***Title Page**

Ecofriendly calcium phosphate and calcium bicarbonate biogrouts

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Abstract

An important direction of construction biotechnology is production and application of the biogrouts to decrease the hydraulic conductivity of soil or fractured rocks. The most popular biogrout is a mixture of calcium chloride, urea, and either ureolytic bacteria or enzyme urease. A disadvantage of this biogrout is the release of a large quantity of toxic ammonia to air, as well as harmful ammonium and hydroxide ions to water. The aim of this study was evaluation of two new biogrouts that release less of harmful by-products to environment. First biogrout was a mixture of acidified hydroxyapatite solution from the bone powder with an addition of two times

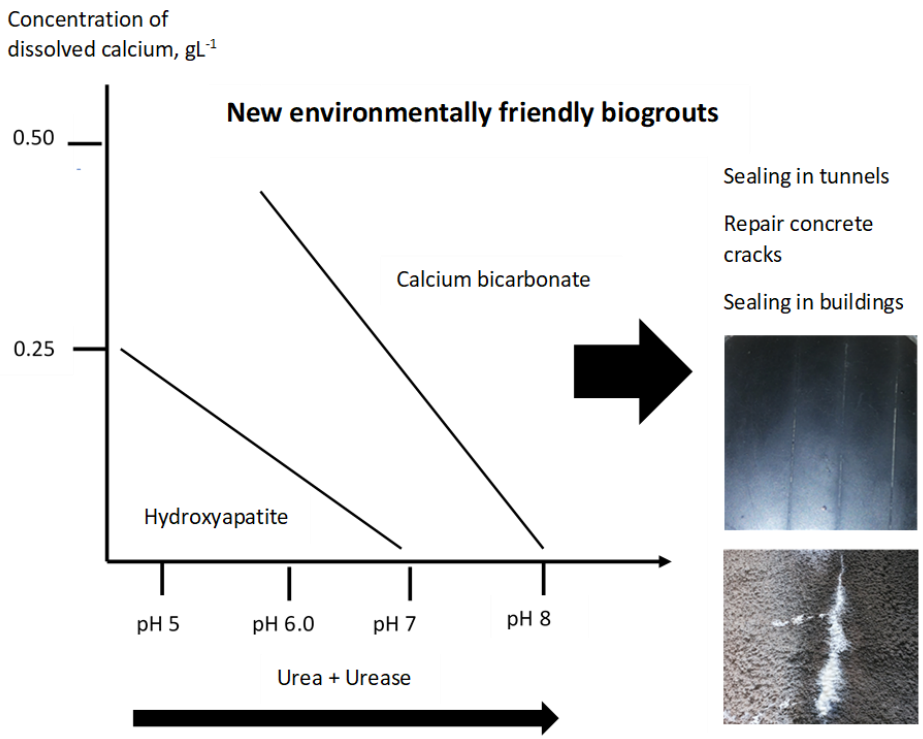
less urea that is used in conventional biogrout. Final pH was 7. Second biogrout was a solution of calcium bicarbonate produced from chalk powder and carbon dioxide with an addition of urea and acidotolerant ureolytic bacteria. This biogrout released to environment four times less ammonium and ammonia than conventional biogrout. Final pH was 8. Precipitation of about 2 g of calcium carbonate per kg of the cracked stone diminished the water seepage through the specimen by three orders of magnitude. Geotechnical or environmental engineering applications of these environmentally friendly biogrouts are limited by the consumption of a large volume of the grouting solution. It is due to low concentration of dissolved calcium, about 200 - 400 g m^{-3}, in these liquid biogrouts. So, these biogrouts could be most effective for the sealing and strengthening of the materials with low porosity such as the fractured rocks or cracked concrete.

Key words: biogrout; soil stabilization; fractured rocks sealing; calcium bicarbonate; calcium phosphate; hydroxyapatite.

Highlights

- environmentally friendly biogrout can be produced from hydroxyapatite of the bones;
- environmentally friendly biogrout can be produced from limestone and carbon dioxide;
- new biogrouts can be used for the sealing of the fractured rocks and cracked concrete.

Graphical abstract



1. Introduction

Construction Biotechnology, i.e. applications of biotechnology to civil and environmental engineering, includes production of the biogrouts (Stabnikov et al., 2015; Ivanov and Stabnikov, 2017). The microbial grout has low viscosity and deeper penetration of the grouting solution in soil or fractured rocks than any conventional chemical grout. Its application produces *in situ* insoluble crystals that bind or clog the porous material. The biogrout can be used in geotechnical and environmental engineering for the ground improvement, soil stabilization, soil desaturation and mitigation of soil liquefaction after earthquake, stabilization of the slopes and dams, control

of the wind and water erosion of soil, to diminish erosion of a shore, to diminish seepage in the channel, pond, landfill, reservoir, tunnel, and dam, to immobilize chemical, radioactive, and biological pollutants of soil (DeJong et al., 2013; Ivanov and Stabnikov, 2017; Haouzi and Courcelles, 2018). There are known at present several hundred experimental papers on these topics as shown in the reviews (DeJong et al., 2013; Stabnikov et al., 2015) and in a monography (Ivanov and Stabnikov, 2017). However, a few practical scale applications of biogrouts are known at present (DeJong et al., 2013; Haouzi and Courcelles, 2018).

The major reason of limitation for practical applications of the biogrouts is environmental harm of the most popular biogrouting process, which often is called as "microbially-induced calcite (or carbonate) precipitation" (MICP). This process has disadvantage, which is formation and release of ammonia to atmosphere, as well as ammonium and hydroxide ions to water. It is performing according to Equations 1-3:

$$(NH_2)_2CO + 2\ H_2O \rightarrow 2\ NH_4^+ + 2\ OH^- + CO_2 \qquad (1),$$

$$CO_2 + 2\ OH^- \leftrightarrow CO_3^{-2} + H_2O \qquad (2),$$

$$Ca^{2+} + CO_3^{-2} \rightarrow CaCO_3\downarrow \qquad (3).$$

Theoretical molar ratio urea:Ca in biogrout is 1:1. However, the real molar ratio urea:Ca in biogrout in majority of experiments was from 1.5 to 2 (Ivanov and Stabnikov, 2017) because a significant portion of urea must be hydrolyzed to increase the pH *in situ* to 8.5 - 9.5 for effective crystallization of calcium carbonate. This increase of the pH also shifts equilibrium between ammonium and ammonia to formation of gaseous toxic ammonia (Equation 4):

$$NH_4^+ + OH^- \leftrightarrow NH_3\uparrow + H_2O \qquad (4).$$

The major enzyme involved in the biogrouting process is urease of bacterial (Ivanov and Stabnikov, 2017; Krajewska, 2017) or plant origin, usually from jack bean (*Canavalia*

ensiformis) (Dilrukshi et al. 2018). Because of high cost of pure enzyme urease, it is replaced by either biomass of urease-producing bacteria of pure and identified culture (Ivanov and Stabnikov, 2017) or potentially unsafe enrichment culture of non-identified indigenous soil bacteria (Burbank et al., 2011; Gomez et al., 2017; Rajasekar et al., 2017).

To avoid environmental harm during biogrouting, an addition of the zeolite to the conventional grout to adsorb ammonium ions (Keykha et al., 2017), retention of ammonium ions near cathode during electro-biostabilization of soil (Keykha and Asadi, 2017), or absorbance of released ammonia by sulphuric acid (Ivanov and Stabnikov, 2017) were proposed.

Alternative solution is biogrouting due to precipitation of calcium phosphate compounds from inorganic phosphates (Akiyama and Kawasaki, 2012; Dilrukshi et al., 2018) or organophosphates (Roeselers and van Loosdrecht, 2010) to produce insoluble and strong crystals of hydroxyapatatite (Akiyama and Kawasaki, 2012; Yu and Jiang, 2018).

Therefore, one goal of this study was evaluation of the low cost, environmentally friendly biogrout based on hydroxyapatite precipitation (see Table 1, Equation 11). It is well known that at pH 4.5 calcium concentration of dissolved hydroxyapatite is about 0.25 g L^{-1}, while at pH 7.0 it is about 0.001 g Ca L^{-1} (see Table 1). Precipitation in this method could be due to enzymatic hydrolysis of urea that increases the pH from 4.5 to 7.

