Chapter

Antimicrobial Efficiency of Metallurgical Slags Suitable for Construction Applications

Július Strigáč, Nadežda Števulová, Jozef Mikušinec, Ľudovít Varečka and Daniela Hudecová

Abstract

The chapter deals with studying antimicrobial efficiency of granulated blast-furnace slag with fineness of 340 (1Sa) and 520 m^2/kg (1Sb), air-cooled blast-furnace slag (2S), demetallized steel slag (3S), calcareous ladle slag (4S) and copper slag (5S), respectively. The efficiency has been tested on G^+ bacteria—*Staphylococcus aureus*, *Bacillus subtilis*, *Micrococcus luteus*; G^- bacteria—*Pseudomonas aeruginosa*, *Escherichia coli*, *Serratia marcescens*; yeasts—*Rhodotorula glutinis*, *Candida albicans*; filamentous fungi—*Penicillium funiculosum*, *Aspergillus niger*, *Alternaria alternata*, *Chaetomium globosum*, *Cladosporium herbarum*, *Trichoderma viride*. The efficiency has been determined by dilution methods in agar media for that reason the resulting concentration of slags has been 10, 20, 40 and 60%, respectively. The antibacterial efficiency decreased as follows: S4 > S3 > S2 > S1a = S1b > S5, whereas anti-yeast efficiency decreased as follows: S4 > S1a = S1b = S3 > S2 > S5. Filamentous fungi were selectively sensitive to slags, that way there is approximate order of efficiency S4 > S3 = S1a = S1b > S5 > S2. Application of metallurgical slags into construction materials provides them increasing biodegradation resistance.

Keywords: metallurgical slags, antimicrobial efficiency, bacteria, yeasts, filamentous fungi

1. Introduction

Biocorrosion of construction materials is a significant problem wherever the conditions suitable for microorganisms occur. Biocorrosion is caused by varied biogenic acids, as well as by H_2S and NH_3, which result from metabolic activities of microorganisms [1–4]. These corrosive metabolites react with calcareous components of construction materials, finally leading to the biodeterioration.

The effects of different compositions of concrete and added nutrients on fungal colonization have been studied in [5]. Fungal strains belonging to *Cladosporium*, *Alternaria*, *Epicoccum*, *Mucor*, *Fusarium*, *Penicillium*, *Trichoderma*, and *Pestalotiopsis* were isolated from fouled concrete structures and used to inoculate mortar tiles varying in cement composition, water-to-cement ratio, supplementary cementitious material additions, and surface roughness. The strong positive relationship has been observed between the tile water-to-cement ratio and the amount of biofouling.

Blast-furnace slag has been added in different amounts of 10, 25, and 50%, and the results showed, that it possessed only a small antifungal effect [5].

To the contrary, it has been determined [6] that granulated blast-furnace slag (GBFS), as well as blast-furnace cements with GBFS ≥65 wt.% have fungistatic properties. Therefore, the utilization of fungistatic GBFS, blast-furnace cements CEM III/A 32,5 N (with GBFS content of 65 wt.%), CEM III/B 32,5 N, and CEM III/C 32,5 N provides long-term fungistatic protection [6].

Effect of pig iron slag (IrS) particles on soil physicochemical, biological and enzyme activities, was studied in [7]. Contamination of IrS particles altered these soil properties. While the pH value of soil increased slightly, the electrical conductivity, phosphorus, potassium and carbon contents increased substantially in polluted soil. Soil contamination with pig IrS caused substantial decrease in microbial population as well as cease of enzyme activities such as dehydrogenase and protease. Soil is a dynamic system in which continuous interaction takes place between soil minerals, organic matter and organisms. Each of these three major soil components influences the physicochemical and biological properties of terrestrial system. Since soil enzyme activities are very sensitive to pollution, enzymes have been suggested as potential indicators or monitoring tools to assess soil quality and bioremediation activities. The aim in the study [7] was to determine the effect of pig IrS on soil physicochemical, biological and enzymatic activities of dehydrogenase, protease, cellulase and amylase. Soil microbial populations such as including bacterial and fungal populations were enumerated by serial dilution technique. The observations revealed that contamination with IrS particles led to increase in pH of the test soil. This indicates the alkaline nature of the disposed waste contaminants. Reduced bacterial and fungal population in polluted soil may be due to the toxic effects of IrS particles on microbial population. This may be because of oxidative stress caused by iron sledge particles or their interference in osmotic balance [7]. Pollution of the soil environment with heavy metals also negatively influenced the soil microbial properties such as basal soil respiration rate and enzyme activities depending on the soil pH, organic matter content and other chemical properties. Enzymes are strongly connected with important soil characteristics such as organic matter, physical properties, microbial activity or biomass. They are the sensitive indicators of soil quality. The study clearly indicated that dumping of pig IrS on soil, altered its physicochemical and biological properties and inhibited enzymatic activities such as dehydrogenase and protease. Very significant inhibition of dehydrogenase and protease activities in polluted soil indicates the alterations in oxidation-reduction activities of enzymes released from microorganisms [7].

The use of steelmaking slag for sewage sludge stabilization was studied in [8]. The objective was the examination of the stabilization of sewage sludge by the addition of a steel slag as an alkaline material and the investigation of the effect of stabilization time on the properties of the produced mixtures. The alkaline material used was a by-product from steelmaking refining processes in ladle furnaces, the steelmaking slag that presented high calcium content. The mixtures of sewage sludge and slag in various ratios (2.5–20%) were prepared and stabilized for 48 days. The determination of pathogens removal rate, pH, moisture content and mineralogical phases present was carried out for monitoring of the mixtures properties. The slag addition resulted in increasing mixture pH (exceeding 12), in the increase of total solids content and in the decrease of volatile percentage, which was more pronounced at the highest alkaline slag dosage. In addition, effective pathogens removal was observed in the mixtures containing more than 10% slag, due to the high pH values of mixtures. The addition of slag at lower dosages was not as effective as the highest dosage. Sewage sludge has been utilized for agriculture and horticulture for several years and represents a good source of nutrients for

plant growth and soil conditioner to improve soil physical properties. One of the alternative methods for sludge hygienisation is chemical stabilization with lime at a pH value equal or above 12 for 3 months. Lime maintains high pH values of mixtures, removing microbial communities in sludge. An alternative alkaline agent for sludge stabilization, other than lime, is the residue produced during mechanical treatment of steel in steelmaking processes (steelmaking slag). Steelmaking slag is a by-product from steel refining processes in ladle furnaces and contains a high calcium amount, representing a medium with strong alkaline properties. The steelmaking slag used represents a high alkaline material with the following average composition, on a dry basis: CaO 53 wt.%; SiO_2 18 wt.%; MgO 4.5 wt.%; Al_2O_3 3 wt.%; MnO 2 wt.%; iron oxides 7.5 wt.% [8]. The alkaline agent addition in sewage sludge is supposed to contribute to sewage sludge stabilization through the pH value increase toward highly alkaline values (>11), which results in the destruction of pathogens. Furthermore, calcium cations may react with sulfur containing substances in the sludge, resulting simultaneously to odor reduction. The raw sewage sludge presented high moisture content, reaching up to 83% and pH values in the neutral range. The pH values of alkaline sewage sludge mixtures were higher than the pH values of raw sludge sample, throughout the whole period of 48 days. The highest pH values (exceeding 12) were measured for alkaline sewages with the highest content of alkaline medium, i.e., containing 10 and 20% slag. Lower pH values, from about 7.5 up to 11.0, were measured for the samples with the lower content of the stabilization alkaline material. In general, the higher the slag content, the higher the pathogens removal rate and the lower the required time for stabilization. The sample with slag addition of 20% presented negligible microbial content from the early stabilization days. The lower stabilization efficiencies were measured in the sludge mixtures containing slag contents lower than 5%; however, extended stabilization times resulted in the efficient removal of pathogens even for the samples with a low slag content [8]. In conclusion, the low-cost steelmaking slag, can be used as the efficient alkaline medium alternative to lime, for the sewage sludge stabilization. High slag additions, up to 20%, were able to offer a mixture with a high pH, and a low content of moisture, volatiles and pathogens. The produced mixtures could have several applications, i.e., soil amendment, daily cover in sanitary landfills, restoration of abandoned mines, etc. Comparison of slag to lime and limestone revealed that slag may be as efficient as the most conventional alkaline mediums for sludge stabilization; however, the benefits of the latter over lime, such as integrated use of a solid waste, availability and low cost, indicate that steelmaking slag could become an efficient medium for sludge stabilization [8].

