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Letter to Editor

Ashes as Effective Biocides of Microbiological Processes

L. Domka, K. Szymańska, K. Seifert, F. Domka

Faculty of Chemistry, Adam Mickiewicz University,
ul. Grunwaldzka 6, 60-780 Poznan, Poland

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Abstract

The influence of fly ash from the "Karolin" heat and power producing plant in Poznan on the kinetics of processes taking place with involvement of the bacteria *Desulfotomaculum ruminis* and *Thiobacillus ferrooxidans* has been studied under laboratory conditions.

The results indicate that the presence of fly ash has an inhibitory effect on the processes related to the microbiological conversion cycle of sulphur compounds and can be used as biocides in the processes of microbiological corrosion of materials.

Keywords: bacteria, *Desulfotomaculum ruminis, Thiobacillus fetrooxidans,* fly ashes, biocides

Introduction

Problems related to storage and utilisation of fly ash and flue dust produced by coal combustion still remain the subject of concern mostly in the aspect of environmental protection. Prior to looking for any solutions there should be a thorough recognition of physical and chemical properties of the ash and dust and the ways of their interaction with the environment. Chemical analysis of fly ash [1] has proven that besides the compounds making the so-called matrix (SiO_2, $A1_2O_3$, CaO) they also contain elements built into the silica skeleton (called dominant elements) and trace amounts of the elements sitting on the surface of the ash molecules. The dominant and trace elements occur mostly in compounds, whose chemical composition depends on the kind of coal and conditions of combustion [2]. As follows from the results of [3], at coal combustion temperature some elements disperse into the atmosphere and some accumulate on the surface of ash and dust molecules [4, 5] as a result of adsorption or condensation. These elements pose the greatest ecological threat. Incorrect conditions of their storage can lead to their wash out by precipitation and entering the water circulation system. Moreover, flue dust molecules may be inhaled and in this way enter living organisms. Recognition of the above threats has stimulated many attempts at developing methods of safe disposal of coal combustion products [6].

Encouraged by our earlier study on inhibitory properties of fly ash [7], an attempt has been made to check their behaviour as biocides in processes taking place with involvement of bacteria [8]. The subject of our concern in this work were processes very important in microbiological corrosion of construction materials and devices [9]. The preliminary results it presented are the basis for future use of fly ash as biocides inhibiting the multiplication of sulphide-reducing bacteria of the genus *Desulfotomaculum* and bacteria of the genus *Thiobacillus* oxidising iron and sulphur. In these two processes the inorganic materials containing sulphates undergo destruction accompanied with release of acid radicals and other substances (e.g. H_2S) initiating corrosion processes [9]. The results were also expected to allow an estimation of the inhibitory effect of fly ash on the cycle of microbiological conversions of sulphur compounds in the natural conditions.

Correspondence to: Prof. F. Domka

Experimental

The fly ash and flue dust samples were collected at the heat and power producing plant in Karolin. The fraction to be studied was obtained after sieving through mesh size 0.4 mm.

The bacteria reducing sulphates were isolated and identified as *Desulfotomaculum ruminis* by the method described earlier [10]. The kinetic studies were conducted at 37°C in anaerobic conditions (helium) at pH varied from 6.8 to 7.2, in sealed glass reactors containing 50 cm^3 of the modified Starkey medium of the composition [in g/dm^3]: $MgSO_4$ x H_2O = 2.00, Na_2SO_4 = 2.66, NH_4CI = 1.00, K_2HPO_4 = 5.00, $CaCl_2$ = 0.13, Mohr salt = 0.006, sodium lactate = 25 and microelements [11]. Selected amounts of the ashes tested: 20, 40, 60,100,150 g/dm^3, were added into the medium and then, after deoxidisation of the samples, they were inoculated by 4% vol of the inoculum taken from the phase of logarithmic growth (after 24h of incubation). The reaction rate was measured as the rate of reduction of sulphates to sulphides, measured at certain time intervals.

The bacteria *Thiobacilus ferroxidans* were isolated from the waters in the Siersza colliery. Standard growth of the bacteria was conducted on the Silverman iron-medium 9K, at 37°C and pH 2.2 [12].

