

Application of X-ray diffraction for studies on microbially induced metal corrosion

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Introduction

Metals and their alloys often undergo corrosion. In some cases it is related to activity of microorganisms forming biofilm on the metal surface. Such a situation is also typical for drinking water supply system. Cells and the organic matter they produce collect in a places, where water continuously acts on pipe walls. Such conditions promote the growth and proliferation of microbes [1 – 3]. Microbes present in water mains are adapted to low content of nutrients in drinking water (less than 2 mg of organic matter per l of water) and are very hard to manage [4]. The presence of bacteria in water supply networks brings a number of problems, e.g. increased consumption of disinfectants, which means higher water treatment costs [5, 6]. The biofilm may also serve as a reservoir of pathogenic microbes [7], including coliforms. Search for their potential source has lead to the conclusion they must have been present in biofilms formed on pipe walls of drinkable water supply system. When released from the biofilm, they entered the water and were detected as suspended (planctonic) bacteria [7]. The bacteria also contribute to the corrosion of piping [8, 9]. This results in decrease of their strength, as well as generation of favourable conditions for growths of other microorganisms on the surface of the corroded porous surfaces [3, 6]. Pipe corrosion among others leads to releasing iron compounds into water, which causes its turbidity and deterioration of organoleptic properties [10, 11]. Bacteria may have direct effect on the quality of supplied water by changing its colour related to the content of compounds containing iron and/or other metals (their presence may be a result of bacterial metabolic activity, e.g. *Galionella* genus) as well as unpleasant odour and taste (e.g. due to the presence of hydrogen sulphide produced by sulphate reducing bacteria; RBS) [3, 10, 12]. Biofilm generation in water supply network may also lead to hydraulic losses due to the increase of pipe wall roughness and deposition on them insoluble compounds of iron and other metals [5, 12, 13].

Microbiologically induced/influenced corrosion (MIC) occurs also in tanks and pipelines used for transportation of waste water, oil, gas and diesel oils. The same problem applies also to components of water construction, waste water treatment plants, many components of power plants, cooling systems and fire sprinkler systems [14 – 18].

X-ray Diffraction (XRD) is a research technique applied both in structural and applied radiography. Applied X-ray analysis includes both qualitative and quantitative phase analysis, measurements of lattice parameters, determination of dimensions of crystallites and mosaic blocks, determination of macro- and microstrains, deformations of crystal lattice, as well as texture testing [19]. In the MIC studies, XRD is employed for determination of individual crystalline phases forming part of corrosion products formed on metal surfaces.

The aim of this paper is to present the current state of knowledge on XRD in terms of the use of this technique to study the corrosion induced by microorganisms.

X-ray Diffraction (XRD)

XRD jest a research technique enabling analysis of structure and defects of crystalline substance, as well as analysis of varieties of amorphous substance and forming various materials, including geological and construction materials, industrial wastes (ashes, dust), useful materials and substances (metal alloys, pharmaceuticals). The majority of naturally occurring substances has crystalline structure (the atoms are arranged in space in a regular and orderly manner). Crystalline substances occurs as monocrystals or polycrystals, i.e. technical materials such as metals and ceramics [20]. Depending on the type of tested material, various techniques of X-ray diffraction are used. It uses principle of interference reflection from set of lattice plane of defined interplanar spacings (Fig. 1) using X-ray diffraction on crystal lattice. The technique takes into account observations of Laue, Braggs and Wulf. They noticed that X-ray reflection from crystal planes occurs only some, specific angles of incidence.

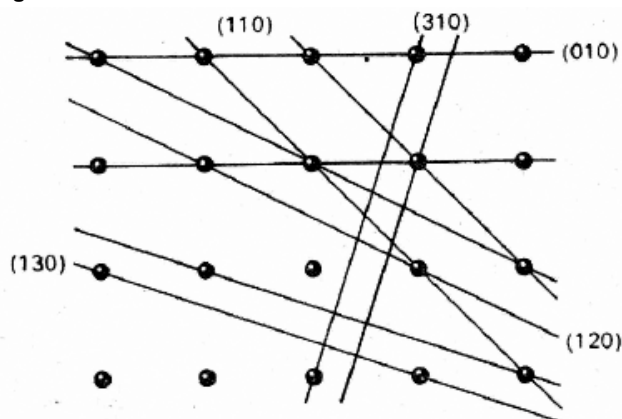


Fig. 1. Diagram of placement of atoms in crystal lattice with marking of different groups of mutually parallel planes [20]

Each atom plane serves as a kind of semipermeable mirror that partially reflects incident X-rays. X-ray are much more penetrating than visible light, thus they can penetrate into the crystal and reflect also from the external crystal planes. If X-ray beam is incident on crystal at specific angle θ , it reflects interferentially from parallel lattice planes of interplanar spacings d . Incident and reflected ray form with each other angle 2θ (Fig. 2).