The effect of metallurgical slag on microbiological activity in Pseudogley was studied in [9]. The aim was to investigate the effect of Ca-containing metallurgical slag from iron-producing factory, comparing to the other lime materials and fertilizers on microbiological activity in acid pseudogley type of soil. Metallurgical slag and certain melioration measures did not show significant effect on number of microorganisms in Pseudogley, although the activity of dehydrogenase was significantly high in combined treatments with slag. Based on the obtained results [9], the studied metallurgical slag can be utilized for increasing microbiological activity and fertility of acid soils. The main task of every agricultural production is increasing and maintaining soil fertility. For the majority of pseudogley soils, characterized by high soil acidity, it is necessary to apply Ca-containing materials—calcifiers. Agricultural liming materials increase soil pH and thereby affect the activity and composition of microbial populations. In acid soils, liming can create better environmental conditions for the development of acid-intolerant microorganisms resulting in increased microbial activity. Along with other lime materials (ground limestone, saturated lime, etc.), metallurgical slag can be of great

importance [9]. The presence of slag influenced on the decrease of microorganisms number in Pseudogley. Activity of dehydrogenase (DHA) is higher in neutral soils. Higher values of DHA from the studied soil comparing to its control indicate that application of melioration measures and the slag increased its activity. Treatments with slag showed significant activity of DHA in the soil (three times higher comparing to control). Higher activity of DHA indicates higher intensity of respiration, thus, higher intensity of mineralization of soil fresh organic matter and humus. Liming increases soil pH and has a positive and significant effect on microorganisms growth and DHA activity in soil, especially in spring. Thus, the changes in soil pH significantly affect the rate of soil C and N cycling and soil productivity. Based on the obtained results, the studied metallurgical slag of the standardized chemical properties can be utilized for increasing microbiological activity and fertility of acid soils [9].

The mortar composition contains cement, a latent hydraulic material, a pozzolan material and aggregate and further contains non-iron metal refining slag aggregate as a part of the whole of the aggregate to provide a mortar having both excellent acid resistance and anti-microbial action suppressing the activity of sulfur oxidizing bacteria and also excellent in adhesion and crack resistance [10]. The latent hydraulic material is GBFS. The pozzolan material contains one or more kinds of fly ash, pulp sludge incineration ash, silica fume, waste glass powder and sewerage sludge incineration ash. The non-iron refining slag aggregate is one or more kinds of zinc, copper and lead slag.

Metallurgical slags can be advantageously used as an agricultural liming material, as a favorable source of minor nutrients. Fertilizers are primarily valued for their ability to supply nutrients. Plants use these nutrients to make components for plant growth. The main chemicals must be supplied to plants that are called primary nutrients are nitrogen, phosphorus, and potassium [11]. A fertilizer containing all three nutrients is a balanced fertilizer. Plants also require the secondary nutrients, calcium, magnesium, and sulfur, plus very small amounts of the micronutrients boron, copper, chlorine, iron, manganese, molybdenum, and zinc. The results for the use of blast-furnace slag with (N, P and K) seem encouraging regarding the replacement of commercial fertilizers. The use of blast-furnace slag should be enhanced in fertilizer making to reduce the cost of fertilizer manufacturer. The modified slag can be used as fertilizer for agricultural purpose and soil conditioner for acidity corrector of the soil and make it as valuable products to protect environment [11]. Steel slag contains fertilizer components CaO, SiO_2, and MgO [12]. In addition to these three components, it also contains components such as FeO, MnO, and P_2O_5, so it has been used for a broad range of agricultural purposes. Its alkaline property remedies soil acidity. The converter slag is used to produce siliceous fertilizer, phosphorus fertilizer and micronutrient fertilizer [12].

Blast furnace, converter or ladle slags can be used for producing silicate liming materials [13]. Utilization of silicate liming materials neutralizes soil acidity and supplies the soil with plant nutrients. Using blast-furnace lime or converter lime promotes yields, plant quality and soil fertility. The main minerals contain CaO, MgO, SiO_2, Mn and other valuable micro nutrients. The solubility of silicate from slags is often higher than from many other silicate containing soil improvers or rock powders. The basicity of the calcium and magnesium compounds in the slags improves soil pH. The use of steel slags in agriculture produces not only economic but also ecological advantages [13].

The chapter of this book aims to study and to evaluate the antimicrobial efficiency of metallurgical slags for enlarging their construction applications regarding to higher biodegradation resistance. The antimicrobial efficiency of slags was mutually compared as well as compared with a commercial biocide based on

antimicrobial silver Ag. The chapter partially originates from the published results in article [14] and contributes to increase the metallurgical solid wastes recovery possibilities and for the utilization of metallurgical slags in ensuring the biological resistance of building materials and products against bio-degradation and bio-deterioration.

2. Materials and methods

The tested kinds of metallurgical slags were as follows: granulated blast-furnace slags with the fineness of 340 m^2/kg (S1a) and 520 m^2/kg (S1b); air cooled blast-furnace slag (S2); demetallized steel slag (S3); calcareous ladle slag (S4); and copper slag (S5). The tested slags were ground to the fineness of 400 m^2/kg, except for S1a and S1b. Their chemical composition was determined by X-ray fluorescence analysis (XRF) using SPECTRO X-LAB 2000 device, according to EN 196-2. The chemical composition of slags is shown in **Table 1**.

The mineralogical composition of the tested slags was analyzed by the XRD method using BRUKER AXS D8 Advance apparatus. The identified mineralogical composition of the slags is as follows: S1a and S1b—glassy phase, melilite C_2AS–C_2MS_2; S2—melilite C_2AS–C_2MS_2, brownmillerite C_4AF, quartz SiO_2; S3—wüstite FeO, brownmillerite C_4AF, free lime CaO, portlandite $Ca(OH)_2$, larnite β-C_2S, quartz SiO_2; S4—free lime CaO, larnite β-C_2S, shanonite γ-C_2S, gehlenite C_2AS, C_3A, gypsum $CaSO_4{\cdot}2H_2O$, quartz SiO_2 and S5—fayalite Fe_2SiO_4, anortite CAS_2, pyroxene type $CaAlAlSiO_6$. Biostat is the commercial biocide additive based on silver Ag bonding to an Al_2O_3 support (Al_2O_3 99.62 wt.%), with the Ag concentration of 20 mg/g. Biostat is added in a proportion of 0.5 wt.% in the thin layer building applications, such as plastering mortars and rendering and of 1.0 wt.% in rough, coarse layer building applications, such as bricklaying and masonry mortar, to achieve an antimicrobial effect.

2.1 Determination of free calcium oxide CaO_{free} content in ground metallurgical slags and pH of slag water leachates

The determination of free calcium oxide CaO_{free} content in the metallurgical slags was performed using the hot ethylene glycol titration method. The slag sample was ground in the laboratory's vibratory mill to a fineness with particle size under 0.1 mm sieve. Then 1.0 g of the slag sample was diluted with 75 mL of ethylene glycol in a filter flask. Afterwards, the solution was heated for half an hour with occasional stirring and then washed with 50 mL of denatured ethanol. Approximately 2–3 drops of alpha-naphthol phthalein were added to the solution as an indicator. The solution was then titrated with 0.1 N HCl, and the titration continued until the color changed from blue to colorless. The CaO_{free} content in the slag sample was calculated as follows: wt.% CaO_{free} = (the volume of 0.1 N HCl used for the titration; in milliliters) × 0.28. The CaO_{free} content in the slag samples is shown in **Table 2**. The ground slag samples were leached in distilled water for 24 h at 20°C. The pH was determined in the slag water leachates by an Agilent Technologies 3200 P pH Meter with an electrode reference system. The measured pH values of the slag water leachates are given in **Table 2**, as well.