The kinetic study was carried out in glass reactors of 50 cm^3 in capacity, containing 20 cm^3 of 9K medium and 10% vol. of the inoculum (10^7 $cells/cm^3$) from the phase of the logarithmic growth (after 22 hours of incubation). The reactors were placed in a thermostated shaker Elpan, 357, shaking at a frequency of 150 r.p.m., with an amplitude of 9. The ash tested was added to the reactors in concentrations from 5 to 125 g/dm^3. The process was conducted at 35°C at pH 2.2, and its rate was established by measurements of iron (II) ions concentration at certain time intervals [13].

Analogous experiments were carried out in the same conditions using the reference samples (without the ash) and the results were averages obtained in three experiments. In this way in assessment of the experimental results we could eliminate the effects of chemical processes. The instruments and media used in the experiments were sterilised for 20 minutes at 120°C.

The Methods

The concentration of sulphides was determined by the iodometric method in the samples after precipitation of CdS [14].

Iron (II) ions were determined spectrophotometrically (Beckman DU 640) with 1,10-phenantroline [15] at the wavelength of 512 nm. Iron (III) ions were also determined spectrophotometrically by the rhodanate method [15] at a wavelength of 495 nm.

Results and Discussion

Microorganisms developing on the surface of inorganic materials educe acid anhydrides and other substances, e.g. H_2S, initiating processes of chemical corrosion, known as corrosion induced by microbes [16]. The species known to initiate such processes are anaerobic bacteria from the genus *Desulfovibrio* and *Desulfotomaculum* and aerobic bacteria from the genus *Thiobacillus.*

Fig. 1 presents results of the kinetic study illustrating the influence of the ashes tested on the process of dissimilatory sulphates reduction taking place with the involvement of *Desulfotomaculum* bacteria. Although the experiments were performed throughout a relatively short time, the kinetic curves obtained clearly suggest that the multiplication of these anaerobic microorganisms is inhibited in the presence of the ash. With increasing concentration of the ashes, the degree of the conversion of sulphates to sulphides significantly decreases. For example, at the presence of 100 g of the ashes in 1 dm^3 of the medium, the degree of reduction after about 45 hours drops to 30%.

A further increase in ash concentration leads to total inhibition of dissimilatory sulphates reduction. The con-

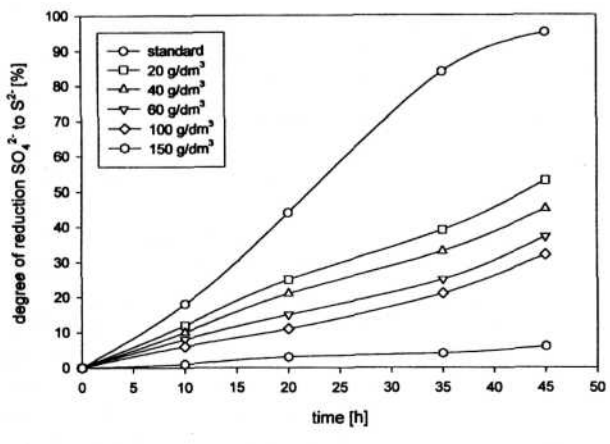

Fig. 1. The effect of the ash tested on the process of dissimilatory sulphates reduction taking place with the involvement of *Desulfotomaculm ruminis* bacteria, at 37°C, pH from 6.8 to 7.2.

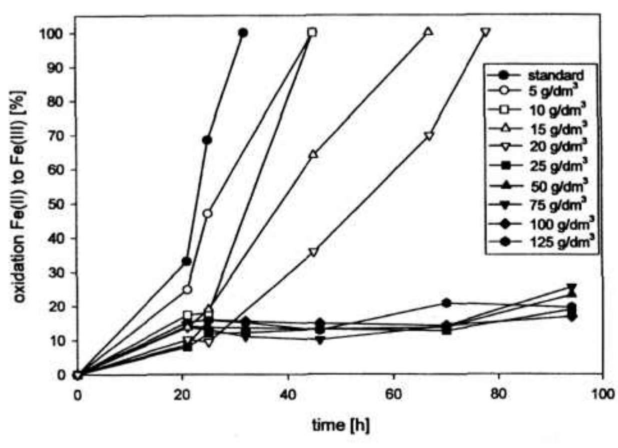

Fig. 2. The effect of the ash tested on the process of Fe(II) ions oxidation taking place with the involvement of *Thiobacillus ferrooxidans* bacteria, at 35°C, pH 2.2.

