density of non-cohesive samples of 80%	Crystal newgrowths	[78]

the mixture, a binary or multicomponent combination) ranked in the order of increasing frequency of use (Fig. 4): ammonium sulfate → calcium nitrate / calcium hydroxide → calcium acetate / calcium lactate → ammonium chloride → calcium chloride → urea.

Effect on strength characteristics and prolongation of materials resistance.

The result of the interaction of the bacteriological inoculum, precursors and individual components of the concrete mixture in the building material is the improvement of the operational properties of the concrete obtained from these mixtures. The influence of carbonate biomineralization on the final characteristics of building materials is studied on the 28th day by many authors (according to regulatory documents on binders) and can last up to a year.

The declared increase in strength on the 28th day ranges from 2% [62] to 40% [48]. A longer test period (up to 150 days) demonstrates the increase in compressive strength by ~ 26% [63]. The increase in strength indicators is explained by a fixed decrease in porosity – up to 50% [48], which also leads to the decrease in the coefficient of water absorption – up to 57% [37].

Biomineralization processes in concrete contribute to increase and / or prolongation of the resistance of materials to the action of external aggressive factors. One of the most aggressive types of corrosion is sulfate corrosion of concrete. The destruction mechanism in this case can be described passing several stages. At the initial stage, the soluble calcium hydroxide “exits” into the pore space of the concrete. Then, under the influence of the external environment, free calcium ions interact with sulfate ions and form two-water calcium sulfate (gypsum), as well as calcium hydrosulfoaluminate.

The crystallization of new compounds occurs in the pore space of the material and is accompanied by a significant increase in volume. This leads to the increase in the volume of cement stone and the increase in stresses in the volume of the material, which leads to cracking of concrete, a decrease in its strength and the deterioration of service. It can be assumed that the biocarbonation of concrete will help increase its resistance to corrosion as a result of physical and chemical factors. The physical factor is associated with the deposition of carbonates in the pore volume of concrete and the clogging (filling) of free space in the composite material. The chemical factor is reasoned by the formation of insoluble stable calcium compounds that do not enter into a chemical reaction with sulfate compounds and, as a result, prevent the formation of “bacilic” compounds, leading to mechanical damage of composite matrix. According to the published results, sulfate corrosion resistance of concrete samples is recorded during the year [52].

The assessment of the effectiveness of self-healing of concrete containing bacterial agents, as well as finished products treated with bacterial compositions, showed that the monolithization of cracks is visualized (regardless of biomineralization technology) for 14–28 days or more.

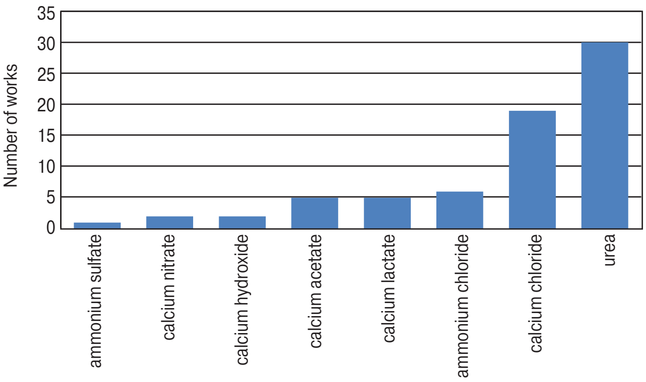

Fig. 4. Usage precursor ranking

Рис. 4. Ранжирование прекурсоров по частоте использования

Characterization of the features of phase formation products.

The study of the phase composition and morphology of crystals formed under the action of microbial enzymatic activity is interesting from the point of view of the determination of mechanisms of biomineralization and evaluation of its effectiveness when filling the pore space of a cement matrix as a result of the interaction of relict and newly formed minerals of various generations in a cement stone; biocementation of cracks; the formation of protective (restoration) carbonate films, etc.