No free lime CaO_{free} content was detected in slags S1a, S1b, and S5, very low CaO_{free} content in slags S2 and S3 and the highest CaO_{free} content was measured in slag S4 on the level of 3.30 wt.%, respectively. The pH values of the slag water leachates were in the range of 9.34–12.93. The highest pH values >12 were measured in leachates of slags S3 and S4, caused by the high free lime CaO_{free} content, and the lowest pH < 11 in leachates of slags S2 and S5. The water leached slags of S3 and S4 agglutinated into

Slag	Unit	S1a and S1b	S2	S3	S4	S5
L.O.I[1]	(wt.%)	0.95	0.09	6.02	5.32	+ 4.30[2]
SiO_2	(wt.%)	42.17	40.57	12.81	13.97	27.26
Al_2O_3	(wt.%)	6.87	8.12	1.64	17.77	7.01
Fe_2O_3	(wt.%)	0.32	2.81	29.78	1.90	46.64
CaO	(wt.%)	41.92	41.73	52.30	58.97	7.48
TiO_2	(wt.%)	0.42	0.11	0.34	0.14	0.21
MgO	(wt.%)	10.39	8.44	2.54	3.30	1.90
K_2O	(wt.%)	0.60	0.72	0.04	0.06	0.40
Na_2O	(wt.%)	0.17	0.19	0.07	0.07	1.07
SO_3	(wt.%)	1.84	2.39	0.28	1.98	0.15
MnO	(wt.%)	0.68	2.31	3.54	0.38	0.61
P_2O_5	(wt.%)	0.05	0.14	0.48	0.05	1.26
Cl	(wt.%)	0.0173	0.0112	0.0138	0.0017	0.0012
V	(ppm)	27.0	32.0	298.0	54.0	41.0
Cr	(ppm)	69.6	65.0	981.0	419.0	5740.0
Co	(ppm)	21.1	21.5	98.0	37.6	307.0
Ni	(ppm)	1.9	3.4	9.9	9.8	1893.0
Cu	(ppm)	1.2	1.5	10.1	4.9	7273.0
Zn	(ppm)	98.1	18.7	41.3	12.3	50,341.0
As	(ppm)	0.7	1.4	3.2	22.1	66.65
Cd	(ppm)	11.6	12.0	24.3	22.0	5.0
Sb	(ppm)	1.5	1.8	2.0	26.7	50.2
Hg	(ppm)	2.9	1.9	6.6	4.2	28.5
Tl	(ppm)	1.5	3.3	5.6	6.0	19.0
Pb	(ppm)	4.0	17.6	3.2	7.5	9203.3

[1]*L.O.I: Loss on ignition.*
[2]*Increment on ignition (caused by, e.g., oxidation of sulfides, metallic particles, etc.).*

Table 1.
The chemical composition of the tested slags.

lumpy granules in water due to the hydration of free lime CaO_{free}, β-C_2S, and in the case of slag S4 by the hydration of C_3A with gypsum, as well. The hydrated lumpy granules were mechanically disaggregated to increase the leaching surface contact.

2.2 Determination of antimicrobial efficiency of metallurgical slags under *in vitro* conditions

The antimicrobial activity of the metallurgical slags was tested on selected species of Gram-positive bacteria (G^+), Gram-negative bacteria (G^-), yeasts and filamentous fungi. Microbial strains (bacteria and filamentous fungi) used in the study were either from the Czech Collection of Microorganisms, T. G. Masaryk University, Brno, Czech Republic (CCM) or yeasts from the Collection of Microorganism of the Institute of Biochemistry and Microbiology, Slovak University of Technology, Bratislava, Slovak Republic. Following microorganisms were used: G^+ bacteria—*Bacillus subtilis* CCM 178, *Staphylococcus aureus* CCM 3958,

Slag	Unit	S1a and S1b	S2	S3	S4	S5
CaO free	(wt.%)	0.00	0.06	0.95	3.30	0.00
pH	–	11.71	9.34	12.87	12.93	10.44

Table 2.
The content of free calcium oxide CaO_{free} in the slag samples and pH values of the slag water leachates.

Micrococcus luteus CCM 410; G^- bacteria—*Escherichia coli* CCM 3988, *Pseudomonas aeruginosa* CCM 3630, *Serratia marcescens* CCM 8587; yeasts—*Candida utilis*—*1a*, *Rhodotorula glutinis*—*1*; microscopic filamentous fungi—*Aspergillus niger* CCM-F 384, *Penicillium funiculosum* CCM 8080, *Chaetomium globosum* CCM 8156, *Alternaria alternate* CCM F-128, *Trichoderma viride* CCM F-534, *Cladosporium herbarum* CCM F-534.

2.2.1 Growth media

1. Meat-peptone bouillon (broth) for inoculation of bacteria (containing in 1000 mL of distilled water, 5 g peptone for bacteriology, 5 g meat extract and 2.5 g NaCl, with the pH adjusted from 7.2 to 7.4);

2. Meat-peptone agar for cultivation of bacteria (containing in 1000 mL of distilled water, 5 g peptone for bacteriology, 5 g meat extract, 2.5 g NaCl and 20 g agar, with the pH adjusted from 7.2 to 7.4);

3. Sabouraud's glucose bouillon (broth) for inoculation of yeasts (containing in 1000 mL of distilled water, 10 g peptone for bacteriology and 20 g glucose, with the pH adjusted to 6.5);

4. Malt agar for cultivation of yeasts and filamentous fungi (containing in 1000 mL of distilled water, 20 g agar and 60 g malt extract, with the pH adjusted to 6.5).

2.2.2 Solutions

For diluting the inoculum of bacteria and yeasts, saline solution (0.85% NaCl) was used, and for preparation of the spore suspension (filamentous fungi), a 0.1% water solution of Tween 80 was used.

2.2.3 Preparation of the inoculum of bacteria and yeasts

A total of 25 mL of meat-peptone bouillon (broth) in a 100 mL Erlenmeyer flask was inoculated with a three-day-old culture of model bacteria using a bacterial loop, and 25 mL of Sabouraud's glucose bouillon (broth) in a 100 mL Erlenmeyer flask was inoculated with a three-day-old culture of yeasts using an inoculation loop. The microorganism cultures were incubated for 15 h at 30°C using shaking apparatus (at a vibration frequency 4 Hz). The grown microorganism cultures were aseptically filtered through three-ply gauze in order to remove possible clusters of cells. The obtained cell suspensions were diluted 100-fold with sterile saline (aqueous physiological solution) and were used for inoculation of the growth media (the cells concentration was 10^6 cells per mL) in order to determine the antibacterial and anti-yeast activity of the slag samples, respectively.

2.2.4 Preparation of spore suspension of filamentous fungi

A total of 8 mL 0.1% water solution of Tween 80 was aseptically added to the sporulated cultures of filamentous fungi, which had been cultivated on slant malt agar for 21 days. Spores from mycelium were loosened by bacterial loop, and filtered as above. The filtered spore suspension was diluted by sterile saline (the spores concentration was 2×10^7 spores per mL) and used for inoculation of the growth media in order to determine the antifungal activity of the slag samples.