centration of 150 g/dm^3 of the ash was found lethal towards the bacteria *Desulfotomaculum ruminis.*

This inhibitory effect of the ash presence on the multiplication of bacteria is undoubtedly related to the synergetic activity of metal ions on the surface of the ash particles. The metal ions occur at higher concentrations and different oxidation degrees than, e.g., in coal. The synergetic effect of different metal ions on the growth of the *Desulfotomaculum* bacteria has been observed in other situations [17]. The surface of the ash particles in direct contact with the environment can be easily washed with water. This increased concentration of trace elements on the surface of ash and dust particles [4, 5] is an important problem from the point of view of environmental protection. On the other hand, increased concentration of ash may prove useful for protection against the destructive activity of microorganisms, in particular corrosion of construction materials being in direct contact with water or soil [9, **15].**

The fly.ash and dust could therefore be used in production of different concrete-based materials like pavement blocks or other products in contact with water and soil, as additives inhibiting corrosion processes [7].

Apart from bacteria from the genus *Desulfotomaculum* and *Desulfovibrio,* the bacteria initiating corrosion of metals and construction materials also include those from the genus *Thiobacillus,* which oxidise sulphites, sulphur (IV) and thiosulphates, and a number of polysulphate compounds to sulphates. In this way they stimulate formation of strong sulphur acids [18]. Corrosion of iron and steel is also related to the oxidation of reduced iron (II) compounds, therefore, in this work we studied the influence of ashes and dusts on the development of *Thiobacillus ferrooxidans* bacteria [19].

As follows from the results, illustrated in Fig. 2, the process of multiplication of these bacteria in a medium containing ash in a concentration higher than 25 g/dm^3 is strongly inhibited. After 80 hours of conversion, the multiplication of bacteria cannot be restarted. This means that in these conditions, the ash play the role of a microbiocide inhibiting oxidation of iron (II) and the reduced sulphur compounds, so inhibiting the multiplication of *Thiobacillus ferrooxidans* bacteria. The process of biooxidation leads to the formation of sulphuric acid, initiating further oxidation with the involvement of iron (II) ions, which after oxidation to iron (III) ions are responsible for chemical oxidation of sulphides. The mechanism of these conversions has been presented in detail in [20] by D.G. Lundgren and L. Silver. The ash tested have been used in production of cement blocks which were then placed in a substrate containing sulphates and bacteria; in one experiment *Desulfotomaculum ruminis* and in another one *Thiobacillus ferrooxidans.* The preliminary results obtained indicate that the development of the microorganisms was inhibited. Moreover, the cement blocks tested, containing about 10% of the ash, placed in a substrate first with the bacteria *Desulfotomaculum ruminis* and then *Thiobacillus ferrooxidans* for 60 days, were characterized by much higher resistance to breaking than the reference samples. Table 1 presents the results of testing the strength of the blocks after freezing, and they confirm our earlier results [7].

Table 1. The influence of deep-freezing on the durability of cement block containing tested ash.

Content of ash [%]	0 standard	5	8	10
Weight loss of block [mg] (average from 2 experiments)	17.5	11.7	9.25	8.2

The results of the preliminary tests performed in laboratory conditions indicate that fly ash and flue dust can be successfully used as additives inhibiting the processes of microbiological corrosion. Because of the problems related to collection of flue dusts, the study on samples of fly ash - particles retained by dust catchers are treated as representative.

Conclusions

As follows from the results of our study, fly ash could be utilised by the cement industry as a component inhibiting microbiological corrosion. A particular concentration of the ash in construction materials should be established in experiments for specific purposes. The practical use of fly ash requires more laboratory and pilot studies, but it seems that the benefits are worth the cost - the difficult waste product will be utilized and the processes of microbiological corrosion will be inhibited.