Another new environmentally friendly biogrout with similar pH-dependent mechanism of precipitation could be soluble calcium bicarbonate that is transforming *in situ* to insoluble calcium carbonate (Equation 5) due to increase of the pH from 6.0 to 8.0 during hydrolysis of urea:

$$Ca(HCO_3)_2 + OH^- \rightarrow CaCO_3\downarrow + HCO_3^- + H_2O \qquad (5).$$

Theoretical molar ratio urea:Ca for this biogrout is 0.5, which is 3 - 4 times lower than usual urea:Ca ratio in the conventional MICP. Therefore, solution of calcium bicarbonate was also evaluated in this study as environmentally friendly biogrout. Increase of the pH from 6 to 8 that is required for decay of calcium bicarbonate can be performed by addition of urease-producing bacteria and urea.

The aim of the research was testing of new environmentally friendly biogrouts based on the precipitation of either hydroxyapatite or calcium carbonate.

2. Materials and Methods

Production of hydroxyapatite grout was from the acidified bone powder. Production of calcium bicarbonate biogrout was from limestone and carbon dioxide. Their use for the sealing of the porous materials requires an addition of biomass of acidotolerant urease-active bacteria and urea.

2.1. Production and use of hydroxyapatite biogrout

To produce this biogrout, 10 g of commercial feed bone meal and 15 mL of concentrated HCl were added to 1 L of tap water. Tap water was used because the concentrations of the biogrout components exceed concentrations of inorganics in tap water for one or two orders of magnitude. So, theoretically there will be no effect of tap water on the results of biogrouting. Additionally, parallel experiments on biogrouting/biocementation made in Kyiv, Ukraine, or in Singapore, showed that the results, for example specific growth rate of bacteria, are the same

using tap or deionized water in these cities because concentrations of medium components are too high in comparison with the concentrations of water components. So, there is no need to account properties of tap water for geotechnical experiments on biogrouting. The bottle was rolled for 5 h at 20°C, then the pH of extract was adjusted to pH 4.5 and solution of hydroxyapatite was decanted.

Hydroxyapatite biogrout contained two liquid components: 1) 1 L of hydroxyapatite solution with about 0.25 g Ca L^{-1} and 0.1 g urea L^{-1} at pH 4.5; 2) 0.1 L of suspension of acidotolerant urease-producing bacteria. The biogrouting process was performed in two steps: 1) addition of bacterial suspension to the cracked concrete or stone cylinder with 10 cm diameter and 10 cm height, coated and bound by the plastic tape, with the rate of 10 mL min^{-1} for adhesion of bacterial cells on the surface of the cracks; 2) the solution of hydroxyapatite with urea was supplied and recycled by the peristaltic pump with the rate about 10 mL min^{-1} ensuring clogging of the cracks after two days of the treatment. Effluent from the cracked cylinder was collected to measure flow rate, pH and concentration of ammonium.

2.2. Production and use of calcium bicarbonate biogrout

To produce this biogrout, 1 g of calcium carbonate powder was added to 1.5 L plastic bottle with 1 L of tap water and 0.5 L CO_2 at atmospheric pressure. The bottle was rolled for 5 h at 20°C. After 1 h of sedimentation, the solution of calcium bicarbonate was decanted.

Calcium bicarbonate biogrout contained two components: 1) 1 L of calcium bicarbonate solution with pH 6.0 - 6.3, containing 0.40 g Ca L^{-1} and 0.2 g urea L^{-1}; 2) 0.1 L of suspension of urease-producing bacteria *Bacillus sp.* (*Sporosarcina pasteurii*) VS1 (Ivanov and Stabnikov,

2017). The biogrouting process was performed in two steps: 1) addition of bacterial suspension *Sporosarcina pasterii* VS1 with the rate of 10 mL min^{-1} to the cracked concrete or stone cylinder with 10 cm diameter and 10 cm height, confined by the plastic tape. It was performed for adhesion of bacterial cells on the surface of the cracks; 2) the solution of calcium bicarbonate with urea was supplied and recycled with the rate about 10 mL min^{-1}. It was performed to clog the cracks after two days of the treatment. Effluent from the cracked cylinder was collected to measure flow rate, pH and concentration of ammonium.

2.3. Selection of acidotolerant urease-producing bacteria

Acid urease is needed for the precipitation of hydroxyapatite due to increase of the pH from 4.5 to 7. Several samples of acidic soil were taken in the park with the pine trees and lawns fertilized with carbamide and used for preparation of the enrichment culture of acidotolerant urease-producing microorganisms. Growth medium for the enrichment culture was of the following content: Tryptic Soya Broth, 30 g L^{-1}; urea, 20 g L^{-1}; NaCl, 15 g L^{-1}, , $NiCl_2$, 24 mg L^{-1}, pH indicator bromocresol purple, 20 mg L^{-1}, 0.1 N HCl to adjust the pH to 4.5, soil sample, 10 g L^{-1}, distilled water, 1 L. Cultivation of soil inoculum was in 70 mL of this medium in 300 mL flasks on the shaker at 200 rpm at 25°C for 72 hours. Change of medium color from yellow-green to violet indicated urease activity that increased pH from 4.5 to 6.8. Obtained enrichment culture was used for pure culture isolation. Aliquots of 0.1 mL from ten-fold dilutions in sterile 1.5% NaCl solution were spread on Petri dishes with growth medium shown above and solidified with 2.0% Bacto Agar (Difco). Petri dishes were incubated after inoculation at the room temperature for 5 days. The urease-positive colonies changed color of the surrounding medium

from yellow-green at pH 4.5 to violet at pH 6.8 due to urease activity and increase of the pH. Pure culture, isolated from one colony, marked as strain AU1, was grown in the liquid medium shown above, for three days at 25°C on the shaker for aeration and then was used for biogrouting as described above.

2.4. Measurements

The seepage through the cracked stone or concrete was used to evaluate effects of the grouting. The seepage was measured at the constant head 5-10 cm.

The surface of the treated material was observed using a scanning electron microscope Quanta 250 FEG (FEI Corporation, Oregon, USA) with a light-element X-ray detector and an Indium X-ray Fluorescence (IXRF) Systems EDS analysis system.

Calcium concentrations in the liquid before and after biogrouting were determined using standard ethylene diaminetetraacetate (EDTA) titrimetric method 2340C with Eriochrome Black T indicator (APHA, 1999). Liquid sample after filtration through 0.2 µm membrane, 50 mL, was placed in a 100 mL conical flask and 1 mL of a buffer solution was added to maintain the pH of 10.0, then few drops of Eriochrome Black T indicator were added as an indicator. The sample was titrated with 0.01 M solution of EDTA until the color was changed from purple to blue.

Concentration of ammonium in effluent after treatment was measured by the APHA standard 4500-NH_3 F Phenate method (APHA, 1999). An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite, and phenol catalyzed by sodium nitroprusside. To measure ammonium, add 1 mL of 10% (v v^{-1}) phenol solution in ethanol, 1 mL of 5% (w v^{-1}) sodium nitroprusside solution, and 2.5 mL oxidizing solution (a mixture of 25 mL

5% (w v^{-1}) sodium hypochlorite with 20 g of trisodium citrate, 1 g of sodium hydroxide and 100 mL of deionized water) to a 25 mL sample in a 50 mL Erlenmeyer flask, let color develop at room temperature (22 to 27°C) for 1 h, and measure light absorbance at 640 nm.

All control and experiments, as well as chemical analysis have been done in triplicate. Mean values ± standard deviations are shown for the data comparisons.

3. Results and Discussion

To produce and apply new biogrouts, the strain of acidotolerant urease-producing microorganism was selected and a choice of inorganic component for environmentally friendly biogrout based on calcium phosphate precipitation has been done. The testing of environmentally friendly biogrouts for the sealing of the solid materials has been done at laboratory conditions.

3.1. Selection of acidotolerant urease-producing microorganism

The strain AU1 was identified using PCR and sequencing of 16S rRNA gene (Ivanov and Stabnikov, 2017). The full-length sequence was compared with all other sequences available in the NCBI Genbank database using BLAST (http://blast.ncbi.nlm.nih.gov). Strain AU1 that produced acid urease was identified as *Staphylococcus saprophyticus* with 100% identity with the 16S rRNA gene sequences of four other strains of this species.

Biosafety is important question in soil biogrouting and soil bioremediation. Prokaryotes are classified into four risk groups (RG) according to German Technical Rules for Biological Agents on classification of prokaryotes into risk groups (TBRA 466, 2010). Microorganisms from RG 1

are unlikely to cause human disease, and microorganisms from RG 2 can cause human disease but unlikely to spread to the community.

