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ISOLATION AND IDENTIFICATION OF MICROBIAL STRAINS INVOLVED IN INDUSTRIAL SYSTEMS MATERIALS CORROSION IN AQUATIC ENVIRONMENT

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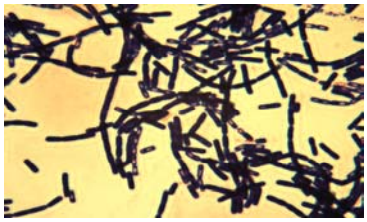
The accumulation of microorganisms and metabolic products on metal surfaces contributes to the corrosion process, which reduces the lifetime of industrial materials. Corrosion is rarely related to a single mechanism or to one species of microorganisms. The experimental investigation has been done with the aim to identify the bacterial community grown on metallic surfaces of industrial aggregates immersed in aquatic environment. The isolation and identification procedure finally provided four predominant bacterial strains as *Pseudomonas koreensis, Pseudoxanthomonas mexicana, Brevibacillus parabrevis* and *Bacillus pseudomycoides*



INTRODUCTION

Industrial sectors use various materials to ensure the transport of water, gas or other fluids. Besides the efforts to design new protective materials these aggregates and pipelines are suffering corrosion process. Corrosion is a complex phenomenon induced by physical, chemical, and biological water quality parameters, as well as hydrodynamic conditions imposed by operation conditions.

Microbial induced or influenced corrosion (MIC) is defined as the deterioration of a material, usually a metal, by processes which occur either directly or indirectly, as a result of bacterial activity.[1] Due to the enormous losses produced by microbial induced corrosion, acquiring of new information are fundamental for understanding and minimizing the negative effect of microbial community growing on surface materials.

In aquatic environment, metal surface are colonized by biofilms, a complex microbial structure with stratified structure having relative uniformity and different thickness. According to Stratman *et al*.,[2] biofilms are dynamic systems consisting in living cells trapped in a heterogeneous matrix that contain extracellular polymeric substances (EPS), adsorbed organic and inorganic substances, and interspersed with interstitial voids. Biofilm is characterized by porosity, strength, hydrodynamics and mass transportation phenomenon and mediates the interaction between metal surfaces and the aqueous solution leading to modifications of the metal/solution interface.

The presence of microorganisms modifies the chemistry of near-surface environment and may also interfere with the electrochemical processes occurring at the metal-environment interface.

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Environmental conditions (physical and nutritional) and interbiotic factors influence the composition of microbial layer, but there are similarities in existing consortia of species. Among the microorganisms, bacteria, fungi, algae, yeasts and even protozoa are present, but predominant are considered to be fast growing bacteria such as species of *Pseudomonas, Aerobacter, Alcaligenes, Arthrobacter, Proteus, Bacillus* and others.[3-5]

The aim of this study was to identify the predominant microbial strains from biofilm growing on surface of stainless steel aggregates. Microbial samples were collected from a hydroelectric industrial unit affected by microbial corrosion and presenting biofilm formation. The characterization of microorganisms that influence corrosion has significant implications for distribution system management and material corrosion control.

EXPERIMENTAL

Reagents and chemicals

K_2HPO_4 – UCB, Belgium; $MgSO_4.7H_2O$ – Merck, Germany; $CaCl_2.2H_2O$, $NaNO_3$, NH_4NO_3 and $FeSO_4$ – Chimopar, Roumania; ammonium iron citrate – Merck, Germany; HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer – Sigma, Germany; peptone – Oxoid Limited, England; yeast extract- Sigma, Germany.

Microorganism isolation

Microbial samples were taken in sterile conditions from three industrial work sites situated on running water.

Microorganism cultivation

The samples were cultivated on Winogradski and MPSV liquid and solid media. The cultivations on liquid media were carried out in agitated flasks on orbital shaker (Fig. 1).