Another conclusion of our study is that the presence of the ash tested significantly disturbs the natural cycle of sulphur conversion.

References

1. WHITE H.J.: Electrostatic precipitation of fly ash. J. Air Poll. Cont. Assoc, **27,** 114, **1977.**
   BLOCK H.C., DAMS R.: Study of fly ash emission during combustion of wal. Envir. Sci. Technol., **10(10),** 1011-1017, **1976.**
2. PACHOWSKI J.: Popioly lotne i ich zastosowanie w budownictwie drogowym. WKL Warszawa, **1976.**
3. NATUSCH D.F.S., WALLACE J.R., EVANS C.A. Jr.: Toxic trace elements. Preferential concentration in respirable particles. Science, **183(411),** 202-204, **1974.**
4. GAVRILOVIC J.: Identification of thin surface films on small particles. Microscope, **25(2), 1977.**
5. LINDFORS P.A., HOVLAND C.T.: Surface chemical analysis of particles by Auger electron spectroscopy and ESCA. Envir. Sci. Res., **13** (Envir. Poll.), 349-363, **1978.**
6. CULLINARE M.J.Jr., JONES L.W., MALONE P.G.: Handbook for Stabilization - Solidification of Hazardous Waste. EPA/540/2/86/001. Cincinnati OHIO, June **1986.**
7. GASIOREK J, DOMKA F., DOMAGALA Z.: Inhibitujace dzialanie popiolow i pylow kominowych na rozwoj bakterii siarkowych. Cement, Wapno, Gips, **12,** 265, **1986.**
8. DOMAGALA Z., DOMKA F.: Desulfurikacja i jej niektore aspekty ekologiczne. Wiad. Chem., **48,** 1-2, **1994.**
9. ZYSKA B.: Mikrobiologiczna korozja materialow. Wyd. Nauk.-Techn., Warszawa **1977.**

10. DOMAGALA Z., DOMKA F.: Estimation of effect of *Desulfotomaculum ruminis* bacteria on the process of degradation of simple organic substances. Envir. Prot. Eng., **17,** 83, **1991.**
11. DOMKA F., GASIOREK J.: Influence of the concentration of phosphorus potassium, calcium and iron compounds on the microbial reduction of sulphates. Acta Microb. Polon., **25(1),** 57-64, **1976.**
12. NOWACZYK K., DOMKA F.: Attempts of microbiological utilization of rubber wastes. Pol. J. of. Envir. Stud., 8(2), 101-106, **1999.**
13. BRYCKI B., SZYMANSKA K., WALIGORSKA M., SEIFERT K., DOMKA F.: The effect of MMPP on the microbiological processes related to conversion of nitrogen and sulphur. Pol. Journ. of Envir. Stud., **vol.** 7, No 2, 65-69, **1998.**
14. WILLIAMS W.: Oznaczanie anionow. PWN, Warszawa, **1985.**
15. MARCZENKO Z.: Kolorymetryczne oznaczanie pierwiastkow. Wyd. Nauk.-Techn., Warszawa, **1967.**
16. HEITZ E.Ed., FLEMMING H.C. SAND W.: Microbially influenced corrosion of materials. Springer, Berlin, Heidelberg, **1996.**
17. SZYMANSKA K., DOMKA F., MIKUTANIEC A.: The effect of interaction between heavy metal ions on the desulfurication process. Pol. Journ. of Envir. Stud., 7(3), 181-184, **1998.**
18. GASIOREK J., DOMKA F.: Utlenianie zredukowanych nieorganicznych zwiazkow siarki przez bakterie *Thiobacillus thioparus,* w swietle badan laboratoryjnych. Cement, Wapno, Gips, **4,** 101, **1984.**
19. ZYSKA B., Mikrobiologiczna korozja materialow. Wyd. Nauk.-Techn., Warszawa **1977.**
20. LUNDGREN D.G. and SILVER L.: Ore leaching by bacteria. Ann. Rev. Microbiol., 34, 262-238, **1980.**