COMPARISON OF DIFFERENT METHODS FOR EVALUATING THE ANTIMICROBIAL ACTIVITY OF GEOPOLYMER COMPOSITES CONTAINING METAL MICROPARTICLES

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Abstract

This paper investigates the antimicrobial effect of geopolymer composites with metal microparticles (metal powder). The geopolymer matrix was modified with silver, copper, or nickel microparticles in the concentration of 1, 2, 3, or 4 %. The indirect observation included the leaching test, the Kirby-Bauer method, and the cultivating bacteria in a bioreactor with a geopolymer. The direct observation and analysis of bacterial growth were based on the print bacteria from the geopolymer surface on an agar plate or fluorescence assessment of bacterial viability with microscopy (so-called LIVE/DEAD™ BacLight™ bacterial viability Kit). Two types of bacteria cells were tested, *Escherichia coli* (gram-negative) and *Micrococcus luteus* (gram-positive). Our study aims to verify and compare different geopolymer composites' antimicrobial activity methodologies. Moreover, we uncovered the influence of the metal microparticle concentration (1-4%) as an additive in the geopolymer matrix.

Keywords: Geopolymer, antimicrobial activity, metal microparticle, silver, copper, nickel

1. INTRODUCTION

Geopolymers (GP) are inorganic polymers formed by polycondensation of various precursor materials, including fly ash [1], metakaolin [2], and granulated blast furnace slag [3], in a strongly alkaline environment [4]. Their superior mechanical properties, further enhanced by various types of fibers and aggregates [5], resistance against high temperatures [6] and high chemical resistance [7] make them a potential alternative to Ordinary portland cement (OPC) as a binder for concrete production. However, similarly to OPC-based materials [8], GPs are also susceptible to microbially induced degradation (MIB) [9]. While having strong antimicrobial properties in a dry state, in a humid environment, their surface may be colonized by alkali-resistant bacteria, such as sulphur-oxidizing bacteria, which then produce acidic compounds (such as hydrogen sulphide), which cause deterioration and lower surface pH [10], allowing other microorganisms (other bacteria, fungi [11], lichen [12], algae [13], etc.) to colonize the surface and further degrade the material. Various methods for antimicrobial protection of GP and OPC-based concrete surfaces were investigated; these include antimicrobial additives, such as metal nanoparticles [14], and surface coatings, such as epoxy resins [15]. Various types of nanoparticles, including silver, copper or copper oxide are investigated as antimicrobial additive [16-17]. However, nanoparticles may be highly toxic for the environment, with the risk of biomagnification in the food chain [18-20]. For these reasons, it is viable to investigate alternative antimicrobial additives. In a previous study, 4 wt.% of silver, copper, and nickel microparticles (cheaper and less toxic alternative to nanoparticles) were investigated by the method of leaching test on agar medium (disk diffusion test) with gram-negative *Escherichia coli* and gram-positive *Micrococcus luteus* bacteria. Geopolymers with silver and copper microparticle additives have reached significant antibacterial effects against *E.coli*, even comparable to antibiotic control samples [21].

In this study, additional methodologies of measuring the antimicrobial effect of GP modified with metal microparticles are investigated, both to verify the antimicrobial effect of microparticles, compare the results of

different methods to results from the leaching test, and to examine the applicability of additional methodologies for antimicrobial GP research. The effect of microparticle content is also measured, with 1-4 wt.% of microparticles (measured in weight proportion to GP base).

2. MATERIALS AND METHODS

2.1 Geopolymer samples

Geopolymer (GP) samples were prepared following an identical procedure as in the previous study [21]. The GP samples were prepared from locally sourced Baucis L_k metakaolin GP base and potassium-based activator, both manufactured by České Lupkové závody, or ČLUZ, a.s, a Czech company specialized in metakaolin production [22].