The growing microbial strains were cultivated in Petri plates on solid media at 37° C for 10 days. Composition of Winogradski medium (g/L):[6, 7] K_2HPO_4 0.5; $MgSO_4.7H_2O$ 0.5; $CaCl_2.2H_2O$ 0.2; $NaNO_3$ 0.5; NH_4NO_3 0.5; ammonium iron citrate 6.0. The pH was adjusted at 4.8. Composition of MPSV medium[7] (g/L): HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid buffer) 10mM; peptone 2.0; $FeSO_4$ 0.001; yeast extract 0.5; $MnSO_4.4H_2O$ 0.2; pH 7. For solid medium 20 g/L agar was added. The first growth elements on solid medium were observed after 24 hours of incubation and these were used for further purification. A serial dilution was carried out on relevant microbial samples. About 1 g of sample collected from biofilm was introduced in 90 mL of distilled water and shaken thoroughly to obtain a homogenous mixture (aliquot). About 1 ml of aliquot was then transferred into 9 mL of distilled water and shaken until homogeneity. This process was repeated in few successions to obtain adequate diluents used for cultivations. After 10 days the discrete colonies were analyzed by Gram staining/coloration. All the determinations were done in triplicate.

Optical observations

The morphological analysis of microbial cultures were done using microscope Olympus BX 51.

Microorganism identification

Isolated colonies were identified by System for taxonomic identification of the microorganisms, MicroLog™ MicroStation™ from BIOLOG Inc. (USA) with GEN III MicroPlate (for both Gram-negative & Gram-positive bacteria).

RESULTS AND DISCUSSION

Most metals and their alloys (including stainless steel, aluminum and copper alloys), polymers, ceramic materials, and concrete can be attacked by microorganisms. Installations buried or immersed in water environments can be expected to suffer badly from microbiological corrosion. Microorganisms are able of depositing iron/manganese hydroxides (rust) on metallic materials and facilitate the degradation and corrosion, especially in case of metal passivity.

a) b)

Fig. 1 – Cultivations of microbial isolates on liquid media:
a) microbial cultures performed in Erlenmayer flasks; b) orbital shaker Heidolph Unimax 1010.

Microbial corrosion phenomenon was observed in all three investigated sites indifferent of flow conditions. Several microbial samples were collected from relevant points of aggregates, such as corner sites, welded points or joints (Fig. 2).

The synergistic effect of different microbes and the degradation produced by corrosion processes, including MIC, electrochemical process etc, are presented in the Fig. 2: biofilm adherent to metallic surface (Fig.2 a-d), formation of pitting zones (Fig. 2 e, f), caves and holes.

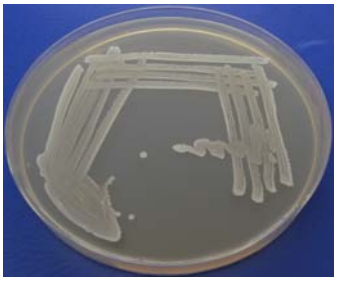
Fig. 2 – Photographs of biofilms (microbe slimes) grown on metallic surfaces: a) biofilm grown on surface of padle wheel; b) biofilm grown on surface of padle wheel; c) biofilm grown on aggregates (white arrow – colecting point); d) biofilm grown on tappered aggregate (site III) (white arrow – colecting point); e) corroded zones of hydro-aggregates; f) damages of as a result of corrosion (white arrow – "tubercules"); g) biofilm and pit scraped from colecting point of aggregates; h) biofilm and pit scraped from colecting point of aggregates.

a)

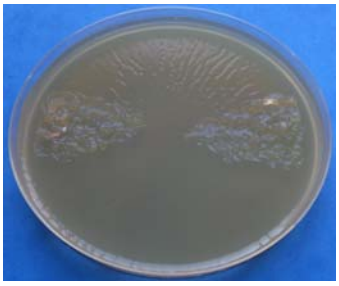
b)

c)

d)



e)



f)

g)