2.2.5 Determination of antimicrobial activity

Meat-peptone bouillon for inoculation of bacteria, meat-peptone agar for cultivation of bacteria, Sabouraud's glucose bouillon for inoculation of yeasts and malt agar for cultivation of yeasts and filamentous fungi were used as growth media. Saline solution was used for diluting the inoculum of bacteria and yeasts, and a water solution of Tween 80 was used for preparation of the spore suspension. Sterilization of growth media and solutions was realized in the laboratory autoclave at 120°C for 20 min. The tests were performed in the laboratory incubator at temperatures of 30°C for bacteria, 28°C for yeasts and 25°C for filamentous fungi at a relative humidity of 95%. Antimicrobial activity was determined by dilution methods in agar growth media, so that the resulting concentration of tested slags in growth media was 10, 20, 40, and 60%; further, the concentration of Biostat in growth media was 1.0 and 0.5%, respectively. The pH of the growth media with the addition of the slags was strongly alkaline (pH 11); thus, half the samples of each slag was tested at this pH, and the second half of the samples was tested at a modified pH (bacteria pH 7.2, yeasts, and filamentous fungi pH 6.6). The first half of the samples with the original pH represented real conditions for growth of microorganisms in concrete; the second half of the samples with a modified pH represented optimal conditions for growth of the microorganisms *in vitro*.

After sterilization, meat-peptone agar and malt agar with added slag samples and Biostat (at original pH and modified pH) were cooled down to 60°C. The slag samples and Biostat were equally dispersed throughout growth media and subsequently they were divided and 6 mL of each was placed in the Petri dishes Ø 60.0 mm. Sterile paper discs (Ø 5.0 mm) were placed on the surface of solidified growth media, which were inoculated by 5 μL from spore suspension of model filamentous fungi on malt agar. The inoculation of bacteria and yeasts was done by direct pipetting the suspension on the surface of agarized media. Each 10 μL of suspensions were pipetted from the model bacteria onto meat-peptone agar or model yeasts on the malt agar.

The microorganisms were incubated in a thermostat at temperatures of 30°C for bacteria, 28°C for yeasts, 25°C for filamentous fungi at a relative humidity of 95% for four days. The growth intensity of bacteria and yeasts was compared with the growth of bacteria and yeasts in the control growth media without slags. The growth of filamentous fungi in the presence of slags was monitored by the measurement of average diameter of growing colony at regular time intervals and was compared with the filamentous fungi growth in the control growth media without slags. In the case that no filamentous fungi growth with the presence of slags was observed, the paper discs with spores were transferred on fresh growth media. After 96 h incubation at 25°C, the inhibiting effect of slags was inspected: fungistatic (spores germinate and subsequently the mycelium grows broader) and fungicidal-lethal (fungi do not grow; they are dead).

3. Results

The antimicrobial efficiency of metallurgical slags on selected species of G^+ bacteria, G^- bacteria, yeasts and filamentous fungi was determined and then mutually compared. The antimicrobial efficiency results are given in **Tables 3**–**8**. The antibacterial efficiencies of slags are given in **Tables 3** and **4**; the anti-yeast efficiencies are shown in **Table 5**; and the antifungal efficiencies are given in **Tables 6**–**8**, respectively. The antimicrobial efficiencies of slags are reciprocally compared as well as compared with the antimicrobial efficiencies of the commercial biocide additive—Biostat. The growth extent of bacteria and yeasts is expressed as the signs (–, +, ++, +++), and the growth degree of filamentous fungi is expressed as the percentage (%) of sample surface covered by fungi colonies (measured against a K—control growth media without slags; with the expression of s—fungistatic effect and c—fungicidal effect). The tests were carried out at N—neutral pH of growth media and A—alkaline pH of growth media, as well.

S1aA	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	+++	+++	+++	+++	+++	+++
20%	+++	+++	+++	+++	+++	+++
40%	+++	++	–	++	+++white	+++
60%	+++	++	–	+	++white	+++
S1aN	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	+++	+++	+++	+++	+++	+++
20%	+++	+++	+++	+++	+++	+++
40%	+++	+++	–	+++	+++	+++
60%	+++	–	–	+++	+++	+++
S1bA	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	+++	+++	+++	+++	+++white	+++
20%	+++	+++	+++	+++	+++white	+++
40%	+++	+++	–	++	+++white	+++
60%	+++	+++	–	+	++white	+++
S1bN	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	+++	+++	+++	+++	+++	+++
20%	+++	+++	+++	+++	+++	+++
40%	+++	+++	–	+++	+++	+++
60%	+++	++	–	–	+++	+++
S2A	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	++	+	+	++	++white	++
20%	++	–	–	++	++white	++
40%	++	–	–	++	++white	++
60%	–	–	–	–	–	–
S2N	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	+++	+++	–	+++	+++	+++
20%	+++	+++	–	+++	+++	+++

40%	+++	+++	–	+++	+++	+++
60%	+++	+++	–	++	++	++
S3A	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	+++	+	+	+++	+++	+++
20%	+++	–	–	+++	+++	+++
40%	+++	–	–	++	+++	+++
60%	+++	–	–	+	+++	+++

Notes: –, no growth of bacteria; +, the growth of bacteria is negligible; ++, the growth of bacteria is gradual; +++, the growth of bacteria is intensive comparable with the control growth media; A, alkaline pH of growth media; N, neutral pH of growth media; white, the colony of Serratia marcescens—loss of red pigment by slag impact.

Table 3.
Antibacterial efficiency of slags regarding bacterial growth inhibition.

3.1 Antibacterial efficiency of metallurgical slags

The test results of antibacterial efficiency of slags and Biostat on the selected model G^+ bacteria and G^- bacteria are shown in **Tables 3** and **4**. From the results, it is evident that the inhibitory effect of slags differs. Slag S4 had the highest antibacterial activity and intensely inhibited growth of G^+ and also G^- bacteria. It was proven already at the lowest concentration of slag S4: 10% in growth media. The bacteria, except *M. luteus*, did not grow at higher slag S4 concentrations, whereas the pH values of growth media (neutral, alkaline) did not affect the intensity of bacterial growth inhibition.

The slag S3 inhibited the growth of G^+ bacteria *S. aureus* and *B. subtilis* already at the concentration of 10%, and at higher slag S3 concentrations the growth was completely inhibited. However, slag S3 did not affect the growth of G^- bacteria, except for *E. coli*, at the highest concentration of 60%.

Bacterial growth inhibition with slag S2 was more intensive in the alkaline pH of growth media. The complete inhibition of the growth of G^+ bacteria *B. subtilis* and *S. aureus* was measured at slag S2 concentrations of 20, 40, and 60% and, inhibition of the growth of G^- bacteria *S. marcescens, P. aeruginosa* and *E. coli* was detected at the slag S2 concentration of 60%. Slag S2 only completely inhibited the growth of *B. subtilis* at a neutral pH of growth media and at all tested concentrations. Slag S1a caused total inhibition of *B. subtilis* growth at concentrations of 40 and 60% at both a neutral and alkaline pH of growth media. The growth of *S. aureus* was completely inhibited at slag S1a concentration of 60% at a neutral pH of growth media. The 40 and 60% slag S1b concentration at neutral as well as alkaline pH of growth media caused the total inhibition of growth of *B. subtilis*. No growth of *E. coli* was measured at slag S1b concentration of 60% at neutral pH of growth media. Slag S5 did not significantly affect the growth of bacteria. Slags S1a, S1b, S2 and S5 brought about a change in *S. marcescens* growth at alkaline pH. The bacteria *S. marcescens* lost its red pigment and grew as a white colony. Biostat at concentration of 1.0% caused 100% growth inhibition of all bacteria at neutral and alkaline pH of growth media. Only negligible or no bacterial growth was observed at Biostat concentration of 0.5% in growth media, and pH had no effect on their growth. Based on the measured results, it is evident that the antibacterial efficiency of slags decreased in the order: S4 > S3 > S2 > S1a = S1b > S5. The testing arrangement in Petri dishes regarding the bacterial growth inhibition with slag S2 at neutral pH (N) and alkaline pH (A) of growth media is given in **Figure 1A**.