In addition, three types of microparticles were used to prepare GP samples, namely silver, copper, and nickel. The microparticle size was: silver (made by PkChemie) and copper (made by Fischema) were both sized below 45 µm in diameter and nickel microparticles (made by Selkat ireneusz Katarzynski) in the range of 3-7 µm. Each sample set contains one microparticle type, except for the control sample. The microparticles were used with 1, 2, 3, and 4 %, measured in weight proportion to the GP base (metakaolin), with 90% alkaline activator. After hardening, the samples were cut to roughly 3×3×1 cm. **Figure 1** shows the GP sample surface with 4 wt.% of microparticles in the structure, which are visible on the cut surface of the GP**.**

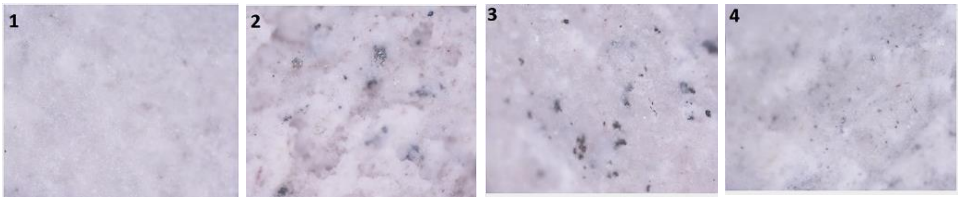

Figure 1 Images of geopolymer surface with 4 wt.% of microparticles, with 20x magnification, 1 = Pure GP, 2 = GP + Ag-microparticles, 3 = GP + Cu-microparticles, 4 = GP + Ni-microparticles [21].

2.2 Antimicrobial activity assessment tests

Gram-negative *Escherichia coli* and gram-positive *Micrococcus luteus* bacteria cells were bought from the Czech microorganism's collection and were incubated for 24 hours at 37 °C before assays. Each bacterial inoculum was prepared with a concentration of about 10^5 CFU/mL (colony-forming units per millilitre). Antibiotics (Gentamicin 10 µg) were used as a positive control (with expected inhibition) in the form of antibiotic-infused paper discs; pure bacteria cells in saline solution were used as a negative control sample (with no inhibition). The GP surface was sterilized with UV light for 30 minutes for all tests. In some experiments, the procedure of pH stabilization of GP surface was used (GP pieces 3×3×1 cm were immersed in tap water and slowly shaken for about two months, whereas each second day was water replaced to reach a pH of about 8); originally pH is 11-12. Fundamental statistical analysis was calculated.

2.2.1 The leaching test

The antimicrobial activity of GP with added microparticles was tested as leach in saline solution (0.9% NaCl). The leachate was prepared five days in advance. The leachate properties and procedures detailed description are specified in our previous study [21].

2.2.2 The Kirby-Bauer test

Bacterial inoculum (see above) was individually applied onto an agar (Mueller-Hinton) surface in the Petri dish and spread using a sterile glass spreader. The bacteria cells were incubated on an agar for 15 minutes at

25 °C. The tested samples were applied in the middle of the Petri dishes (either GP itself or 20 µL of 4 wt.% microparticles in sterile tap water) and incubated for 24-48 hours (according to microorganism type) at 37 °C; each measurement was repeated three times. The antimicrobial activity is proven when a visible inhibition zone around the tested sample exists; the area without cells (colony-forming units) may be easily measured. Image analysis software ImageJ (the National Institute of Health) was used to measure the diameters of the inhibition zones around the tested sample.

2.2.3 The imprint method of bacteria from the geopolymer surface onto an agar plate

Each type of bacteria inoculum was separately applied on a sterilized GP surface with a sterile swab. The cultivation of inoculum was ongoing directly on the GP surface at 25 °C for 10 minutes, then the GP surface was imprinted (after a gentle rinse with physiological saline solution) on Mueller-Hinton agar in a Petri dish and left there for about 5 minutes, then GP sample was removed. All Petri dishes were incubated for 48 hours at 37 °C, and finally, the parameter of colony-forming unit was assessed.

2.2.4 The cultivation of geopolymer in a bioreactor

Both bacteria type together were applied in a bioreactor with Nutrient Broth and geopolymer samples. For every kind of micro-particle (pure or incorporated into GP) and for each concentration, a separate reactor was used and the reactors were shaken to ensure oxygen supply. After 24 hours 40 µL of suspension was placed in Petri dish and poured with Mueller-Hinton agar. All Petri dishes were incubated for 48 hours at 37 °C, and finally, the colony-forming units were assessed.

