Evaluation of the antimicrobial efficiency of slag based composites

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Abstract. Degradation of cement mortar composites occurs due to a variety of causes such as corrosion (sulphate or chloride attack) cracking or shrinkage. Chemical agents often play a major role in corrosion processes, however recent research has revealed the importance of biological agents. Microbiological corrosion is caused by the presence and activities of microorganisms, including algae, bacteria, yeasts and fungi. These microorganisms produce substances like biogenic organic and mineral acids that can disrupt the matrix of mortar composites. The most effective method for improving the resistance of cement based composites is the addition of pozzolans like blast furnace slag. Slag is often used in preparation of cement composites to improve the acid resistance, impermeability and crack resistance of mortars. This article presents the investigation of antimicrobial efficiency of blast furnace slag regarding the selected species of bacteria (*Pseudomonas aeruginosa, Micrococcus luteus*), yeasts (*Candida albicans, Saccharomyces cerevisiae*) and algae (*Chlorella vulgaris*). Antimicrobial properties were studied on mortars with different share of slag (65% - 95%). Antimicrobial efficiency for the tested species of microorganisms was found moderate to low.

1. Introduction

Building materials are progressively and inevitably subjected to biological colonization [1,2]. For some years now, it has been well known that microbiological contamination and growth on building materials may lead to the severe issues, including the structural degradation of materials (biodeterioration) [3,4]. More especially, the sewers become more and more bioreceptive due to their exposition to waste water [2,5].

Prolonged exposure of any solid surface to water results in a series of physical, chemical, and biological events at the surface that form a community of microorganisms referred to as biofilm. The process starts with initial settlement of free floating microorganisms on the surfaces followed by the accumulation of an organic material layer, generally the extra-cellular polymeric substances (EPS) excreted by the attached microorganisms [6].

Several biological organisms can be involved in the biocolonization of sewer network, such as bacteria, cyanobacteria, algae, fungi and yeast. Among these microorganisms, cyanobacteria and algae are predominant in the biofilm formed on the substrates, and they are the first colonizers after bacteria [2,7]. The intensity and kinetics of biological colonization of building surfaces depends on the environment and substrate [2,5,8,9]. Physical (roughness, porosity) and chemical (mineralogical/chemical composition, surface pH) characteristics of the substrate influence the biological colonization [2,5,10].
In attempts to prevent or limit microbial development on building materials, various solutions have been studied like using of biocide or using pozzolanic materials like blast furnace slag, fly ash or silica fume, to improve the quality of these materials [4,11].

Recently, there has been a growing trend for the use of supplementary cementitious materials, whether natural, waste, or by-products, in the production of composite cements because of ecological, economical, and diversified product quality reasons. Blast furnace slag, a by-product of the transformation of iron ore into pig-iron in a blast furnace, is one of these materials whose use in cement manufacture goes back to as far back as 1880 [12]. Since then its use has expanded because it has various advantages over other cemenitious materials. Firstly, blast furnace slag has a relatively constant chemical composition compared to fly ash, silica fume etc. Besides, it has advantages like low heat of hydration, high sulfate and acid resistance, better workability, higher ultimate strength, etc. [1,13].

It was shown that cement mortars with the addition of blast furnace slag exhibits a greater resistance than ordinary Portland cement to inorganic or organic acids produced by microorganisms [2,14]. These properties are beneficial for specialised objects associated with water and waste water management such as hydroelectric dams, sewer networks, large bridges and harbours [13].

The aim of the present study is to investigate the potential antimicrobial efficiency of slag based cement mortars regarding the selected species of bacteria (Pseudomonas aeruginosa and Micrococcus luteus), yeasts (Candida albicans and Saccharomyces cerevisiae) and algae (Chlorella vulgaris).

2. Material and methods

2.1. Mortar composites
Five different cement composite mixtures were investigated in the bacterial experiment. The R represented the reference sample with 100 wt.% of Portland cement CEM I 52.5 R, with the content of main components as follows: 57.15 wt.% of CaO, 18.11 wt.% of SiO₂, 4.02 wt.% of Al₂O₃, 2.69 wt.% of Fe₂O₃, 1.49 wt.% of SO₃, 1.37 wt.% of MgO, 1.12 wt.% of K₂O, 0.33 wt.% of P₂O₅, 0.18 wt.% of TiO₂, 0.06 wt.% of Cl.