As it is known, calcium carbonate forms three anhydrous polymorphic modifications – calcite, aragonite and vaterite; and two hydrated crystalline phases - monohydrocalcite ($CaCO_3 \times H_2O$) and ikaite ($CaCO_3 \times 6H_2O$). The studies showed that vaterite and calcite are the most common bacterial polymorphs of calcium carbonate, but they do not always form idiomorphic crystals [30, 49, 58, 69]. There are homogeneously distributed biocrystals in the form of needles, as well as rhomboid and rhombohedral crystals. The authors of the work [49] suggest that high ammonia content contributes to the stable formation of the rhombohedral crystal form. The spherical shape of the new growths is explained by the presence of acetate ions [58]. However, despite the occasional attempts to identify morphostructures, to establish some patterns of influence of the composition of precursors and the cemented matrix of the building composite on the morphology of newly formed carbonates, their interaction with binder hydration products and relic particles of the concrete mixture (aggregate grains, non-hydrated clinker minerals), these aspects remain understudied, i. e. the field for research remains very extensive.

Methods of the introduction of bacterial cultures and precursors.

The analysis of publications demonstrating the results of experimental studies on the technology of the use of the carbonate biomineralization method from the point of view of subsequent replication allows distinguishing the following three methods:

I. Introduction of carbonate mineralization precursors and a bacterial agent or agent association into the concrete mix (Table 1).

II. Local processing of ready-made (in plant conditions) or exploited (for example, during repair or restoration works) concrete products with solutions containing carbonate mineralization precursors and a bacterial agent or association of agents (Table 2).

III. Creation of new materials using biocarbonate synthesis (biocarbonate cementation) (Table 3).

Method I. The introduction of carbonate mineralization precursors and a bacterial agent or association of agents into the concrete mix is carried out at various stages of production (Fig. 5):

1. The introduction of a bacterial inoculum (microbial monoculture or bacterial association) or bacterial lyophilisate into mixing water with diluted precursors, followed by introduction of the resulting solution into a binder, followed by compounding with the remaining components of the concrete mixture (aggregate) [44–51];
2. Mixing a bacterial inoculum or bacterial lyophilisate with precursors, introducing this solution into water, subsequent mixing with the obtained binder solution and mixing with aggregate [32, 37, 39, 52–60];
3. Mixing a binder with dry precursors of carbonate mineralization initiation, adding mixing water, introducing a bacterial inoculum or lyophilisate and a filler at the last stage [61];
4. Adding immobilized (fixed) bacterial cultures to the concrete mixture, which includes nutrient components [61].

Depending on the method of immobilization used, this technological solution can be implemented in 2 options:

a) Absorption – immobilization of the inoculum of bacterial cultures in porous materials (carrier-absorbent) with a high sorption capacity [36–40, 42, 62–65];

b) Encapsulation – the introduction of an inoculum or lyophilisate of a bacterial culture into capsules made of special materials such as hydrogel, melamine, perlite, etc. [33–35, 66]. These capsules are introduced into the concrete mixture and opened because of stresses (cracking of the capsules) or dissolution (soluble capsule shell) when cracks appear in the exploited concrete.

The mineral component acting as a carrier on which the immobilization of microorganisms takes place may differ in particle size, type of mineral raw materials (alumina [37, 39, 40, 42], microcrystalline cellulose [38], pumice, zeolite [36], expanded clay [63], diatomite, hydrogel [66], etc.), its initial functional purpose in concrete mix (aggregate, pozzolanic additive, stabilizer, etc.). Immobilization in absorbent carriers occurs due to their high porosity and sorption capacity, which allows the inoculum to be absorbed together with the nutrient solution.

The processes of lysis of bacterial cells in building material also affect the degree of calcification. These problems have been solved by some authors using methods of encapsulation in various protective materials, followed by release under certain external conditions. The encapsulation of bacterial cultures is a complex and time-consuming process, but, from the point of view of protecting bacteria it is the most effective from the adverse environmental conditions of the concrete mixture and cytotoxic substances. It prevents outwashing of the culture, thereby prolonging the processes of carbonate biomineralization in building products during the operational period. This type of technology also requires additional research.

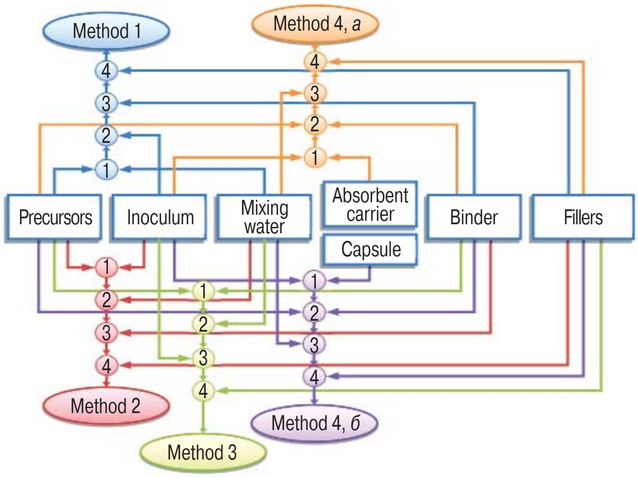

Fig. 5. Methods of the introduction of a bacterial agent and carbonate mineralization precursors in concrete mix