There are known safe and unsafe strains of *Staphylococcus saprophyticus.* For example, *Staphylococcus saprophyticus subsp. bovis* belongs to RG1, while *Staphylococcus saprophyticus subsp. saprophyticus* belongs to RG2. The strain of *Staphylococcus saprophyticus* was selected not only in our study on biogrouting but also was selected by urease activity among numerous isolates (Ghezelbash and Haddadi, 2018).

Generally, according to the main Koch's postulates the pathogenic microorganism must be found in abundance in all organisms suffering from the disease, but should not be found in healthy organisms, and the cultured microorganism should cause disease when introduced into a healthy organism. However, *Staphylococcus saprophyticus* is a representative of common gastrointestinal flora in pigs and cows. It is also is a part of the normal human flora that colonizes the perineum, rectum, urethra, cervix, and gastrointestinal tract. Over 40% of all young women contain *S. saprophyticus* as part of their normal genitourinary flora. However, at the same time there are known strains of this species that were isolated from patients with urinary tract infections associated with *S. saprophyticus* (Ehlers and Merrill, 2018; Martins et al., 2018). So, *Staphylococcus saprophyticus* could probably cause disease in a host whose immune system is not functioning properly or after accident.

Most active bacteria that are currently used in soil bioremediation and municipal wastewater treatment are similar microorganisms, i.e. they are representatives of normal human microflora but cause disease occasionally or in the immune-compromised patients. Therefore, it is a common environmental engineering practice to use representatives of normal human microflora. However, it could be safer to replace any live bacteria with dead but urease-active

cells (Ivanov and Stabnikov, 2017). It is safer also to use enzyme urease instead of urease-producing bacteria, but this is limited by the high cost of enzyme. However, future industrial-scale production of crude acid or neutral urease could diminish cost of enzyme to the practically acceptable level.

There are also known other acidotolerant urease-producing bacteria such as *Arthrobacter mobilis*, which produce acid urease that has been used to remove urea from sake and wine (Miyagawa et al., 1999; Panesar and Marwaha, 2010), fish pathogen *Edwardsiella ictaluri* (Booth et al., 2009), as well as lactic acid bacteria that are using in yogurt, probiotics, fermented milk, or cheese production such as *Lactobacillus reuteri*, *Streptococcus salivarius*, *S. thermophilus* (Mora and Arioli, 2014). Application of lactic acid bacteria as a source of acid urease could be most interesting approach to replace *Staphylococcus saprophyticus* or other sources of acid urease.

3.2. Choice of inorganic component for environmentally friendly biogrout based on calcium phosphate precipitation

The main condition for precipitation of hydroxyapatite from different calcium phosphates (see Table 1) is increase of the pH, which is caused by enzymatic hydrolysis of urea (Eq. 1).

Table 1. Precipitation of hydroxyapatite due to transformation of calcium phosphate compounds shown in Equations 6 - 12 (modified from Tung, 1998; Pan and Darvell, 2010; Akiyama and Kawasaki, 2012; Kuroda and Okido, 2012)

Compound	Ca/P	Urea/Ca	Solubility at 25°C, g L^{-1}	Reaction of precipitation
Monocalcium phosphate	0.5	2.10	20	$5\ Ca(H_2PO_4)_2 + 21\ OH^- \rightarrow Ca_5(PO_4)_3OH\downarrow + 7\ PO_4^{3-} + 21\ H_2O$ (6)
Dicalcium phosphate	1.0	0.60	0.2	$5\ CaHPO_4 + 6\ OH^- \rightarrow Ca_5(PO_4)_3OH\downarrow + 6\ PO_4^{3-} + 6\ H_2O$ (7)
Octacalcium phosphate	1.33	0.22	0.28 - 0.56	$5\ Ca_8(PO_4)_6H_2 + 18\ OH^- \rightarrow 8\ Ca_5(PO_4)_3OH\downarrow + 6\ PO_4^{3-} + 18\ H_2O$ (8)
Tricalcium phosphate	1.5	0.10	0.64 g CaL^{-1} at pH 4.5 0.008 g CaL^{-1} at pH 7.0	$5\ Ca_3(PO_4)_2 + 3\ OH^- \rightarrow 3\ Ca_5(PO_4)_3OH\downarrow + PO_4^{3-} + 3\ H_2O$ (9)
Hydroxyapatite	1.67	0.40	0.25 g CaL^{-1} at pH 4.5	Dissolution of hydroxyapatite at low pH due to formation of slightly soluble dicalcium phosphate: $Ca_{10}(PO_4)_6(OH)_2 + 8\ H^+ \rightarrow 4\ Ca^{2+} + 6\ CaHPO_4 + 2\ H_2O$ (10) Crystallization of hydroxyapatite *in situ* due to increase of the pH by urease:

Compound	Ca/P	Urea/Ca	Solubility at 25°C, g L^{-1}	Reaction of precipitation
			0.001 g CaL^{-1} at pH 7.0	$4\ Ca^{2+} + 6\ CaHPO_4 + 8\ OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2\downarrow + 6\ H_2O$ (11)
Tetracalcium phosphate	2.0	0.17	0.02	$3\ Ca_4(PO_4)_2O + 3\ H_2O + 4\ OH^- \rightarrow 2\ Ca_5(PO_4)_3OH\downarrow + 2\ Ca(OH)_2$ (12)

As can be seen from the Table 1, dissolved hydroxyapatite at pH 4.5 could be the best component for grouting among other calcium phosphate compounds. The molar ratio urea:Ca for this biogrout is 1:1, which is 30% - 50% lower than urea:Ca ratio in conventional MICP. Due to neutral pH, the main harmful product of this grouting will be ammonium. The release of ammonia will be low, so it could be relatively safe to use this biogrout in the confined space of the tunnels or buildings.

3.3. Environmentally friendly biogrout based on hydroxyapatite and its use for biogrouting

Stability of the hydroxyapatite biogrout depends on the pH only, so the solution of hydroxyapatite components will be stable at the pH 4.5. The rate of the biogrouting based on precipitation of hydroxyapatite can vary in the wide range because it depends on many factors such as: 1) porosity of the material; 2) the number of bacterial cells adhered to the surfaces of the cracks; 3) the urease activity of these bacterial cells; and 4) the retention time for the biogrout in

the pores. For the contents, dosages, and flow rates shown in the Materials and Methods section the clogging of the cracked concrete or stone typically can be done for 2 days of the treatment. Seepage through the cracked specimen depended on the quantity of precipitated calcium phosphate. To diminish the seepage through the specimen of the cracked concrete from 1×10^{-4} $m^3\times m^{-2}$ of the cracked surface$\times s^{-1}$ by three orders of magnitude it was necessary to precipitate about 20 g of Ca in form of hydroxyapatite per 1 kg of the cracked concrete. Crystals formed in the grouting space were rod-like and plate-like microparticles (Fig.1).

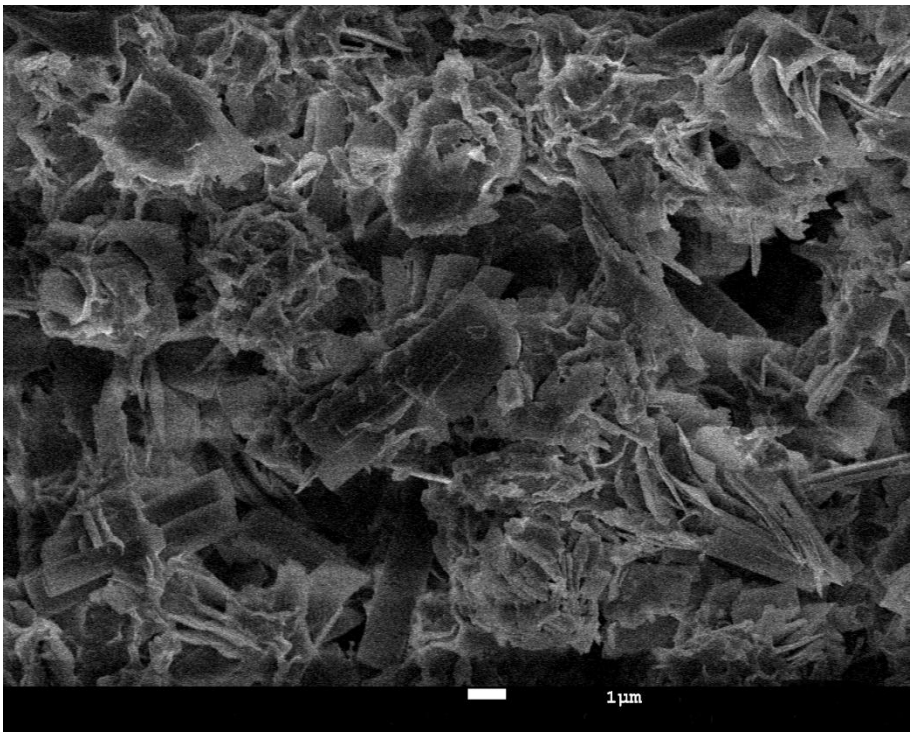


Figure 1. Crystals formed in the grouting space.

