Microbial Diversity of Biofilms on Metallic Surfaces in Natural Waters

Case Study in a Hydropower Plant on Amazon Forest

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***Abstract*-The Balbina Hydroelectric Power Station is located in the central Brazilian Amazon Forest and is vulnerable to biofouling and biofilm formation on immersed metal surfaces because of the high ambient temperature near equatorial line. Microbiologically influenced corrosion (MIC) comprises organic and inorganic processes that occur simultaneously or separately and enhance the harmful effects of the dissolution of metal surfaces. Several types of microorganisms are involved in this process, and microbial colonisation of surfaces through biofilm maturation leads to biocorrosion. Each stage of MIC has characteristic microorganisms, and the present study aimed to characterise the different microorganisms found in samples collected from carbon steel, stainless steel, and copper alloy coupons exposed to the water reservoir of the Balbina Hydroelectric Power Station. Microorganisms described in the literature as important for MIC were detected using microbiological tests and molecular tools. In Brazil, 72% of the energy-based power generation is derived from hydropower plants; hence, it is important to study the origin of corrosion on metallic surfaces exposed to the water of the reservoir.**

Keywords- Biofilm; Amazon Biodiversity; Biocorrosion; Biofouling

I. INTRODUCTION

The northern region of Brazil has suitable climatic conditions for the development of biofilms on surfaces immersed in natural waters [1]. Biofilms are generated by the growth of surface-associated microbial consortia and production of extracellular polymeric substances (EPS) by these microorganisms; it is estimated that almost all species of microorganisms on Earth live, at least for a period in their life cycles, in such communities [2]. This phenomenon often leads to irreversible attachment of cells to the surface, which comprises inorganic precipitates derived from the bulk aqueous phase and corrosion products of the metal substratum. Microorganisms that form biofilms and are involved in biocorrosion often have diverse in characteristics and physiology, and they pose a threat to metal alloys [3].

The first stage of biofilm development is the adsorption of organic material on metallic surfaces [4-7]. The second stage involves the transport of cells and nutrients to sites where bacterial adhesion has already initiated. The genus *Pseudomonas* is widely described as a biofilm precursor, as it generally secretes exopolymers that permit its adhesion to different surfaces [5, 7]. These bacteria are also involved in an oxidative metabolism, which can be associated with biofilm formation [8]. Fe^{2+} release on mature, established biofilms permits iron-oxidizing bacteria to grow in this environment. Sulphate-reducing bacteria (SRB) are only capable of developing later, when anaerobiosis is established through the development of micro-environments. Iron-oxidizing bacteria decompose iron oxides and produce corrosion spots, thereby generating micro-environments that lack oxygen and are finally occupied by SRB [9, 10].

These precursor bacteria can produce a suitable environment for the adhesion of other microorganisms such as fungi, which are widespread in nature and can grow on several substrates and environments [11]. These microorganisms are regarded as colonisers in biofilm establishment on solid surfaces [12, 13]. Many fungal species metabolise organic compounds such as wood and paint and rubber polymers [14], thereby producing organic solvents as acids and alcohols that can promote biocorrosion.

The identification of microorganisms in biofilms established on immersed metals is essential for assessing the microbial diversity in this region of Brazil, and it can help elucidate the biocorrosion process, which is common in hydroelectric power stations. The corrosion and weathering triggered by the formation of biofilms can lead to a reduced efficacy of heat exchangers, unexpected corrosion of stainless steel, and premature destruction of mineral materials [3].

Biocorrosion affects several industrial sectors such as: general industries (i.e., Shipbuilding, petrochemical, bioprocess, chemical, refineries), buried pipes, seals fuel tanks on airplanes and ships, power generation plants (i.e., Thermoelectric, hydroelectric, nuclear). Yet it is estimated that 20% of the deterioration of metallic surfaces is originated from biological

processes related to factors inherent to electrochemical corrosion [3]. The biofilm development and the control of biocorrosion process in hydroelectric power station in Brazil are based on systems that use chemical compounds as chlorine to avoid clogging of the cooling system which can interrupt the electricity generation as a result of the overheat. Nowadays many companies are searching substitutes for these compounds because the contact of them with organic material shall influence the formation of trihalomethanes, which are a group of organic compounds derived from methane. The trihalomethanes are composed of three molecules of hydrogen replaced by the same number of halogen atoms such as chlorine, bromine and iodine [15-18]. These compounds are known for their carcinogenic and toxic potential [19, 20], therefore it is extremely important to choose the correct microbiological control agent [20, 21]. It is also important to characterize the type of biofilm formed in the environment which the hydroelectric power station is operating in order to ensure the efficiency of the cooling system and consequently maintaining a good water quality of the regional rivers that are used by the population.