2.2.5 The fluorescence assessment of bacterial viability

The bacterial viability was observed with Olympus Life Science IX73 microscope and fluorescence staining (LIVE/DEAD™ BacLight™). Both bacteria type was applied together directly on the GP surface; contact with the surface was about 15 minutes. The excess suspension was removed before staining to evaluate adherent cells only. The photographs of the cell surface were taken (more than 10 fields for each sample), where cells with a disrupted membrane are considered dead or dying and turn red; cells with an intact membrane (alive) turn green. Image analysis in Matlab (The Mathworks, Inc.) was applied to evaluate the proportion of living/dead cells in the image.

3 RESULTS AND DISCUSSION

The graphs below show the comparison with the positive control (100 % inhibition corresponds to Gentamicin 10 µg), and negative control (equal to 0 % inhibition). The following abbreviations are used in the charts below: EC = *Escherichia coli*, ML = *Micrococcus luteus*; Control = Negative control sample; Gentamicin = Positive control sample (Gentamicin 10 µg).

3.1 The leaching test

Additional leaching test has confirmed results from the previous study [21]. While the inhibition effect of leachates from pure microparticles and geopolymers with 4% microparticle additive against *E.coli* is significant and comparable to Gentamicin etalon, except for nickel microparticles, their effect against *M.luteus* is much weaker. In contrast, the effect of nickel microparticles is negligible.

3.2 The Kirby-Bauer test

While the Kirby-Bauer (contact) test also shows the antimicrobial effectiveness of microparticles and geopolymers with microparticles against *E.coli* and *M.Luteus*, although lower than leaching test, it has also shown high antimicrobial effectiveness of pure geopolymer (with pH at 8 and 11). This effect may be attributed to the release of Na+ or K+ ions from the geopolymer. However, the diminishing of the antimicrobial activity of

geopolymer after pH stabilization (repeated and long-term washing with pure water), indicates the microparticle efficiency deteriorates with long-term use, presumably due to their release from geopolymer (which may have also contributed to high antimicrobial activity of leachate). The higher antimicrobial activity of geopolymers with nickel microparticles in comparison to the leaching test also indicates their effect is more "localized," and they do not significantly release ions or dissolve.

3.3 The imprint method of bacteria from the geopolymer surface onto an agar plate

Similar results were obtained by the Imprint method. At the same time, the tests show an increase of antimicrobial activity with increasing content of microparticles at pH=11, with copper microparticles retaining their high antimicrobial activity with low content. However, the antimicrobial activity of pure geopolymer is also seemingly high. This method has also measured the antimicrobial activity of nickel microparticles as comparable to copper and silver.

3.4 The cultivation of geopolymer in a bioreactor

Similar results were also shown during the cultivation of geopolymer in bioreactor tests. While increasing the content of microparticles leads to significant inhibition of bacteria, including the nickel microparticles (even after the pH is stabilized at 8), the antimicrobial activity of pure geopolymer is also seemingly high.

3.5 The fluorescence assessment of bacterial viability

The observation of viability of bacteria after direct application on geopolymer surface has likewise shown significant antimicrobial activity of pure geopolymer, especially at 11 pH, although the effect was lower at 8 pH. Samples with higher microparticle content were measured to have stronger antimicrobial activity than samples with low microparticle content. Although 4% content of nickel microparticles was measured to have a lower antimicrobial effect than others, which further indicates the effect of different surface properties, which might change due to increase in microparticle content, on the results. This effect is likely more profound for nickel microparticle geopolymers due to the smaller size of nickel microparticles. The strongest effect was reached by 4% copper microparticles content, which achieved the same rate of inhibition as Gentamicin etalon. The results of this test are shown in **Figure 2**.