Concentration of ammonium in effluent was below 50 mg N-NH_3 L^{-1}, pH was in the range 6.8 - 7.4. Therefore, it was no smell of ammonia from the treated cylinder of the cracked

concrete. So, the biogrout for the sealing of porous materials can produced from the bone powder, urea and a source of acid urease.

3.4. Environmentally-friendly biogrout based on calcium bicarbonate

Solution of calcium bicarbonate from calcium carbonate in atmosphere of CO_2 at 1 bar was produced for 3 hours (Fig. 2) with maximum concentration of 0.9 g Ca L^{-1}.

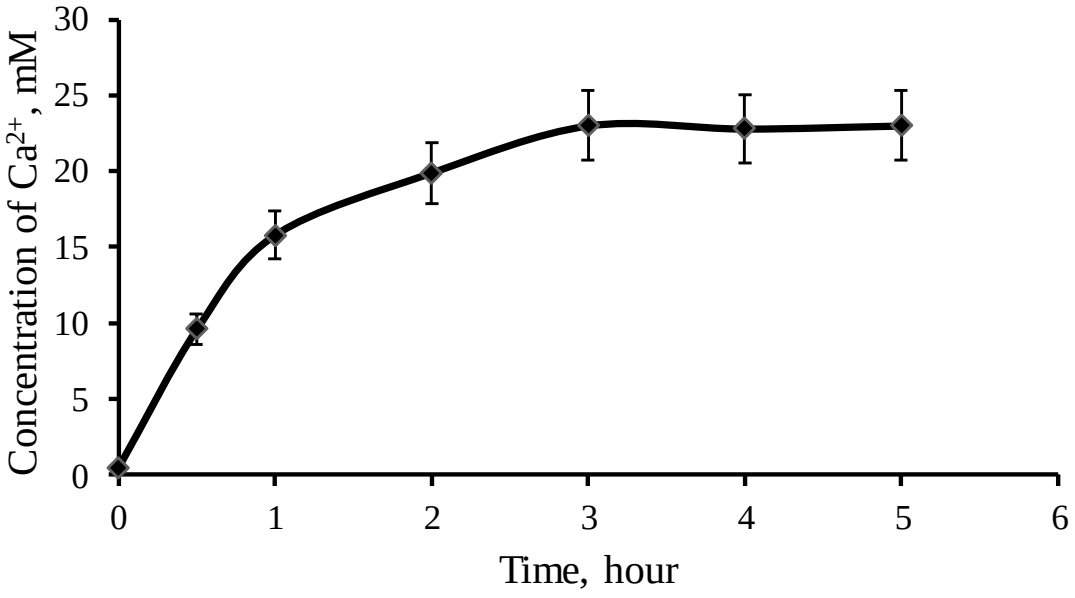


Figure 2. Production of calcium bicarbonate from $CaCO_3$ and CO_2.

The solution of calcium bicarbonate in the atmosphere of CO_2 was stable for at least one week at the pH 6.0 - 6.3. However, calcium bicarbonate decayed with the production of calcium carbonate either under urease-initiated increase of the pH to 8 or even at a low partial pressure of

CO_2, without addition of urea and urease. The average rate of self-decay on air, i.e. the rate of the biogrouting, was 0.84 g Ca×m^{-3} of solution×h^{-1}. The decay of calcium bicarbonate on air is shown in Fig. 3.

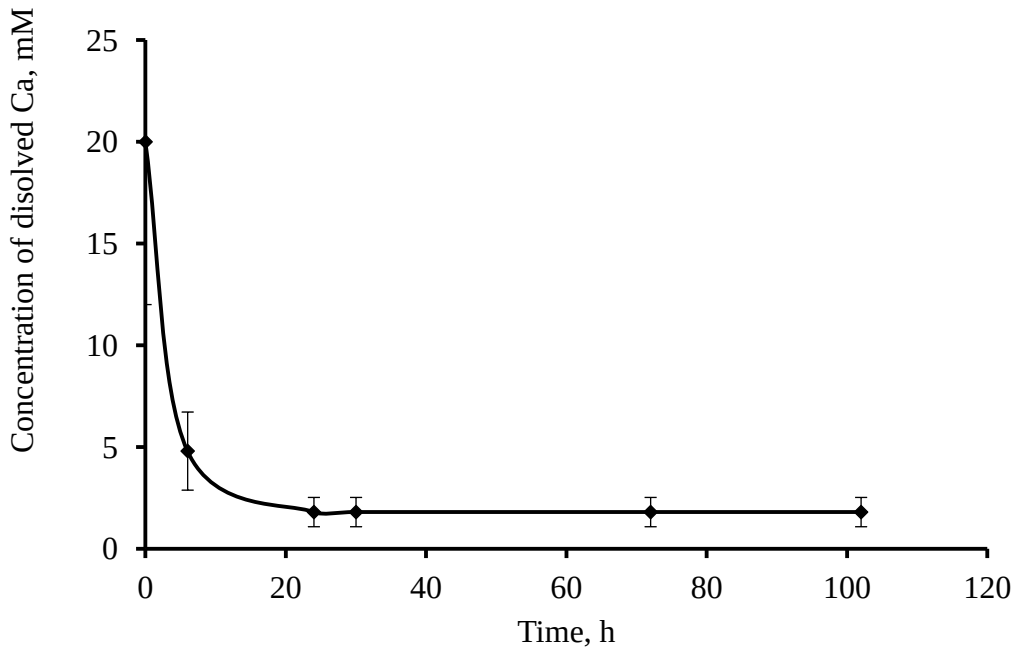


Figure 3. Self-decay of calcium bicarbonate on air.

The biogrouting process was performed by addition of bacterial suspension *Sporosarcina pasterii* VS1 to the cracked concrete or stone cylinder with the rate about 10 mL min^{-1} and then by addition and recycle of the calcium bicarbonate solution with urea with the rate about 10 mL min^{-1} that ensured clogging of the cracks after two days of the treatment.

Generally, the rate of this biogrouting depends on: 1) the partial pressure of CO_2; 2) the pH; 3) the material porosity; 4) the urease activity of bacterial cells on the surface of the cracks; 5) the retention time for the biogrout inside the pores. In our experiments, the clogging of the

cracked concrete or stone can be made for two days of the treatment. Calcium bicarbonate solution produced during clogging the plate-shaped crystals (Fig.4).