Рис. 5. Способы введения бактериального агента и прекурсоров карбонатной минерализации в состав бетонной смеси

Lyophilisates of bacterial cultures do not lose the efficiency of the urease enzyme after the use of freeze-drying and rehydration cycles. It allows bacteria to participate effectively and reproducibly in the structure formation processes of building materials, when they are introduced as a lyophilisate into the concrete mix, where, taking into account dry biomass, known ranges of hydrolysis efficiency and production of calcium carbonate are obtained.

Summarizing the analyzed studies, we note that in most articles the improvement in strength characteristics was observed as a result of the effect of bacterial cultures on a cement stone or product when microbial carbonate biomineralization initiators were introduced into the composition of the raw material mixture, which confirms the effectiveness of biocalcification. However, experimental data show a wide range of physical and mechanical parameters, depending not only on the generic affiliation of microorganisms and precursors, but also on technological conditions (sequence and method of introducing components, temperature conditions, system density, pH environment, etc.). In addition, various authors cite extremely disparate data on the characteristics obtained with almost identical compositions and technologies. In this regard, despite the fact that the potential of this type of biocarbonization technology is considered in various areas of science, complex interdisciplinary research is required for its application at industrial level in the production of building materials.

Method II. Local processing of concrete products can be carried out in the following options (Fig. 6):

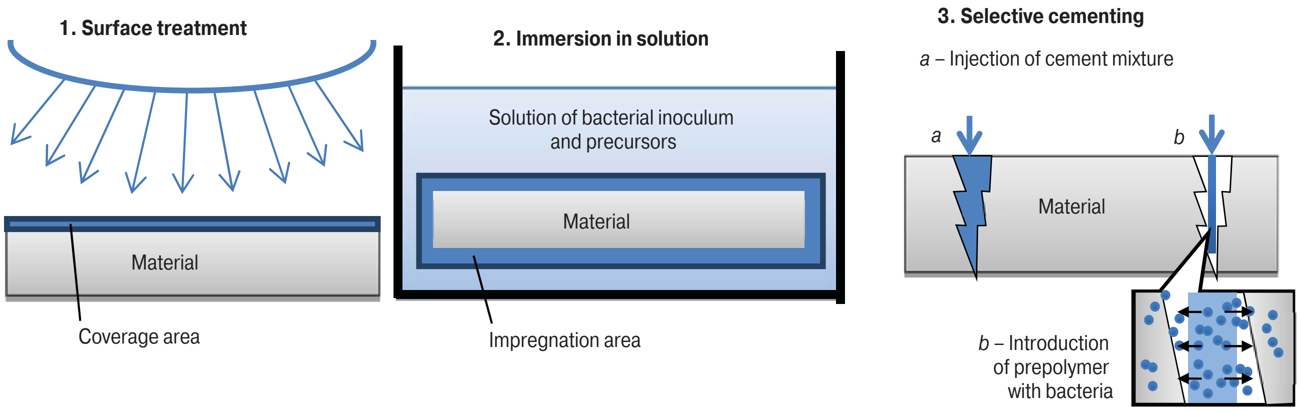

Fig. 6. Methods for local processing of concrete products
Рис. 6. Методы локальной обработка бетонных изделий

1. Surface treatment of materials:

a) Spraying a solution with a bacterial inoculum and precursors [52, 67, 68];

b) Applying a solution with a bacterial inoculum and precursors with a complete coating [31];

2. Immersion of the product in a solution of bacterial inoculum and precursors:

a) Partial immersion of the product [30];

b) Complete immersion of the product [30, 31, 37, 41, 43, 47, 52, 69, 70];

3. Selective cementing:

a) Injection of bacterial cement mixture into the cracks of concrete products [71];

b) Penetration of prepolymeric films with bacteria incorporated into the cracks in the cracks [72].

The treatment with bacterial solutions creates a coating with a hydrophobic effect, i. e. material is preserved and protected from environmental influences [67, 68].