S3N	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	+++	++	++	+++	+++	+++
20%	+++	–	–	+++	+++	+++
40%	+++	–	–	+++	+++	+++
60%	+++	–	–	–	+++	+++
S4A	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	++	++	++	++	+	–
20%	+	–	–	–	–	–
40%	+	–	–	–	–	–
60%	–	–	–	–	–	–
S4N	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	+	+	+	+	+	–
20%	+	–	–	–	–	–
40%	+	–	–	–	–	–
60%	–	–	–	–	–	–
S5A	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	+++	+++	+++	+++	+++	+++
20%	+++	+++	+++	+++	+++	+++
40%	+++	+++	+	+++	+++white	+++
60%	+++	+++	+	+++	++white	+++
S5N	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
10%	+++	+++	+++	+++	+++	+++
20%	+++	+++	+++	+++	+++	+++
40%	+++	+++	+++	+++	+++	+++
60%	++	++	++	++	++	++
BiostatA	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
0.5%	++	–	+	+	+	+
1%	–	–	–	–	–	–
BiostatN	***M. luteus***	***S. aureus***	***B. subtilis***	***E. coli***	***S. marcescens***	***P. aeruginosa***
0.5%	+	–	–	–	+	–
1%	–	–	–	–	–	–

Notes: –, no growth of bacteria; +, the growth of bacteria is negligible; ++, the growth of bacteria is gradual; +++, the growth of bacteria is intensive comparable with the control growth media; A, alkaline pH of growth media; N, neutral pH of growth media; white, the colony of Serratia marcescens—loss of red pigment by slag impact.

Table 4.
Antibacterial efficiency of slags regarding bacterial growth inhibition.

3.2 Anti-yeast efficiency of metallurgical slags

The test results of anti-yeast efficiency of slags and Biostat on the selected model yeasts are shown in **Table 5**. Slag S4 had the highest anti-yeast activity. Growth of all yeasts was totally inhibited at slag S4 concentration as low as 10%, at both alkaline and neutral pH of growth media. Slags S1a, S1b, and S3 caused complete

S1aA	***C. utilis***	***R. glutinis***	**S1aN**	***C. utilis***	***R. glutinis***
10%	+++	+++	10%	+++	+++
20%	–	–	20%	–	–
40%	–	–	40%	–	–
60%	–	–	60%	–	–
S1bA	***C. utilis***	***R. glutinis***	**S1bN**	***C. utilis***	***R. glutinis***
10%	+	+	10%	+	+
20%	–	–	20%	–	–
40%	–	–	40%	–	–
60%	–	–	60%	–	–
S2A	***C. utilis***	***R. glutinis***	**S2N**	***C. utilis***	***R. glutinis***
10%	+++	+++	10%	+++	+++
20%	+++	++	20%	+++	+++
40%	–	+	40%	+	+
60%	–	+	60%	–	+
S3A	***C. utilis***	***R. glutinis***	**S3N**	***C. utilis***	***R. glutinis***
10%	–	+	10%	–	+++
20%	–	–	20%	–	–
40%	–	–	40%	–	–
60%	–	–	60%	–	–
S4A	***C. utilis***	***R. glutinis***	**S4N**	***C. utilis***	***R. glutinis***
10%	–	–	10%	–	–
20%	–	–	20%	–	–
40%	–	–	40%	–	–
60%	–	–	60%	–	–
S5A	***C. utilis***	***R. glutinis***	**S5N**	***C. utilis***	***R. glutinis***
10%	+++	+++	10%	+++	+++
20%	++	++	20%	++	+++
40%	++	++	40%	++	+++
60%	+	+	60%	++	++
BiostatA	***C. utilis***	***R. glutinis***	**BiostatN**	***C. utilis***	***R. glutinis***
0.5%	+++	+++	0.5%	+++	+++
1%	–	–	1%	–	–

Notes: –, no yeasts growth; +, yeasts growth is negligible; ++, yeasts growth is gradual; +++, yeasts growth is intensive comparable with the control growth media; N, neutral pH of growth media; A, alkaline pH of growth media.

Table 5.
Growth inhibition of selected yeasts by metallurgical slags.

growth inhibition of all yeasts from a concentration of 20% at neutral and alkaline pH of growth media, as well. The growth of yeasts was intensively reduced with slag S2 at concentrations of 40 and 60%. Slag S5 partially inhibited the growth of yeasts; however, total growth inhibition was not observed even at the highest slag S5 concentration of 60%. Biostat at concentration of 1.0% caused total growth inhibition of all yeasts at both neutral, as well as alkaline, pH of growth media. However,

Sample	**Growth of selected filamentous fungi (%)**					
S1aA	***A. alternata***	***P. funiculosum***	***A. niger***	***T. viride***	***C. herbarum***	***Ch. globosum***
10%	90	30	0 s	0 s	88	72
20%	78	24	0 s	0 s	80	20
40%	50	0s	0 s	0 s	60	0 s
60%	50	0s	0 s	0 s	40	0 c
K	100	100	100	100	100	100
S1aN	***A.alternata***	***P.funiculosum***	***A.niger***	***T. viride***	***C.herbarum***	***Ch. globosum***
10%	90	30	0 s	0 s	88	72
20%	60	24	0 s	0 s	80	20
40%	30	0s	0 s	0 s	20	0 s
60%	0s	0s	0 s	0 s	0 s	0 c
K	100	100	100	100	100	100
S1bA	***A.alternata***	***P.funiculosum***	***A.niger***	***T. viride***	***C.herbarum***	***Ch. globosum***
10%	80	22	0 s	0 s	80	72
20%	60	14	0 s	0 s	40	60
40%	30	10	0 s	0 s	40	50
60%	0s	0	0 s	0 s	0 s	10
K	100	100	100	100	100	100
S1bN	***A.alternata***	***P.funiculosum***	***A.niger***	***T. viride***	***C.herbarum***	***Ch. globosum***
10%	40	30	0 s	0 s	80	72
20%	40	14	0 s	0 s	60	60
40%	40	10	0 s	0 s	60	40
60%	0 s	0 s	0 s	0 s	0 s	10
K	100	100	100	100	100	100
S2A	***A.alternata***	***P.funiculosum***	***A.niger***	***T. viride***	***C.herbarum***	***Ch. globosum***
10%	80	30	0 s	0 s	80	80
20%	70	26	0 s	0 s	80	60
40%	54	20	0 s	0 s	60	40
60%	52	15	0s	0s	60	40
K	100	100	100	100	100	100

Notes: s, fungistatic effect; c, fungicidal effect; N, neutral pH of growth media; A, alkaline pH of growth media; K, control growth media.

Table 6.
Antifungal efficiency of metallurgical slags regarding inhibition of growth of selected filamentous fungi.

the intensive growth of yeasts was measured at Biostat concentration of 0.5%, and so Biostat did not possess an inhibiting effect on the growth of yeasts at this concentration. Based on the obtained results, it is evident that the anti-yeast efficiency of slags decreased in the order: S4 > S1a = S1b = S3 > S2 > S5. The testing arrangement

in Petri dishes regarding the yeasts growth inhibition with slag S5 at alkaline pH (A) and neutral pH (N) of growth media is given in **Figure 1B**.