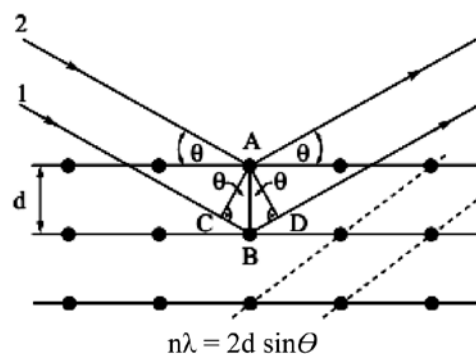


Fig. 2. X-ray reflection from parallel atom planes [21]

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Key:
 d - spacing between adjacent lattice planes in the crystal,
 θ - incident/reflected angle defined as an angle between X-ray beam and crystal plane (differently than in optics),
 λ - X-ray wavelength ($\lambda \leq 2d$),
 n - spectrum order (integer; it shows how many times the wavelength λ is in the path difference ΔS ; $\Delta S = AB + BC$).

Rays reflected from subsequent planes constructively interfere only when the path difference ΔS is equal to integer multiple of X-ray wavelength λ ($\Delta S = n\lambda$, $n = 1, 2, 3, \dots$). Constructive condition occurs when $\Delta S = CB + BD = n\lambda$ and $CB = BD = d \sin\theta$, hence the relationship (1): $n\lambda = 2d \sin\theta$. The formula (1) is known as Wulf-Bragg equation. Knowing the wavelength λ one can calculate the spacing d .

Interference image is a result of superposition of "in phase" reflections of X-rays scattered on several to hundreds of subsequent parallel crystallographic planes. Thus, the total reflected wave is strong enough to cause a measurable result [20]. It must be noted that X-ray radiation does not reflect in the literal sense. Photons are scattering elastically on atoms in all directions. At specific wavelength and angle of incidence, a constructive interference occurs (Fig. 3), but only for specific angles of reflection; one of them is angle of X-ray incidence θ .

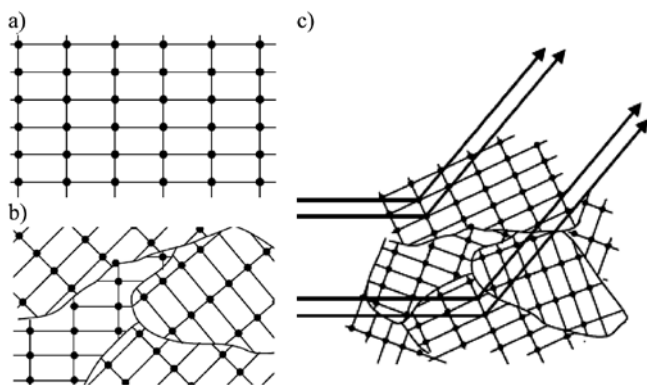


Fig. 3. Lattices of substances: a) forming a monocrystal; b) polycrystalline; c) constructive interference of rays scattered on polycrystalline grains [22]

Monocrystal, in its entire bulk is one lattice (Fig. 3a), where location of any atom can be described as a respective sum of lattice vectors coming from any node of crystal lattice. Polycrystalline substance is a cluster of single crystals – it comprises numerous small crystallites aligned randomly (Fig. 3b). Among numerous crystallites there are always those, which orientation allows to meet condition (1) for specific groups of lattice planes (Fig. 3c). This means that at the specific wavelength λ and interplanar spacing d , angle between direction of incident and reflected beam is equal to 2θ that is determined using equation (1).

Diffraction data is obtained in form of diffraction patterns showing relation between intensity of diffractive reflexes and interplanar spacing d or angle 2θ . Diffraction pattern is specific for substance structure and it is its characteristic and unique image, which is identical both for pure substance and substance present in the mixture. This enables analysis of complex sample composition and determination of phase, in which given substance occurs.

X-ray powder diffraction is employed for analysis of polycrystalline materials. It is used both for phase and structure analysis of polycrystalline substances, including stone, ceramics, construction materials, metal alloys and corrosion products, as well as pigments. As a result it is used in studies of cultural heritage [23]. XRD allows also determination of mineral composition of ashes and other mineral waste (Fig. 4), structural analysis of single crystals, as well as analysis of defects in single crystals and thin layers.