The samples with different share of blast furnace slag were BFS1 (65 wt.% of slag), BFS2 (75 wt.% of slag), BFS3 (85 wt.% of slag) and BFS4 (95 wt.% of slag), respectively. The content of main components of incorporated blast furnace slag was as follows: 39.55 wt.% of CaO, 38.95 wt.% of SiO₂, 10.11 wt.% of MgO, 8.33 wt.% of Al₂O₃, 0.74 wt.% of MgO, 0.57 wt.% of SO₃, 0.54 wt.% of Fe₂O₃, 0.48 wt.% of K₂O, 0.37 wt.% TiO₂, 0.04 wt.% of P₂O₅, 0.02 wt.% of Cl.

2.2. Microorganisms
Microscopic bacteria Pseudomonas aeruginosa and Micrococcus luteus, yeasts Candida albicans and Saccharomyces cerevisiae and algae Chlorella vulgaris were obtained as new isolates from the environment of metal-contaminated sludge ponds after ore extraction [15,16,17].

2.3. Methods
The composite samples were crushed and powdered and then sterilized in sterilizer Stericell (BMT Medical technology, Czech Republic) at 180°C during 1 hour (figure 1). Soya Casein Digest (HiMedia Laboratories, Mumbai, India) represented the growth medium for bacteria. Algae Culture Agar (HiMedia Laboratories, Mumbai, India) was used as a growth medium for algae and for growth medium for yeast the Sabouraud Dextrose Broth (HiMedia Laboratories, Mumbai, India) was used. All of the growth mediums were used for inoculation and cultivation of each microorganism and the composition of each medium is shown in table 1.

Table 1. The composition of used growth mediums.

| Medium                        | Composition (in 1 L H₂O)                       | Final pH of medium |
|-------------------------------|------------------------------------------------|-------------------|
| Soyabean Casein Digest Medium | 17g tryptone, 3g soya peptone, 5g NaCl, 2.5g dextrose (glucose) and 2.5g K₂HPO₄ | 7.3 ± 0.2         |
Sabouraud Dextrose Broth: 20g dextrose (glucose) and 10g special peptone. 
Algae Culture Agar: 1g NaNO₃, 0.25g K₂HPO₄, 0.513g MgSO₄, 0.05g NH₄Cl, 0.058g CaCl₂, 0.003g FeCl₃ and 15g agar.

Sterilization of growth medium was realized in laboratory autoclave at 121°C for 15 minutes. In total, five Erlenmeyer flasks with growth medium were prepared, each for every powdered composite sample. Immediately after the sterilization of growth medium the powdered composite samples were added and thoroughly mixed to the broths and this mixture was then placed in the Petri dishes (with an average Ø 90.0 mm). The antimicrobial activity was determined by dilution methods in agar growth media so that the resulting concentration of tested composite sample in growth media was 10%, by mass.

On the surface of solidified growth media bacteria, yeasts and algae were evenly dispersed (figure 2). Each microorganism was placed on separate Petri dish with growth medium. The duration of experiments was one week for all studied microorganisms.

| Microorganism          | Sample   | R    | BFS1  | BFS2  | BFS3  | BFS4  |
|------------------------|----------|------|-------|-------|-------|-------|
| Sterilization of mortar samples in Stericell. | Figure 1 |   |       |       |       |       |
| Inoculation of tested microorganisms on growth medium with composite samples. | Figure 2 |   |       |       |       |       |

The scale of evaluation of the microorganism growth on building materials is according to [18] by where the degree of the growth is expressed as follows:

- **No growth** - No growth of microorganisms.
- **Poor growth** - The growth of microorganisms is negligible (the colonies of microorganisms are dispersed).
- **Fair growth** - The growth of microorganisms is gradual (numerous small colonies of microorganisms, which cover 25% of the sample surface).
- **Good growth** - The growth of microorganisms is intensive (colonies of microorganisms cover up to 50% of the sample surface).
- **Excellent growth** - The growth of microorganisms is very intensive (colonies of microorganisms cover up to 75% of the sample surface).
- **Full growth** - The sample surface is fully covered by fungi (colonies of microorganisms cover 100% of the sample surface).