3.3 Antifungal efficiency of metallurgical slags

The test results of antifungal efficiency of slags and Biostat on the selected model filamentous fungi are shown in **Tables 6–8**. Growth of *A. alternate* was most intensively inhibited in the presence of slag S4. Total inhibition of *A. alternata* growth was measured at slag S4 concentrations of 40 and 60% at alkaline as well as neutral pH of growth media, particularly at concentration of 20% at alkaline pH. Slag S4 even possessed a fungicidal (lethal) effect on the fungal spores of *A. alternata* at concentration of 60% at neutral pH, while slag S1b hindered the growth of *A. alternata* at concentration of 60% at alkaline as well as neutral pH of growth media. Slags S1a and S3 totally inhibited *A. alternata* growth at concentration of 60%, but only at neutral pH of the growth media. Slag S2 at concentrations of 40 and 60%, and slag S5 at concentration of 60%, partially inhibited the growth of *A.*

Sample	**Growth of selected filamentous fungi (%)**					
S2N	***A. alternata***	***P. funiculosum***	***A. niger***	***T. viride***	***C. herbarum***	***Ch. globosum***
10%	80	40	0 s	0 s	100	70
20%	72	30	0 s	0 s	80	60
40%	64	24	0 s	0 s	70	60
60%	54	20	0 s	0 s	60	50
K	100	100	100	100	100	100
S3A	***A. alternata***	***P. funiculosum***	***A. niger***	***T. viride***	***C. herbarum***	***Ch. globosum***
10%	80	20	0 s	0 s	52	80
20%	70	10	0 s	0 s	40	75
40%	40	0s	0 s	0 s	20	60
60%	20	0s	0 s	0 s	0 s	0 s
K	100	100	100	100	100	100
S3N	***A. alternata***	***P. funiculosum***	***A. niger***	***T. viride***	***C. herbarum***	***Ch. globosum***
10%	60	10	0 s	0 s	60	80
20%	40	0 s	0 s	0 s	40	70
40%	32	0 s	0 s	0 s	28	60
60%	0 s	0 s	0 s	0 s	0 s	0 s
K	100	100	100	100	100	100
S4A	***A. alternata***	***P. funiculosum***	***A. niger***	***T. viride***	***C. herbarum***	***Ch. globosum***
10%	70	0 s	0 s	0 s	60	0 s
20%	0 s	0 s	0 s	0 s	0 s	0 s
40%	0 s	0 s	0 s	0 s	0 s	0 s
60%	0 s	0 s	0 s	0 c	0 s	0 c
K	100	100	100	100	100	100

Sample	Growth of selected filamentous fungi (%)					
S4N	***A. alternata***	***P. funiculosum***	***A. niger***	***T. viride***	***C. herbarum***	***Ch. globosum***
10%	20	20	0 s	0 s	0 s	0 s
20%	10	10	0 s	0 s	0 s	0 s
40%	0 s	0 s	0 s	0 s	0 s	0 s
60%	0 c	0 s	0 c	0 c	0 s	0 c
K	100	100	100	100	100	100

Notes: s, fungistatic effect; c, fungicidal effect; N, neutral pH of growth media; A, alkaline pH of growth media; K, control growth media.

Table 7.
Antifungal efficiency of metallurgical slags regarding inhibition of growth of selected filamentous fungi.

alternata. Based on the comparison of growth intensity of *A. alternata* in the presence of the tested slags, the order of decreasing inhibition efficiency of slags was: S4 > S1b > S1a = S3 > S2 > S5.

Growth of *P. funiculosum* was significantly inhibited by all tested slags. The highest growth inhibition was measured in the presence of slag S4. Based on a comparison of the growth intensity of *P. funiculosum* in the presence of tested slags, the order of inhibiting efficiency of slags decreased as follows: S4 > S3 > S1a > S1b > S5 > S2. Total growth inhibition of *A. niger* was measured in the presence of all tested slags, with the exception of slag S5, at concentration of 10% at both alkaline and neutral pH of growth media. Slag S4 had a fungicidal (lethal) effect on the fungal spores of *A. niger* at concentration of 60% at neutral pH of growth media. The order of inhibiting efficiency of tested slags on *A. niger* growth decreased as follows: S4 > S1a = S1b = S2 = S3 > S5. No *T. viride* growth was measured in the presence of all tested slags, with exception of slag S5, at concentration of 10% at alkaline pH of growth media. Slag S4 possessed a fungicidal (lethal) effect on fungal spores of *T. viride* at concentration of 60% at neutral as well as alkaline pH of growth media. The inhibiting efficiency of the tested slags on *T. viride* growth decreased in the order: S4 > S1a = S1b = S2 = S3 > S5.

Slag S4 had the highest inhibiting efficiency on *C. herbarum* growth. Complete growth inhibition of *C. herbarum* was detected at all tested slag S4 concentrations, with the exception of concentration of 10% at alkaline pH of growth media, when the growth inhibition of *C. herbarum* was on the level of 40%. No growth was detected in the presence of S1b and S3 slags at concentration of 60% at either alkaline or neutral pH and in the presence of S1a at neutral pH of growth media. Partial growth inhibition of *C. herbarum* was measured at slags S2 and S5. The comparison of growth intensity of *C. herbarum* in the presence of the tested slags demonstrated that the inhibiting efficiency of the tested slags decreased in the order: S4 > S3 > S1b > S1a > S5 > S2. Total growth inhibition of *Ch. globosum* was measured at slag S4, even from the lowest concentration of 10%. Similarly, complete growth inhibition was observed in the presence of slags S1a and S5, however, only at high concentration values of 40 and 60%, and as in the presence of slag S3 even at the highest concentration of 60%. Partial growth inhibition of *Ch. globosum* was measured at slags S1b and S2. The comparison of growth intensity of *Ch. globosum* in the presence of the tested slags showed a decreasing inhibition efficiency of slags in the order: S4 > S1a = S5 > S3 > S1b > S2.

From the measured results, it is seen that the model filamentous fungi used were sensitive to the presence of tested slags in various ways. All slags inhibited the growth of filamentous fungi by 40–100% at concentration of 60% slag. The most

sensitive to the presence of all slags were *T. viride* and *A. niger*. Their growth was completely inhibited at 20–60% concentrations of all slags at alkaline and neutral pH of growth media. The growth of these two fungi species was stopped, thus causing a fungistatic effect. The 60% concentration of slag S4 resulted in the inhibition of *T. viride* growth with fungicide effect (killed fungal spores). Slag S4 possessed the most inhibiting activity for all filamentous fungi. It totally inhibited growth of almost all filamentous fungi in concentrations from 20 to 60% at alkaline pH of growth media, with mostly a fungistatic effect. Only *T. viride* growth was inhibited completely at slag S4 concentration of 60% at alkaline pH of growth media with a fungicide effect. The growth of filamentous fungi was inhibited completely at concentrations from 40 to 60% of slag S4 and at slag S4 concentration of 60% with fungicide effect on *A. niger, A. alternate, Ch. globosum* and *T. viride*, at neutral pH of growth media.

A lower inhibiting effect on filamentous fungi growth was measured at slags S1a, S1b and S3, however, they inhibited the growth of all filamentous fungi from

Sample	**Growth of selected filamentous fungi (%)**					
S5A	***A. alternata***	***P. funiculosum***	***A. niger***	***T. viride***	***C. herbarum***	***Ch. globosum***
10%	84	48	20	20	60	60
20%	80	14	0 s	0 s	40	10
40%	80	10	0 s	0 s	40	0 s
60%	56	0	0 s	0 s	40	0 s
K	100	100	100	100	100	100
S5N	***A. alternata***	***P.funiculosum***	***A. niger***	***T. viride***	***C.herbarum***	***Ch.globosum***
10%	80	52	70	0 s	72	80
20%	80	20	0 s	0 s	40	40
40%	80	10	0 s	0 s	40	0 s
60%	50	10	0 s	0 s	40	0 s
K	100	100	100	100	100	100
BiostatA	***A. alternata***	***P. funiculosum***	***A. niger***	***T. viride***	***C. herbarum***	***Ch. globosum***
0.5%	100	100	100	100	100	60
1%	10	70	70	40	50	30
K	100	100	100	100	100	100
BiostatN	***A. alternata***	***P. funiculosum***	***A. niger***	***T. viride***	***C. herbarum***	***Ch. globosum***
0.5%	100	100	100	100	100	60
1%	30	70	70	40	50	30
K	100	100	100	100	100	100

Notes: s, fungistatic effect; c, fungicidal effect; N, neutral pH of growth media; A, alkaline pH of growth media; K, control growth media.