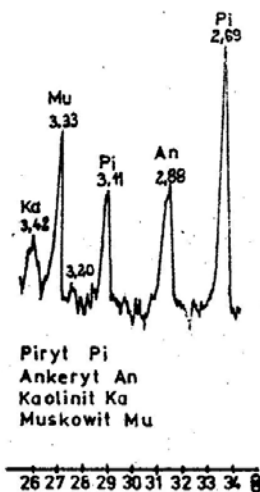


Fig. 4. An example of mineralogical analysis by means of XRD – analysis of mineral composition of waste coal pyrites [24]

Key:
 Pi, pyrite – FeS_2 ,
 An, ankerite – $\text{Ca}(\text{Mg}, \text{Fe})[\text{CO}_3]_2$,
 Ka, kaolinite – $\text{Al}_4[\text{Si}_4\text{O}_{10}](\text{OH})_8$,
 Mu, muscovite – $\text{K}(\text{Al}, \text{Fe}^{3+})_2[(\text{OH})_2\text{AlSi}_3\text{O}_{10}]$,
 Over peaks – values of d ; Å.

Databases are used for identification, mainly ASTM (American Society for Testing and Materials) and JCPDS – ICDD (Joint Committee for Powder Diffraction Standards – International Centre for Diffraction Data).

Diffraction methods can be classified as non-destructive tests, as after the diffraction analysis, the sample can be employed in other measurements. The measurement time is relatively short. Moreover, the sample that not need any special preparation and obtained result can be identified using extensive databases (Powder Diffraction Files). An additional advantage is possibility of conducting tests using microdiffraction technique that allows analysis of few samples, including also ones in the form of e.g. sections prepared for other types of analysis [23].

An example of XRD application for studies of Microbiologically Induced Corrosion

There are numerous methods for diagnosis of MIC. They include: isolation and culture of microorganisms, biochemical testing, testing microbial metabolic activity, applying means of molecular biology, using microscope techniques and chemical analysis of corrosion products [13, 25]. This work focuses on application of XRD for analysis of metal corrosion product influence by microorganisms, mainly bacteria. XRD allows determination of crystal phases of deposits formed at surfaces of corroded metals, which contributes among other to MIC mechanisms (Tab. 1).

The knowledge on MIC mechanisms has increased greatly and has been verified in the recent years. Interesting results were published among others by Enning et al. [26, 27]. By studying corrosion of mild steel products in the presence of BRS, they found mechanism of Electrical Microbially Influenced Corrosion (EMIC). It involves of using electrons from oxidation of metallic iron by some strains of BRS ($\text{Fe}^0 \rightarrow \text{Fe}^{2+} + 2e^-$). The EMIC in the presence of BRS usually generates large quantities of inorganic corrosion products. XRD studies on composition of crystalline phases forming shells on steel sample surface found presence of FeS and other than sulphide minerals, including iron carbonate [26]. The authors [27] ascertain that role of FeS in MIC does not involve its catalytic effect on abiotic cathodic reaction of proton reduction to hydrogen. On the contrary, FeS as semiconductor plays an important role in anaerobic MIC by mediating in the flow of electron stream from metal to BRS cells.

Table I
Indicator minerals in corrosion products of pipelines – for corrosion in soil and laboratory soil tests [25]

Corrosion processes	Corrosion products – colour; chemistry; mineral forms	Corrosion rate, mm/year
Basic corrosion processes		
Abiotic aerobic corrosion (O ₂ serves as electron acceptor in cathodic reaction)	Yellow, orange, brown, black; Fe(III) oxides; lepidocrocite, goethite, magnetite, maghemite, haematite	0.04-0.2
Abiotic anaerobic corrosion (H ⁺ serves as electron acceptor in cathodic reaction)	White, mushy or diffused, Fe(II) carbonate – siderite	0.002-0.01
Anaerobic MIC (caused by BRS; biotic iron sulphides serve as electron acceptors in cathodic reaction)	Black; strongly fragmented; Fe(II) sulphides; amorphous FeS, mackinawite, greigite	0.2 general 0.7 pitting
Secondary changes involving MIC		
Aerobic → Anaerobic MIC (BRS/"FeS")	Fe(II) sulphides; marcasite and pyrite	2-5 ^a
Anaerobic MIC (BRS/"FeS") → aerobic	Elemental sulphur, Fe(III) oxides; residues of anaerobic corrosion products	
^a This very high rate of corrosion applies only to the period of secondary oxidation in the place of anaerobic corrosion		