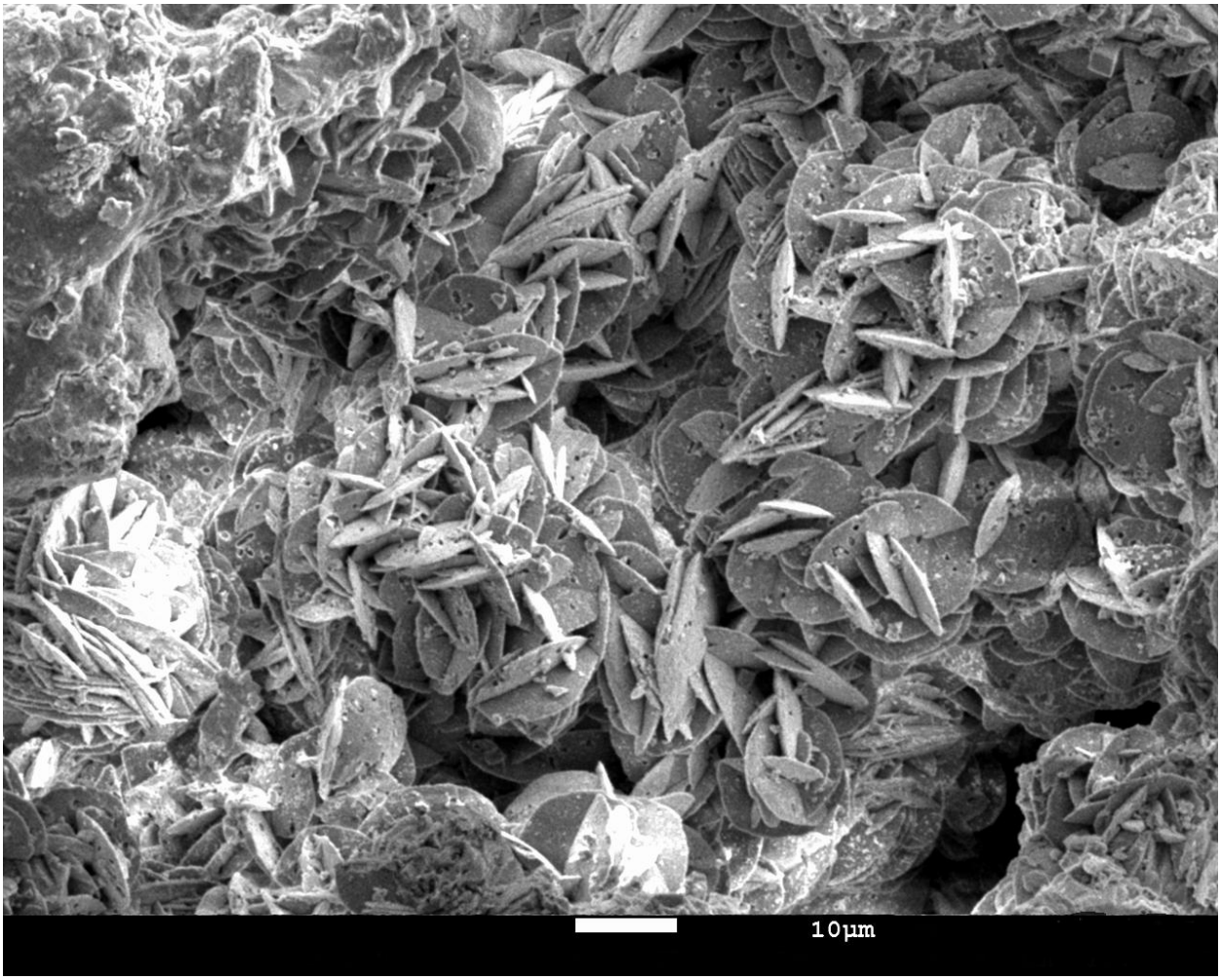


Figure 4. Crystals produced after treatment of the fissures with calcium bicarbonate grout.

These crystals clog the fissures in stone (Fig.5) in linear correlation with the volume of the used grout (Fig.6).

(a)

(b)

c

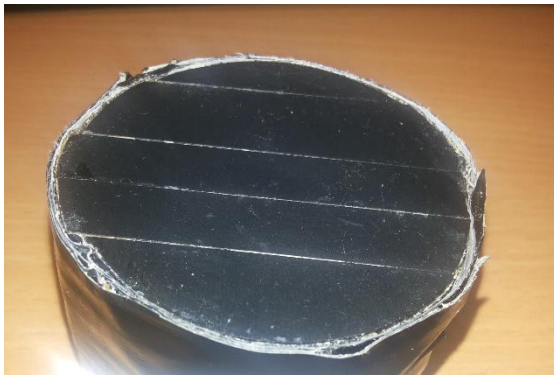


Figure 5a-c. Cracked stone before (a) and after treatment (b), and the treated stone cylinder with the cuts along the axis (c).

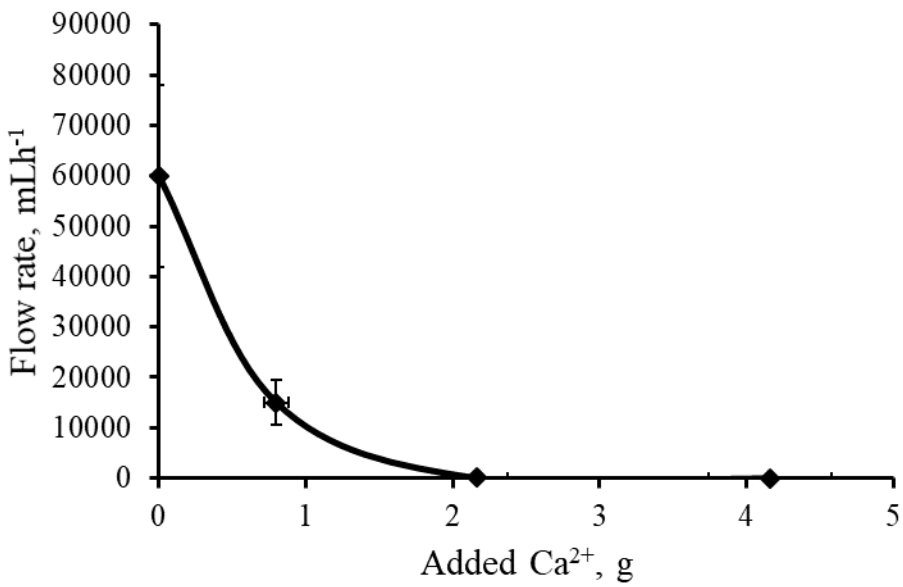


Figure 6. Correlation of seepage (flow rate) through the cracked stone cylinder with the content of precipitated calcium during grouting with calcium bicarbonate solution.

To diminish the seepage of the specimen of the cracked stone by three orders of magnitude it was necessary to use about 2 g of precipitated calcium carbonate per 1 kg of the cracked stone. Self-decay of calcium bicarbonate at low partial pressure of CO_2 is the most environmentally

friendly grouting process. Concentration of ammonium in effluent was below 100 mg N-NH_3 L^{-1}, pH was about 8.0, therefore it was no any ammonia smell from the treated cracked stone cylinder. So, the biogrout for the sealing of porous materials can produced from the limestone powder, carbon dioxide, urea and a source of acid urease.

3.5. Industrial aspects of the hydroxyapatite biogrout production

Economic advantage of hydroxyapatite biogrout is an opportunity to produce it from such sustainable material as the bones of livestock. The bone meal powder is used as a feed component and partially as the fertilizer but about half of the bones that remain after meat processing are incinerated, usually in the cement kilns (Moller, 2015). So, there available million tons of the bone wastes for biogrout production. The calculated cost of calcium from the bone meal powder is US $525 – 1250 per metric ton. This is comparable with the cost of calcium from calcium chloride, US $416 – 832 per metric ton, which is the most popular component for the conventional MICP biogrout. Additionally, the supply of urea is four times lower than in conventional MICP grouting. The cost of calcium from the bone meal was calculated for the feed grade bone meal powder with cost of US $125-250 per metric ton and the content of Ca about 20% (w w^{-1}). The cost of calcium from calcium chloride was calculated for technical grade anhydrous calcium chloride with cost of US$150 - 300 per metric ton and the content of Ca 36% (w w^{-1}).

Production of hydroxyapatite liquid biogrout should include the following stages: grinding of the row bones, extraction of soluble hydroxyapatite from the grinded raw bones or dry bone meal powder by 1M hydrochloric acid to the concentration of dissolved calcium about 0.25 gL^{-1} and pH 4.5, and decantation of acid solution. The precipitating protein part of the bones after

extraction of calcium phosphates could be used as a feed or food product. Concentrated solution of urea and acid urease or suspension of urease-containing bacteria should be added to the acid solution of hydroxyapatite components before the biogrouting procedure. This biogrout is sustainable due to the production from meat-processing waste and more environmentally-friendly than conventional MICP biogrout.

3.6. Industrial aspects of the calcium bicarbonate biogrout production

An economic advantage of calcium bicarbonate biogrout is an opportunity to produce it from cheap limestone powder, chalk powder, or even from the waste cement powder. The calculated cost of calcium from calcium carbonate powder, US $20 - 32 per metric ton, is significantly lower than the cost of calcium from calcium chloride, US $416 – 832 metric ton, which is the most popular component for the conventional MICP biogrout. Additionally, the supply of urea is 3 - 4 times lower than in conventional MICP grouting.

The cost of calcium from the calcium carbonate powder was calculated for the calcium carbonate powder with price of US $ 50-80 per metric ton and the content of Ca 40% (w w^{-1}). The cost of calcium from calcium chloride was calculated for technical grade anhydrous calcium chloride with its price of US $150 - 300 per metric ton and the content of Ca 36% (w w^{-1}).

Production of the liquid calcium bicarbonate grout should include the following stages: grinding of the limestone, stirring of limestone in the rotating reactor under atmosphere of CO_2 to concentration of dissolved calcium about 0.4 g L^{-1}, and decantation of the calcium bicarbonate solution. The concentrated solution of urea and acid urease or suspension of urease-containing bacteria should be added to this solution before the biogrouting procedure.