Table 8.
Antifungal efficiency of metallurgical slags regarding inhibition of growth of selected filamentous fungi.

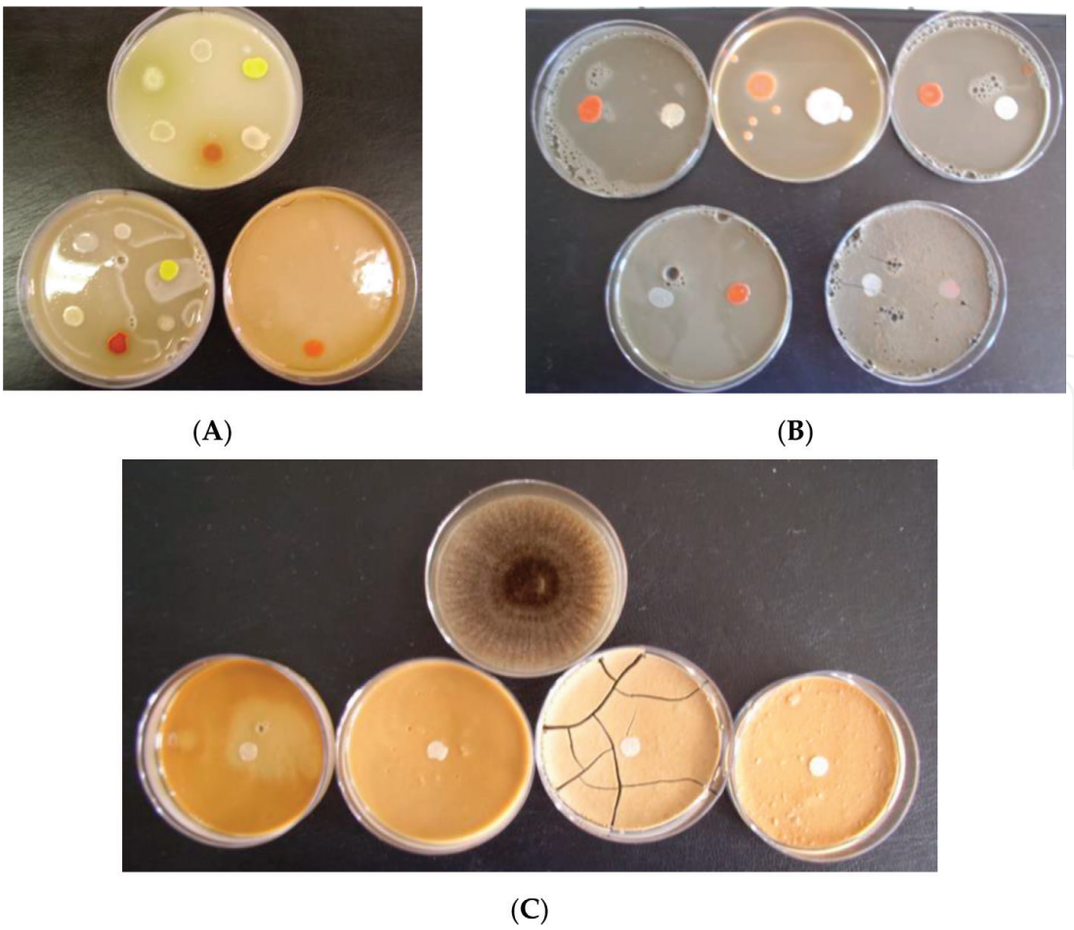

Figure 1.
*The testing arrangement in petri dishes regarding the growth inhibition of selected species of G^+ bacteria, G^- bacteria, yeasts and filamentous fungi in the presence of tested slags: (***A***) bacterial growth inhibition with slag S2 at neutral pH (N) and alkaline pH (A) of growth media (upper line: control (no slag); lower line from left: slag S2 (N)—60%, S2 (A)—60%); (***B***) yeasts growth inhibition with slag S5 at alkaline pH (A) and neutral pH (N) of growth media (upper line from left: slag S5 (A)—10%, control (no slag), slag S5 (N)—10%; lower line from left: slag S5 (A)—60%, S5 (N)—60%); and (***C***) inhibition of Aspergillus niger growth with slag S1a at alkaline pH (A) of growth media (upper line: control (no slag), lower line from left: slag S1a (A)—10, 20, 40, 60%).*

40 to 100% at concentration of 40%. The lowest impact on the growth of filamentous fungi was observed at slags S2 and S5, which affected only the growth of the most sensitive fungi, *A. niger* and *T. viride*. The most resistant was *A. alternata*. Its growth was most intensely inhibited by slag S4, with total inhibition observed at 40–60% of slag concentration range, with mostly a fungistatic effect. Biostat, at concentration of 0.5% did not affect the growth of filamentous fungi, with exception *Ch. globosum*, the growth of which was inhibited by 40%. Biostat at concentration of 1.0% inhibited the growth of filamentous fungi by 30–70%. Based on the measured results, it is evident that Biostat is characterized mainly by antibacterial and anti-yeast efficiency, and affects the growth of model filamentous fungi only to a minimum extent. Regarding the fact that model filamentous fungi were selectively sensitive to the presence of the tested slags, it is possible to determine only an approximate order of inhibition efficiency of the slags to filamentous fungi as follows: S4 > S3 = S1a = S1b > S5 > S2. The pH values of the growth media did not significantly influence the intensity of inhibition of the model microorganisms' growth. The testing arrangement in Petri dishes regarding the inhibition of *A. niger* growth with slag S1a in alkaline pH (A) of growth media is given in **Figure 1C**.

4. Discussion

The results obtained by testing the antimicrobial efficiency of metallurgical slags on selected species of G^+ bacteria, G^- bacteria, yeasts and filamentous fungi can be summarized as follows: the tested slags are characterized by selective toxicity on the model representatives of biodeteriogenic microflora; calcareous ladle slag (S4) has the highest antimicrobial efficiency; slag S4 best inhibits the growth of bacteria, yeasts, and filamentous fungi of all slags; complete growth inhibition of *A. niger* and *T. viride* was measured at all slags at concentration of 10%, except for copper slag (S5); slag (S4) and granulated blast-furnace slag with the fineness of 340 m^2/kg (S1a) had fungicidal (lethal) effect on spores of some filamentous fungi at concentration of 60%; slag (S5) possesses the lowest antibacterial and anti-yeast efficiency, despite having the highest Cu, Hg, As, Pb, Cr, Zn, Co, and Ni amounts from all slags; air-cooled blast-furnace slag (S2) and slag (S5) possess the lowest antifungal efficiency. Biostat at concentrations of 0.5 and 1% inhibited the growth of bacteria, at concentration of 1% inhibited the growth of yeasts, at concentration of 0.5% did not affect the growth of filamentous fungi at all, and at concentration of 1% inhibited the growth of filamentous fungi only to a minimum extent.

In general, slag (S4) is characterized by the highest antimicrobial efficiency and inhibits the growth of bacteria, yeasts and filamentous fungi most of all slags. Slag S4 possesses the highest CaO content of 58.97 wt.% and the highest CaO_{free} content (3.30 wt.%) as well as the highest pH value of water leachate (12.93), in comparison with the other slags. Antimicrobial activity of metallurgical slags, with the very high CaO and CaO_{free} contents, as well as the very high pH value of water leachate, as in the case of slag S4, could be approximately similar to the antimicrobial efficiency of lime [15, 16]. Calcareous metallurgical slags give rise to an increase in the pH values of water leachates up to the range of about 11.5–12.9 due to the high CaO content, e.g., similarly in the case of granulated blast-furnace slag, cements and concretes in the wet state, when they hydrate [1, 2, 6], which affects the viability of microorganisms. This effect is similar to the effect of lime during hygienisation. Disinfection by lime reduces extensively the number of microorganisms when operated at pH 11.2 at a wastewater treatment plant [15]. Lime enables the destruction of all pathogens due to the pH increase (alkaline hydrolysis), combined with the temperature rise that quicklime hydration provides (thermolysis) [16]. The thermolysis effect can be excluded at metallurgical slags in comparison with lime, however, alkaline hydrolysis could be considered as their antimicrobial potential. Alkaline hydrolysis (OH^-) results in the destruction of proteins, nucleic acids (RNA), carbohydrate and lipids and so finally pathogen inactivation [16].