The immersion method is more effective than the application method, since the impregnation forms a denser layer of the mineralizing membrane, which improves acid resistance, frost resistance, light and aging resistance of composite material [52, 68].

Surface treatment of building materials with solutions containing a bacterial inoculum and initiating carbonate mineralization is also effective. This method increases the durability of building materials, products and architectural monuments. This type of technology is already used in restoration work at industrial level.

Selective cementing allows the use of carbonate mineralization in the healing of cracks. Thus, injection of cement mixture containing inoculum with mineral components into the cracks is possible through injection of the solution under pressure [71].

The incorporation (inclusion) of cell fluid into the prepolymer (HYPOL®2000 polyurethane, consisting of a prepolymer (97%) and water-based toluene diisocyanate (3%)) and the creation of tapes and their introduction into cracks are new directions in the technology of concrete restoration. Despite the fact that this method slightly reduces the enzymatic activity of bacteria, the uniform distribution of the polymeric tape over the entire width of the crack opening contributes not only to the protection of bacteria from adverse conditions, but also to their uniform release and carbonization process stabilization [72].

Method III. The creation of new materials using biocarbonate synthesis is carried out by the simulation of formation of calcareous sandstones formed under geological conditions by calcite cementation of fragments of sand particles with different mineral composition (carbonate, quartz, etc.). In the same way, the material is obtained in artificial conditions as follows: aggregate (marble chips [71], quartz sand [42, 73–78]) is impregnated (percolation treatment) or mixed with a mixture of inoculum with carbonization precursors, which, in turn, can be either in the form of solutions or in an immobilized state, as a result of which carbonate cementation of bulk material occurs with the formation of a consolidated composite.

The promising technology of biocarbonate cementation in the production of building materials has a widely used analog in engineering geology. This is microbial carbonate cementation of soils by stimulation of natural biochemical processes. This treatment method involves the introduction of precursors and bacterial agents in a place where lithification of soils is required [12, 14].

Most often, sandy soil compaction is carried out using *Sporosarcina pasteurii* bacterial culture. As a result of the formation of calcium “bridges” between the grains of the soil, the porosity of the samples decreases, their permeability, and stiffness increases.

Percolation treatment of soils with solutions of initiation of crystallization of carbonates leads to the formation of a surface crust, which reduces leakage of liquid into the inner layers [18].

The manipulation of biogeochemical processes in soils by adding precursors and microorganisms in order to strengthen and stabilize slopes and embankments of roads and railways, minimize soil erosion, etc. is also reflected in interdisciplinary research and engineering projects [17].

Despite the fact that the technology of biocarbonate synthesis is similar to natural cementation, for obvious reasons (the duration of geological processes is incomparably longer, the pressure of overlying rock masses, temperature), the researchers have not been able to achieve the same strength indicators as their natural counterparts. In addition, the microbial cementation process is less manageable than traditional chemical methods. However, from the point of view of prototyping nature-like building materials, this technology seems to be very promising.

Conclusion

Scientific research in the field of development and application of microbial carbonate biomineralization as a variety of nature-like technologies oversteps the limits of engineering and technological research, being an outstanding example of interdisciplinary research bringing together experts in the field of biology, chemistry, geology and building materials science. This, of course, has its advantages, in terms of the scope of accumulated knowledge, scientific approaches and methods, as well as disadvantages, in terms of the fragmentation of research and its results and the difficulty in the consolidation of the efforts of scientists working in various fields of science.

Successful commercialization of any of the studied groups of technologies of microbial carbonate biomineralization requires the use of inexpensive precursors and strains of microorganisms, their production in large volumes, the development of appropriate regulatory and technical documentation, which makes this technology at this stage less repeatable in comparison with traditional methods.

At the same time, there is a fundamental justification of the functional role of carbonate biomineralization, based on research results in biology, mineralogy, engineering geology, lithology, paleontology, indicating the phase-forming function of microorganisms in the geological processes of rock formation, their role in the development of the Earth biosphere. A certain amount of empirical data was generated, both by specialists in the field of building materials science and in various related fields, which allows transforming them into scientific concepts, as well as building scientific hypotheses. However, the studies related to the processes of microbial carbonate mineralization in the technologies of the production of building materials are still at the stage of accumulation of experimental data. Therefore, it is important to ensure the methodological unity of their production and evaluation.

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