In case of production of the calcium bicarbonate solution from the waste cement powder and carbon dioxide released during cement production the cost of this grout will be significantly lower than the cost of the conventional MICP biogrout using calcium chloride. So, the best way of the calcium bicarbonate biogrout production could be the use of the waste cement powder and CO_2 of produced on the cement plant.

4. Conclusions

The experiments outlined that environmentally friendly biogrouts can be produced from the cheap sources like bone powder, limestone powder, and carbon dioxide. These biogrouts release to environment 2 or 4 times less toxic ammonium or ammonia than MICP biogrout. The obtained results are important for the sustainable development and cleaner production of the biogrouting materials for civil and environmental engineering. New biogrouts help to overcome the problem of environmental safety of the biogrouts. So, the consequences of the obtained results will be industrial sustainable production and the large-scale use of new environmentally friendly biogrouts.

However, these new biogrouts are not universal ones. Concentration of dissolved calcium in these liquid biogrouts is low, from 200 to 400 mg L^{-1}, so they could be used only under the following conditions: 1) when the release of toxic gaseous ammonia is a big concern due to the confined space in the tunnels or buildings; 2) when porosity of the grouted material is lower than approximately 2%, so low quantity of grout is needed for the sealing; 3) the geotechnical application site is not far from the biogrout production site. For example, the best use of these grouts could be the sealing of the fractured rocks in the tunnels and other underground facilities,

nuclear waste storages, repair of the cracks in concrete and stone, soil dust control, and confinement of the chemical, biological, and radiological agents in soil crust.

Acknowledgements

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Figure captions

Fig. 1. Crystals formed in the grouting space.

Fig. 2. Production of calcium bicarbonate from $CaCO_3$ and CO_2.

Fig. 3. Self-decay of calcium bicarbonate on air.

Fig. 4. Crystals produced after treatment of the fissures with calcium bicarbonate grout.

Fig. 5a-c. The cracked stone cylinder before (Fig.5a) and after treatment (Fig.5b), and the treated stone cylinder with the cuts along the axis (Fig.5c).

Fig. 6. Correlation of seepage through the cracked stone cylinder with the content of precipitated calcium during grouting with calcium bicarbonate solution.

To: Dr. Giorgio Besagni,
Associate Editor
Journal of Cleaner Production

27th of January, 2019

Cover letter

Dear Dr. Besagni,

The reviewers have suggested the acceptance of our submission, but you mentioned in your letter that the manuscript contains some issues to be solved before final acceptance.

On behalf of the authors, I am pleased to submit to the Journal of Cleaner Production the manuscript "Ecofriendly calcium phosphate and calcium bicarbonate biogrouts" revised according to your comments. A list of the answers and actions to all your comments is given in the separate file.

Thank you for your kind and very useful consideration of our manuscript.

Yours sincerely,

Volodymyr Ivanov
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Deputy Rector on International Biotechnological Projects
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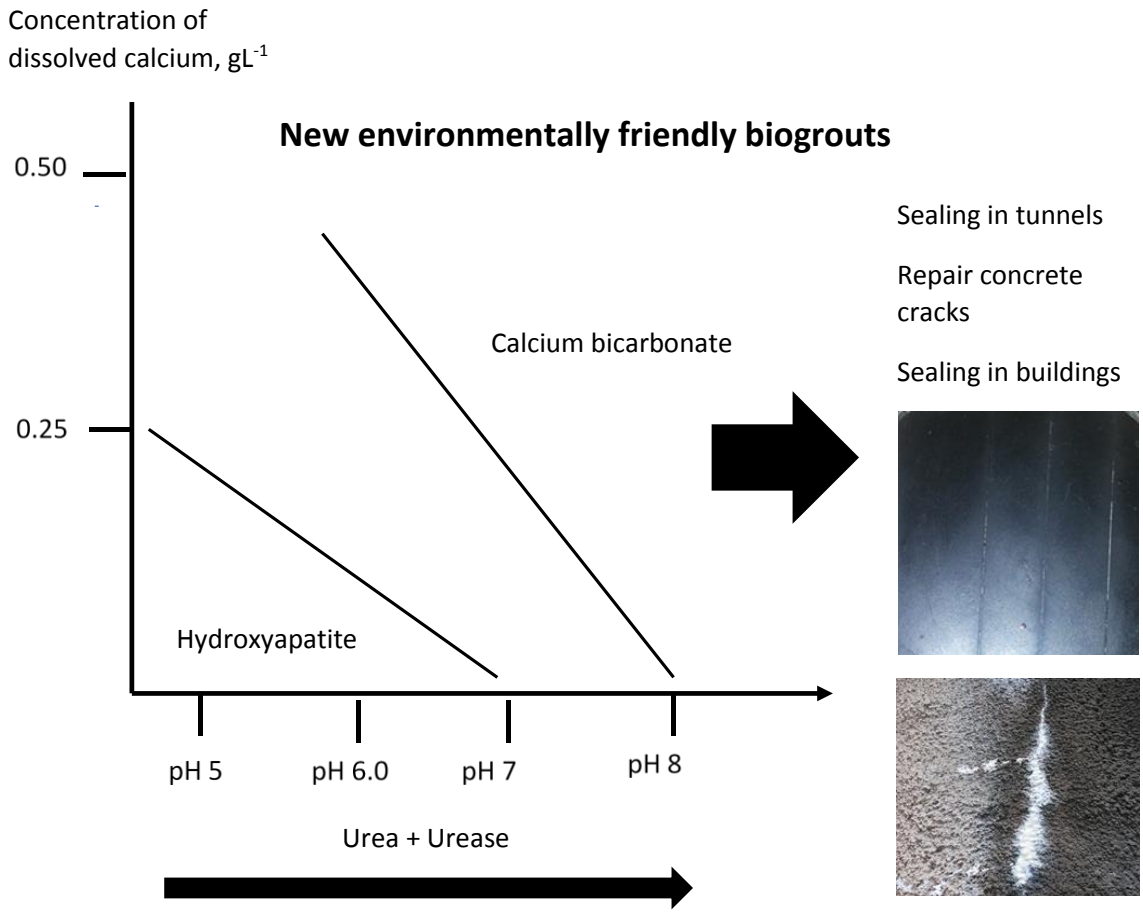
Graphical Abstract



Highlights

- environmentally friendly biogrout can be produced from hydroxyapatite of the bones;

- environmentally friendly biogrout can be produced from limestone and carbon dioxide;

- new biogrouts can be used for the sealing of the fractured rocks and cracked concrete.