5. Conclusions

According to the measured results, it is evident that: the antibacterial efficiency of the tested metallurgical slags decreased in the order: S4 > S3 > S2 > S1a = S1b > S5; the decrease in anti-yeast efficiency was in the order: S4 > S1a = S1b = S3 > S2 > S5; filamentous fungi were selectively sensitive to the tested slags, therefore, it is only possible to determine the approximate order of inhibition efficiency of slags to filamentous fungi: S4 > S3 = S1a = S1b > S5 > S2. Calcareous ladle slag (S4) is characterized by the highest antimicrobial efficiency, while granulated blast-furnace slag (S1a, S1b) and demetallized steel slag (S3) possess medium activity. Air cooled blast-furnace slag (S2) has still lower activity and copper slag (S5) has the lowest activity. Based on the antimicrobial efficiency results, metallurgical slags

possess a great potential for utilization as appropriate antimicrobial components for construction applications.

Acknowledgements

The chapter is partially based on the results and information from the published article [14] "Strigáč J, Števulová N, Mikušinec J, Varečka Ľ, Hudecová D. Antimicrobial efficiency of metallurgical slags for application in building materials and products. Buildings 2018;8:33. DOI: 10.3390/buildings8020033," which was published by MDPI AG, Buildings Editorial Office, Basel, Switzerland (http://www.mdpi.com/journal/buildings/). MDPI AG, Buildings Editorial Office in the published article [14] contributed to the chapter of this book. The chapter is extended review paper of the article [14], with new insights.

Notes/thanks/other declarations

Many thanks to the original publisher—MDPI AG, Buildings Editorial Office, St. Alban-Anlage 66, 4052 Basel, Switzerland (http://www.mdpi.com/journal/buildings/) of article [14].

Author details

Július Strigáč[1*], Nadežda Števulová[2], Jozef Mikušinec[1], Ľudovít Varečka[3] and Daniela Hudecová[3]

1 Povazska Cement Plant Ladce, Ladce, Slovakia

2 Institute of Environmental Engineering, Faculty of Civil Engineering, Technical University of Košice, Košice, Slovakia

3 Institute of Biochemistry and Microbiology, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Bratislava, Slovakia

*Address all correspondence to: strigac.j@pcla.sk

References

[1] Cwalina B. Biodeterioration of concrete. Architecture Civil Engineering Environment. 2008;**4**:133-140

[2] George RP, Vishwakarma V, Samal SS, Mudali UK. Current understanding and future approaches for controlling microbially influenced concrete corrosion: A review. Concrete Research Letters. 2012;**3**:491-506

[3] House MW, Weiss WJ. Review of microbially induced corrosion and comments on needs related to testing procedures. In: Olek J, Weiss J, editors. Proceedings of the 4th International Conference on the Durability of Concrete Structures (ICDCS), 24-26 July 2014; West Lafayette, IN, USA: Purdue University; 2014. pp. 94-103. Available from: https://pdfs.semanticscholar.org/38e9/5fe75db5b0043d05b7615b76221842bbe19d.pdf

[4] Strigáč J, Martauz P, Eštoková A, Števulová N, Luptáková A. Bio-corrosion resistance of concretes containing antimicrobial ground granulated blastfurnace slag BIOLANOVA and novel hybrid H-CEMENT. Solid State Phenomena. 2016;**244**:57-64. DOI: 10.4028/www.scientific.net/SSP.244.57

[5] Giannantonio DJ, Kurth JC, Kurtis KE, Sobecky PA. Effects of concrete properties and nutrients on fungal colonization and fouling. International Biodeterioration and Biodegradation. 2009;**63**:252-259. DOI: 10.1016/j.ibiod.2008.10.002

[6] Strigáč J, Martauz P. Fungistatic properties of granulated blastfurnace slag and related slag-containing cements. Ceramics–Silikáty. 2016;**60**: 19-26. DOI: 10.13168/cs.2016.0003

[7] Anjaneyulu E, Ramgopal M, Narasimha G, Balaji M. Effect of pig iron slag particles on soil physico-chemical, biological and enzyme activities. Iranica Journal of Energy and Environment. 2011;**2**:161-165

[8] Papastergiadis E, Nathanail E, Sampalioti D, Sklari S, Chasiotis A, Lollos K, Karagiannidis A, Samaras P. The use of steelmaking slag for sewage sludge stabilization. In: Albanis T et al. editors. Proceedings of the 10th International Conference on Protection and Restoration of the Environment (PRE 10), 5-9 July 2010; Corfu Island, Greece. 2010;**191**:5-9. Available from: http://ikee.lib.auth.gr/record/251683; https://qa.auth.gr/en/cv/akarag?nopagecache=1

[9] Stanojković A, Pivić R, Maksimović S, Stevanović D, Delić D, Stajković O. The effect of metallurgical slag on microbiological activity in Pseudogley. In: Marić S, Lončarić Z, Florijančić T, Lužaić R, editors. Proceedings of the 45th Croatian and 5th International Symposium on Agriculture, 15-19 February 2010; Opatija, Croatia; 2010. p. 925-929 Available from: file:///C:/Users/Ing%20Strig%C3%A1%C4%8D%20J.PhD/Downloads/Olivera_Stajkovic_Srbinovic_%20tema_BF.pdf

[10] Masayoshi K, Minoru M. Mortar composition. Japanese Patent JP2006315883 A, Publication date 24 November, 2006, Filing date 11 May, 2005, Assignee: Denki Kagaku Kogyo KK, Application number JP2005000137952, [Internet]. Available from: http://www.sumobrain.com/patents/jp/ Mortar-composition/JP2006315883.html [Accessed: 2006-11-24]

[11] Maghchiche A, Naseri R, Haouam A. Uses of blast furnace slag as complex

fertilizer. Journal of Chemistry & Chemical Engineering. 2012;**6**:853-859

[12] Huang Y, Guoping X, Huigao C, Junshi W, Yinfeng W, Hui C. An overview of utilization of steel slag. Procedia Environmental Sciences. 2012;**16**:791-801. (https://doi.org/10.1016/j.proenv.2012.10.108) Available from: https://core.ac.uk/download/pdf/82356046.pdf

[13] Euroslag Technical Leaflet No. 3. Fertilisers from blast furnace and steel slags. Secretary: FEhS- Building Materials Institute. Duisburg, Germany, [Internet]. 2006. Available from: http://www.euroslag.org/fileadmin/_media/images/Research/FACT_SHEETS/Fertiliser_Leaflet.pdf [Accessed: 2006-01-01]

[14] Strigáč J, Števulová N, Mikušinec J, Varečka Ľ, Hudecová D. Antimicrobial efficiency of metallurgical slags for application in building materials and products. Buildings. 2018;**8**:33. DOI: 10.3390/buildings8020033

[15] Grabow WOK, Middendorf IG, Basson NC. Role of lime treatment in the removal of bacteria, enteric viruses and coliphages in a wastewater reclamation plant. Applied and Environmental Microbiology. 1978;**35**:663-669

[16] Remy M. Scientific evidence on the effectiveness of lime. In: Proceedings of the Conference Animal Health and Welfare. A Sustainable Solution from Industry; 18 February 2009; Brussels. Belgium: EuLA c/o IMA-Europe; 2009. pp. 11-21