3. **Results and discussion**
The antimicrobial efficiency of composites samples with blast furnace slag at inhibition of growth of selected microorganisms are given in table 2. The highest antimicrobial efficiency regarding the degree of growth of all tested microorganisms was observed for the reference sample R with no addition of slag and similar results was detected for sample with 65% of blast furnace slag.

| Table 2. The antimicrobial efficiency of composite samples. | Sample |
|----------------------------------------------------------|--------|
| Microorganism                                            | R      | BFS1 | BFS2 | BFS3 | BFS4 |

3
|                              | No growth | No growth | Full growth | Fair growth | Full growth |
|------------------------------|-----------|-----------|-------------|-------------|-------------|
| **Pseudomonas aeruginosa**   | No growth | No growth | Full growth | Fair growth | Full growth |
| **Micrococcus luteus**       | No growth | No growth | Good growth | Good growth | Good growth |
| **Candida albicans**         | No growth | Poor growth | Full growth | Fair growth | Fair growth |
| **Saccharomyces cerevisiae** | No growth | No growth | Good growth | Poor growth | Fair growth |
| **Chlorella vulgaris**       | No growth | No growth | Excellent growth | Poor growth | Fair growth |

Figure 3 shows the effect of *Pseudomonas aeruginosa* on growth media with addition of cement composite with BFS. Samples R, BFS1 and BFS3 were found to have no to minimal amount of dispersed colonies of *P. aeruginosa* and no antimicrobial activity was found for samples BFS2 with 75 wt.% of slag and sample BFS4 with 95 wt.% of slag.

**Figure 3.** Antimicrobial efficiency of composite samples regarding to strain *Pseudomonas aeruginosa*.

Antimicrobial efficiency of mortar samples regarding the strain *Micrococcus luteus* is shown on figure 4. The highest antimicrobial efficiency was found for the samples with ordinary Portland cement R and sample BFS1 with 65 wt.% of slag. For the other 3 samples good growth of microorganisms was detected.

**Figure 4.** Antimicrobial efficiency of composite samples regarding to strain *Micrococcus luteus*.

The results of determining the antimicrobial properties of mortars against strain of yeast *Candida albicans* are given in figure 5. The most resistant sample was the reference sample R. The growth of colonies of *C. albicans* was detected on all of the samples with blast furnace slag, with the most effective antimicrobial properties for the sample BFS1 with 65 wt.% of slag.
Figure 5. Antimicrobial efficiency of composite samples regarding to strain *Candida albicans*.

Best results regarding to the inhibition of growth of microorganism was evaluated for the strain of yeast *Saccharomyces cerevisiae* (figure 6). The growth was determined to be ranging from no growth to fair growth of colonies of yeasts, except for the sample BFS2 with 75 wt.% of slag, where the inhibition was only around 50%.

Figure 6. Antimicrobial efficiency of composite samples regarding to strain *Saccharomyces cerevisiae*.

Figure 7 shows the effects of algae *Chlorella vulgaris* on composites samples, the highest antimicrobial properties was obtained for reference sample (R) and sample with 65 wt.% of slag (BFS1), respectively.

Figure 7. Antimicrobial efficiency of composite samples regarding to strain *Chlorella vulgaris*.

Overall the best results for determining the antimicrobial efficiency had the reference sample R with ordinary Portland cement. For the composites with blast furnace slag, the most resistant was found to be the sample BFS1 with 65 wt.% of slag. Results obtained for the sample BFS2 with 75 wt.% of blast furnace slag showed little inhibition efficiency. This outcome is more likely linked with possible biological fouling of sample during milling process and from this point of view additional experiments regarding this sample are needed.

4. Conclusion
The aim of the paper was the evaluation of possible antimicrobial efficiency of cement composites with different share of blast furnace slag. The inhibition properties were studied on five different strains of microorganisms (*Pseudomonas aeruginosa, Micrococcus luteus, Candida albicans, Saccharomyces*...
According to the results, the addition of blast furnace slag is suitable for achieving microorganism-free environment mostly regarding yeast Saccharomyces cerevisiae and also Candida albicans. The highest inhibition efficiency was found for the reference sample with ordinary Portland cement. For the samples made with slag, the sample with 65 wt.% was found to be most effective for all studied strains of microorganisms. Since in the experiment a low concentration of studied composites in the growth media was used, the mechanism of the antimicrobial effects of blast furnace slag should be the subject of further study.

5. References
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