Figure 1
Click here to download high resolution image

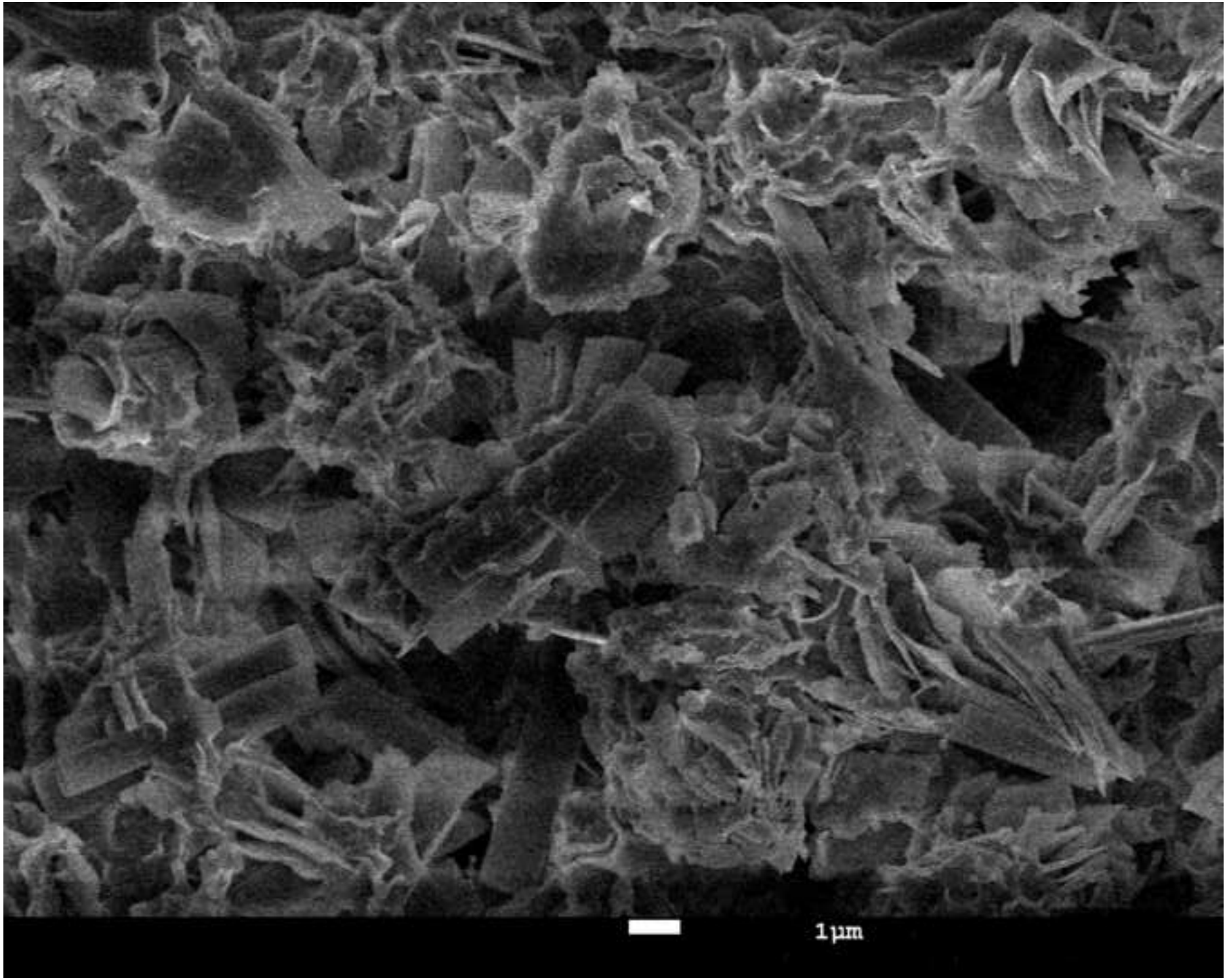


Figure 2

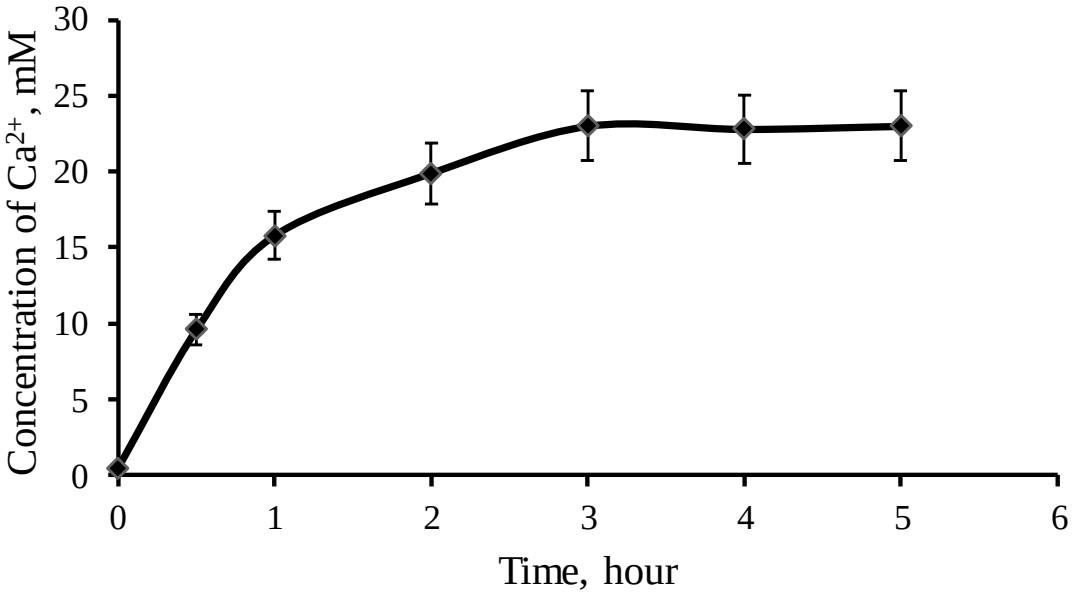
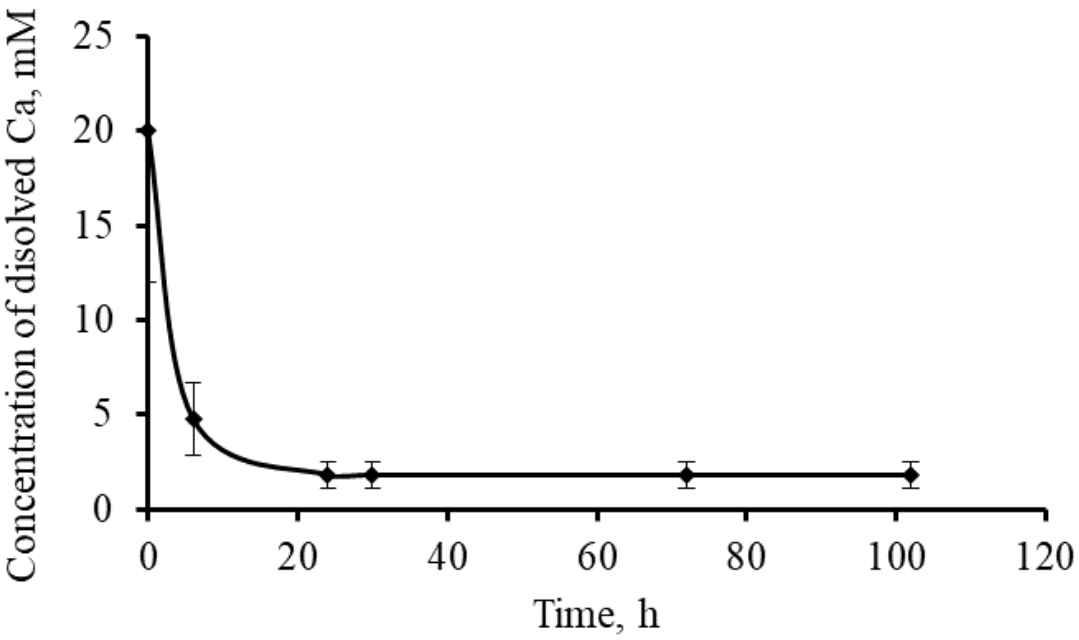


Figure 3


Concentration of disolved Ca, mM
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Time, h

Figure 4
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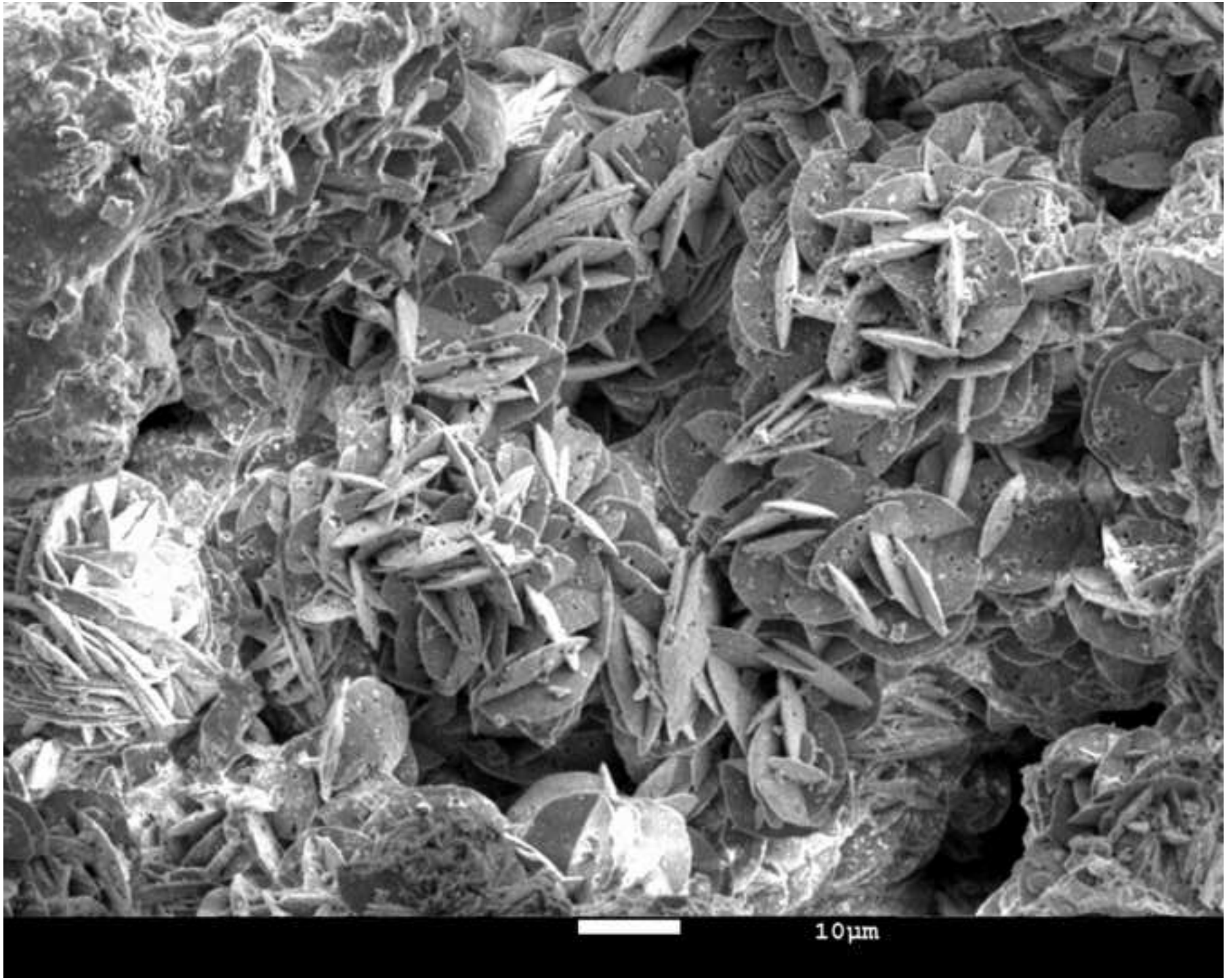