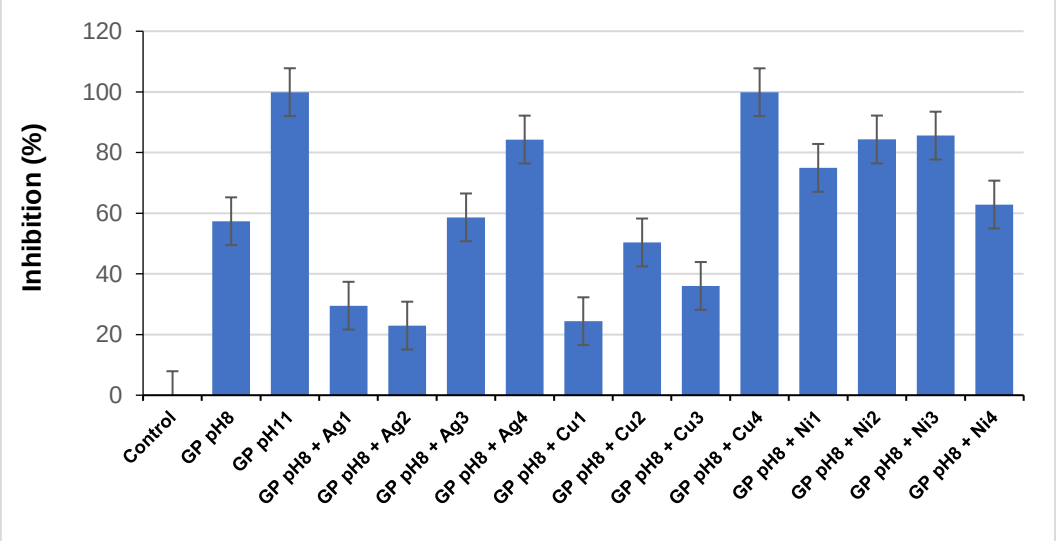

Figure 2 Inhibition effect based on the fluorescence assessment of bacterial cell viability (live/dead). Both microorganisms were applied at one time. The 1-4 % concentration of metal particles was evaluated (only incorporated in the geopolymer). Original pH (11) and stabilized pH (8) were considered only for pure GP; otherwise, only stabilized pH (8) was assessed.

4 CONCLUSION

This paper investigated multiple methods of measuring the antimicrobial activity of geopolymer samples, including samples with microparticles, against *E.Coli* and *M.Luteus bacteria.* These methods include the leaching test, Kirby-Bauer test, imprint method, cultivation in the bioreactor, and measurements of bacterial viability after contact with the geopolymer surface. While all these tests are generally applicable for measuring geopolymer antimicrobial activity, most of them also measured the antimicrobial activity of pure geopolymer as very high, sometimes even higher, than the antimicrobial activity of geopolymers with microparticles of antimicrobial metals, even with pH stabilized at 8 (usually, geopolymer increases the pH of solutions to about 11). There are multiple possible explanations for this. It is possible that microparticles stabilize the geopolymer matrix (as various types of particles are used to reinforce it in geopolymer concrete, including sand or other aggregates or silica fumes), which would lead to pure geopolymers increase the pH faster even after pH stabilization and release more ions from their structure. Alternatively, pure geopolymers have different surface properties, such as porosity or shape, which may have also influenced the test results. However, even if pure geopolymer exhibits high antimicrobial activity, it is still unsuitable for most applications (such as mortar or coating), due to its worse properties and lower durability when compared to geopolymer with aggregates, additives, etc. These problems also show the drawbacks of using types of bacteria without significant resistance to alkaline environments, such as *E.coli* or *M.luteus,* for testing the antimicrobial activity of geopolymers, as changes of pH might destroy them very effectively.

Furthermore, nickel microparticles were shown to have significant antimicrobial activity in other tests than the leaching test, indicating that their antimicrobial activity is more “localized” than the antimicrobial activity of silver and copper microparticles, possibly to lover ion release or dissolving, making them a potentially viable antimicrobial additive to geopolymers, as this type of antimicrobial activity may prevent bacterial colonization of geopolymer surface. Higher microparticle content also increased the antimicrobial activity. From the tested methods, the measurement of bacterial viability after direct contact with the geopolymer surface most closely approximates the expected conditions of the mechanism of geopolymer surface colonization by bacteria.

Further studies should investigate the effect of common geopolymer additives (especially sand and silica fumes) on the antimicrobial activity of geopolymer with and without microparticle additives, as well as the changes in surface properties, which may be caused by these additives and influence the antimicrobial activity. Furthermore, alkali-resistant types of bacteria (especially sulphur-oxidizing bacteria) should be used to perform the tests of antimicrobial activity to prevent changes in pH from influencing the results and to better emulate the conditions for microbial degradation of geopolymer, which is caused by these species of bacteria.

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