Fig. 3 – Solid cultures of collected and isolated microorganisms: a) MS1 sample grown on MPSV solid medium; mucoid and transparent colony with smooth surface and plate profile, white-yellow; b) MS1 sample grown on Winogradski semi solid medium; slime colony colored by rufous pigment; c) MS2 sample grown on MPSV solid medium; mucoid colony with integer edges and smooth surface; brown color in light; d) MS2 sample grown on Winogradski semi solid medium; slime colony colored by rufous pigment; e) MS3 "magnet" sample grown on MPSV solid medium inoculated from culture on Winogradski liquid medium; circular and mucoid colony with rough surface and intact edges; white color; f) MS3 sample grown on Winogradski semi solid medium; insignificant colony growth, pigment absent; g) sample MS 4 grown on MPSV solid medium; mucoid filamentous colony with smooth surface and branched edges, irregular profile, white color.

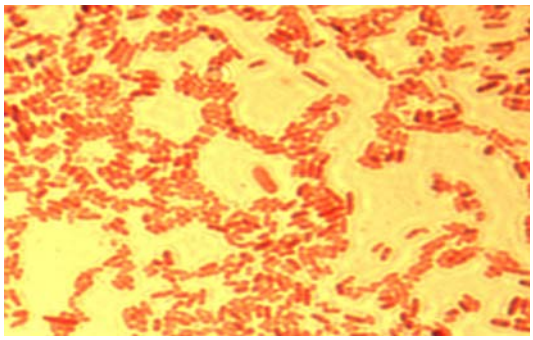
In corrosion process, iron/manganese-oxidizing bacteria are among the most dangerous microorganisms. These bacterial strains are capable of depositing metal, iron/manganese on metal surface producing damages. Hence, isolation for iron oxidizing bacteria was made by Winogradski nutrient medium and, for manganese-oxidizing bacteria with MPSV nutrient medium, respectively. The isolation process leads to 15 microbial isolates collected from different points. Among them, few microbial strains were found to be similar to those obtained from other collecting points or duplicate of its. These strains were excluded from further investigation. Finally, the isolation procedure provided 4 main microbial strains (MS1-MS4 samples; MS4 sample collected by scraping metallic surface with a magnet). These strains were further characterized by the morphological study of culture – color of culture, topography (flat or heaped), texture (granular, velvety, cottony etc.) – and microscopic analysis of developed colonies in Petri plates on solid media. Fig. 3 presents the aspects of isolates cultures grown on solid media in Petri plates.

An attempt was made to identify the four microbial strains. Their metabolic profile was carried out based on the results obtained by using different carbon substrates on Biolog (Biolog, USA) on GIII plates, according to manufacturer's instructions. Differential properties of the isolates suggest the assignment of strains MS1, MS2, MS3 and MS4 to the genera *Pseudomonas, Pseudoxanthomonas*, *Brevibacillus* and *Bacillus,* respectively (Table 1).

Table 1

Identification of microbial strains isolated from corroded metals

Identification	Code	Taxonomical group
Pseudomonas koreensis	MS1	Gammaproteobacteria
Pseudoxanthomonas mexicana	MS2	Gammaproteobacteria
Brevibacillus parabrevis	MS3	Firmicutes
Bacillus pseudomycoides	MS4	Firmicutes



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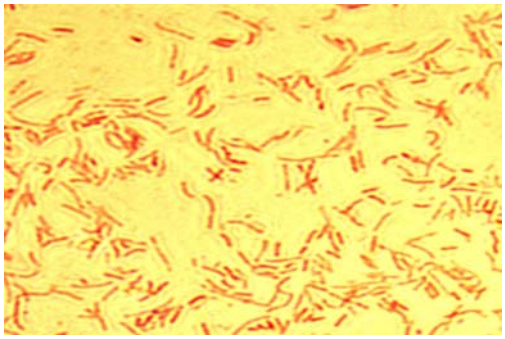