Figure 5

(a)

(b)

(c)

Figure 6

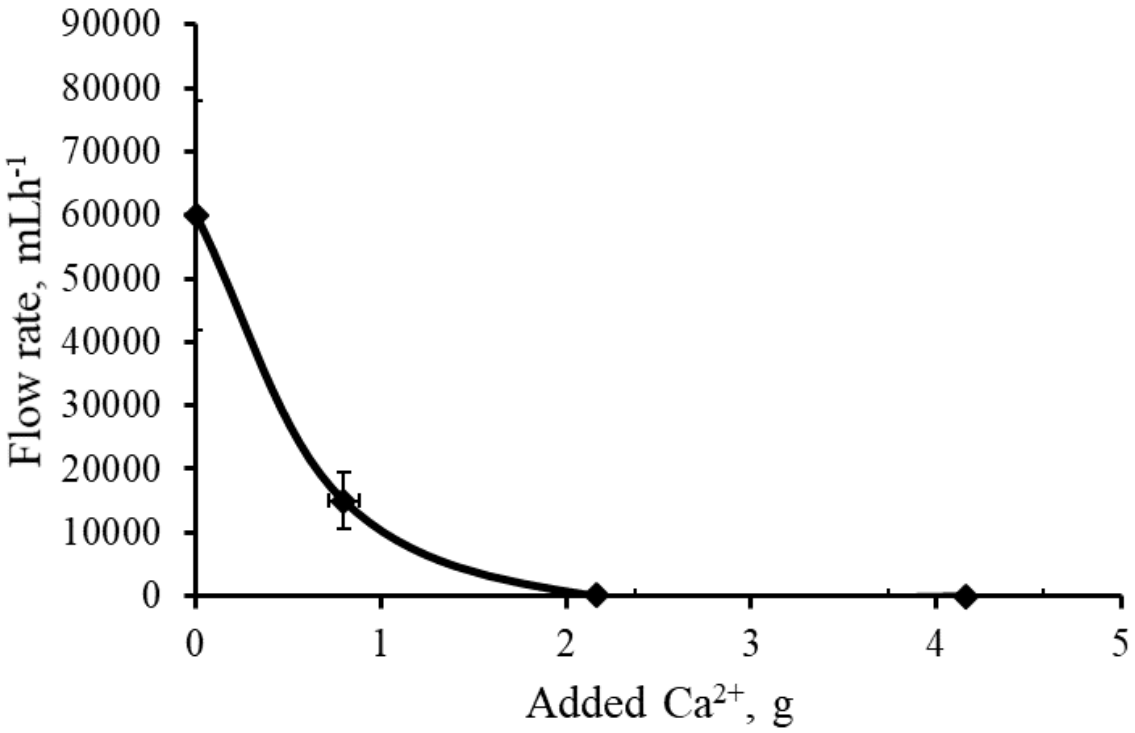
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Table 1

Table 1. Precipitation of hydroxyapatite due to transformation of calcium phosphate compounds shown in Equations 6 - 12 (modified from Tung, 1998; Pan and Darvell, 2010; Akiyama and Kawasaki, 2012; Kuroda and Okido, 2012)

Compound	**Ca/P**	**Urea/Ca**	**Solubility at 25°C, g L^{-1}**	**Reaction of precipitation**
Monocalcium phosphate	0.5	2.10	20	$5\ Ca(H_2PO_4)_2 + 21\ OH^- \rightarrow Ca_5(PO_4)_3OH\downarrow + 7\ PO_4^{3-} + 21\ H_2O$ (6)
Dicalcium phosphate	1.0	0.60	0.2	$5\ CaHPO_4 + 6\ OH^- \rightarrow Ca_5(PO_4)_3OH\downarrow + 6\ PO_4^{3-} + 6\ H_2O$ (7)
Octacalcium phosphate	1.33	0.22	0.28 - 0.56	$5\ Ca_8(PO_4)_6H_2 + 18\ OH^- \rightarrow 8\ Ca_5(PO_4)_3OH\downarrow + 6\ PO_4^{3-} + 18\ H_2O$ (8)
Tricalcium phosphate	1.5	0.10	0.64 g CaL^{-1} at pH 4.5 0.008 g CaL^{-1} at pH 7.0	$5\ Ca_3(PO_4)_2 + 3\ OH^- \rightarrow 3\ Ca_5(PO_4)_3OH\downarrow + PO_4^{3-} + 3\ H_2O$ (9)
Hydroxyapatite	1.67	0.40	0.25 g CaL^{-1} at pH 4.5	Dissolution of hydroxyapatite at low pH due to formation of slightly soluble dicalcium phosphate: $Ca_{10}(PO_4)_6(OH)_2 + 8\ H^+ \rightarrow 4\ Ca^{2+} + 6$

Compound	Ca/P	Urea/Ca	Solubility at 25°C, g L^{-1}	Reaction of precipitation
				$CaHPO_4 + 2\ H_2O$ (10) Crystallization of hydroxyapatite *in situ* due to increase of the pH by urease:
			0.001 g CaL^{-1} at pH 7.0	$4\ Ca^{2+} + 6\ CaHPO_4 + 8\ OH^- \rightarrow Ca_{10}(PO_4)_6(OH)_2\downarrow + 6\ H_2O$ (11)
Tetracalcium phosphate	2.0	0.17	0.02	$3\ Ca_4(PO_4)_2O + 3\ H_2O + 4\ OH^- \rightarrow 2\ Ca_5(PO_4)_3OH\downarrow + 2\ Ca(OH)_2$ (12)

Abstract

An important direction of construction biotechnology is production and application of the biogrouts to decrease the hydraulic conductivity of soil or fractured rocks. The most popular biogrout is a mixture of calcium chloride, urea, and either ureolytic bacteria or enzyme urease. A disadvantage of this biogrout is the release of a large quantity of toxic ammonia to air, as well as harmful ammonium and hydroxide ions to water. The aim of this study was evaluation of two new biogrouts that release less of harmful by-products to environment. First biogrout was a mixture of acidified hydroxyapatite solution from the bone powder with an addition of two times less urea that is used in conventional biogrout. Final pH was 7. Second biogrout was a solution of calcium bicarbonate produced from chalk powder and carbon dioxide with an addition of urea and acidotolerant ureolytic bacteria. This biogrout released to environment four times less ammonium and ammonia than conventional biogrout. Final pH was 8. Precipitation of about 2 g of calcium carbonate per kg of the cracked stone diminished the water seepage through the specimen by three orders of magnitude. Geotechnical or environmental engineering applications of these environmentally friendly biogrouts are limited by the consumption of a large volume of the grouting solution. It is due to low concentration of dissolved calcium, about 200 - 400 g m^{-3}, in these liquid biogrouts. So, these biogrouts could be most effective for the sealing and strengthening of the materials with low porosity such as the fractured rocks or cracked concrete.