Teng et al. [9] employed powder XRD method for studying effect of biofilm on the composition of crystalline phases – corrosion products on surfaces of cast iron pipes used in drinkable water supply networks. XRD tests shown presence of aragonite (CaCO₃), calcite (CaCO₃), maghemite (Fe₂O₃) and goethite [FeO(OH)], while both for samples with and without biofilm, crystalline phases were predominant. Their formation may be related to the metabolic activity of two species of iron bacteria of *Leptospirillum* genus: *L. ferriphilum* and *L. ferrooxidans*. In the presence of biofilm, also change of corrosion rate over time was observed – from its acceleration at the start of experiment to its inhibition after 7 days. This can be a result of change of biodiversity in the biofilm.

The effect of iron bacteria on mineral composition of corrosion products was also studied by Ashassi-Sorkhabi et al. [15]. They worked on carbon steel. The corrosion involved 2 iron oxidising bacterial strains of species *Gordonia* and *Enterobacter*. XRD analysis has indicated presence of iron oxide hydroxide FeO(OH) as a main phase in all test samples, however intensity of diffraction bands and interplanar spacing were different for samples immersed in the medium containing bacteria and for samples immersed in the medium without bacteria. Both properties mentioned above characterise crystal lattice of given phase – hence, in the presence of bacteria or lack thereof different FeO(OH) structures were formed.

While Maruthamuthu et al. [18] studied effect of biocides and MIC inhibitors in pipelines used for transport of diesel oil through northern India. The dried samples of corrosion products collected from various measurement points along the pipeline, as well as taken from lab experiment, were grounded to a fine powder and analysed by means of XRD. It was shown that all the corrosion products contained mainly iron(III) oxides, iron (III) chlorides and composite phases, e.g. iron(III) silicates. However, iron(III) oxide was always the predominant phase, formed due to the presence of iron(II) oxidising bacteria.

AlAbbas et al. [14] employed X-ray diffraction to study effect of BRS (isolated from water samples collected in crude oil shaft) on the corrosion of API 5L X52 steel, which is intended for manufacturing pipes used in transport of crude oil and natural gas. XRD ana-

lysis of corrosion products formed on the surface of studied steel samples showed presence of iron sulphides – FeS and mackinawite (Fe_{1+x}S), as well as siderite (FeCO₃) and iron (III) oxide-hydroxide. The rate of formation of mackinawite was much higher than this would result from typical kinetics of precipitation. This could have been a result of very high activity of H₂ (produced by BRS) towards iron. Mackinawite is not a stable and may dissolve in unsaturated solution. In the case of environment of pH=4-7 (typical for BRS), solutions are usually supersaturated in terms of iron sulphates, hence, mackinawite does not dissolve [28]. In the case of deficiency of hydrogen required for sulphate reduction, BRS can use some carbon sources, such as lactate they convert into pyruvate and further – to acetate and carbonate. Carbonate reacts with Fe(II) ions forming siderite (FeCO₃ – second important corrosion product in the presence of BRS.

The above brief literature review on application of XRD for studies of MIC processes and mechanisms shows that different techniques of X-ray diffraction, mainly powder diffraction, can be useful for obtaining data on MIC.

Summary

Studies of microbiologically induced corrosion (MIC) are essential to cope with its consequences. Losses caused by corrosion in the economy of industrialized countries are enormous. The U.S. Environmental Protection Agency have announced at the end of 20th century that in 20 years costs of service and replacement of only cast iron pipelines in water supply network in the USE will exceed 77 billion dollars [29]. This should be enough incentive to study MIC. Understanding this phenomena is possible among others through experiments involving XRD methods. Application of XRD for MIC studies allows:

- learning MIC mechanisms on the basis of finding crystalline phases being characteristic products of chemical reactions,
- based on the knowledge of crystalline phases present in the sample – determination, whether sample corrosion can be classified as MIC (i.e. if it is a result of microbial metabolic activity),
- determination based on so-called indicator crystalline phases of microorganisms group that may be involved in MIC,
- determination of character of corrosion products formed on the test surfaces, as well as character of protective oxide layers.

The indication of groups of microorganisms involved in the MIC (on the basis of XRD) does not prove the participation of specific microorganisms in corrosion process. These suggestions should be confirmed by microbiological methods and/or methods of molecular biology.

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Dokończenie na stronie 472