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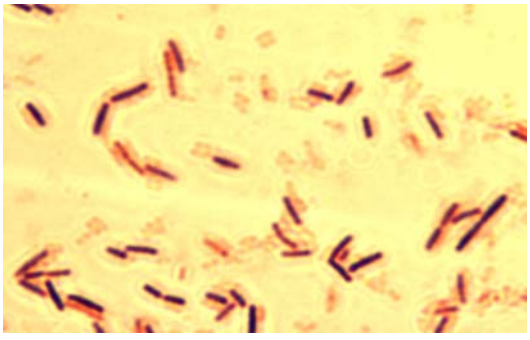

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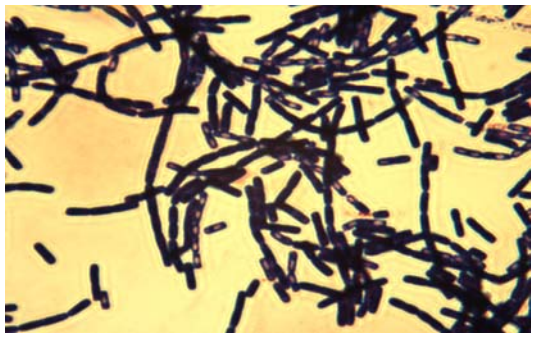

d)

Fig. 4 – Morphology of microbial isolates from biofilm growing on metallic surface: a) microscopic feature of sample MS1 identified as *Pseudomonas koreensis*; Gram negative bacteria*;* x 100; b) microscopic feature of sample MS2 identified as *Pseudoxanthomonas mexicana*; Gram negative bacteria; x 100; c) microscopic feature of sample MS3 identified as *Brevibacillis sp*.; Gram positive bacteria; x 100; d) microscopic feature of sample MS4 identified as *Bacillus pseudomycoides;* Gram-positive with short chains of rods; x 100.

Simultaneously the samples were analyzed by Gram staining/coloration and the morphology observed using optical microscope (Fig. 4).

The microbial strains identified by our work as dominant in biofilms grown on metallic surfaces belong to genera that are often cited in the literature as causing problems in industrial systems. *Pseudomonas* genus is considered to be pioneer colonizers in the process of biofilm formation and often found in the primary stage of biofilm formation in aquatic environments. *Pseudomonas aeruginosa* is present in environmental waters, especially in those associated to human activity and is able to colonize the Ni-Co surfaces immersed in water.[8] *P. aeruginosa* forms a biofilm layer on the Ni-Co alloy surface, even though cobalt is toxic to a variety of microorganisms.[9] A *Pseudomonas* strain was described as dominant bacteria in the micro colony grown on aluminum alloy in natural aqueous environment.[10] The colonization of *Pseudomonas* bacteria on the alloy surface has produced changes in elemental composition as the depletion of iron and the enrichment of chromium as compared with controls.[11] *Pseudomonas koreensis* produces biosurfactants that facilitate the adherence to metallic surfaces in aquatic environment systems.[12]

Pseudoxanthomonas sp was reported as a novel manganese oxidizing bacteria having a key role in the corrosion of carbon steel producing a MnO_2 depot on metallic surface.[13] Also, *Pseudoxanthomonas* was found in bacterial community grown on metallic surface submerged in aquatic environment.[14]

Several bacteria species such as *Meiothermus silvanus, Pseudoxanthomonas taiwanensis, Deinococcus geothermalis*, *Bacillus sp.* and *Brevibacillus sp.* were found in microbial accumulation on steel surface.[15]

CONCLUSIONS

The effective prevention and control of MIC can be achieved through proper characterization of the microorganisms and understanding their specific role in corrosion processes. Involvement of microorganisms and microbial metabolism accelerate the deterioration of industrial water system producing considerable damages, and finally destruction of the industrial water systems. The experimental investigation has been done with the aim to characterize the bacterial community grown on metallic surfaces of industrial aggregates immersed in aquatic environment. The isolation and identification procedure finally provided four predominant bacterial strains*, Pseudomonas koreensis, Pseudoxanthomonas mexicana, Brevibacillus parabrevis* and *Bacillus pseudomycoides*. It can be concluded that identification of microorganisms involved in MIC represent the first step in a complex and complicate attempt to effective control and prevention of the corrosion process.

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