This work aims to identify bacteria and fungi from biofilms on the surface of the metal coupons submerged in the water reservoir Balbina Hydroelectric Power Station in Presidente Figueiredo (AM), Brazil. Evaluate the microbial diversity in this environment, the biological and ecological as well as economic impact. The elucidation of the process biocorrosion can provide strategies to minimize the financial losses [24].

II. MATERIAL AND METHODS

A. Materials and Exposure Conditions

Carbon steel 1045, stainless steel 304L, and copper alloy coupons were settled in acrylic boxes and placed at 2 different locations at the Balbina Hydroelectric Power Station in Presidente Figueiredo City (AM) Brazil: Point 'A' was at a depth of 10 m, located near the bottom of the reservoir, and Point 'B' was at a depth of 10–15 m, located far away from the bottom of the reservoir (Fig. 1). After exposure to water for 75, 150, and 225 days in April 2007, June 2007, and September 2007, respectively, the metal samples were collected and analysed.

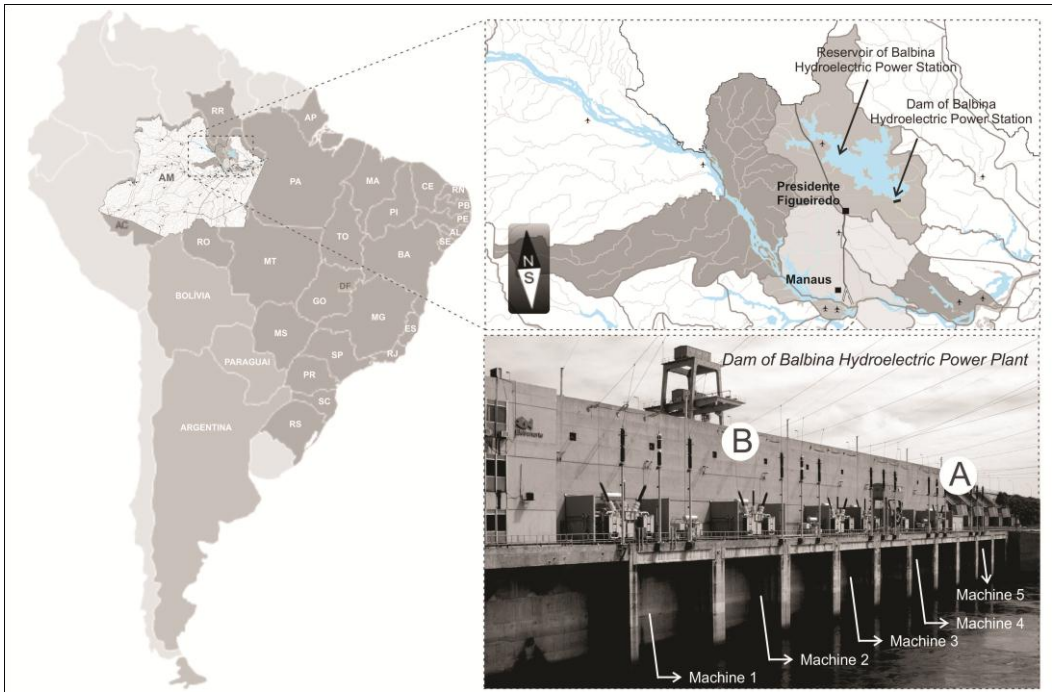

Fig. 1 Balbina hydroelectric power plant, geographical location. Collection Points A and B were located at the dam's hydroelectric power plant, respectively at Machines 2 and 5

B. Inocula Preparation

Corrosion spots on each metallic coupon were separately removed (1 g) using a sterile glass slide, and 9 mL of sterile 0.85% NaCl was added and carefully mixed.

C. Isolation of Microorganisms

Aliquots of the suspensions drawn from the corrosion spots (100 μL) were placed in Petri dishes containing potato dextrose agar (PDA; Acumedia) supplemented with streptomycin (40 μg·mL^{-1}) for fungal isolation and incubated at 28 ℃, for 5 days. For the isolation of aerobic and facultative anaerobic bacteria, the samples were placed in tryptic soy casein medium (Acumedia) and incubated at 28 ℃, for 3 days. The surface spread technique was used, and microorganisms were grown in a B.O.D. incubator. The fungal and bacterial colonies obtained were isolated on fresh PDA and nutrient agar plates (Merck),

respectively, and incubated as described above.

D. Identification of Microorganisms by Morphological and Biochemical Characterization

1) Aerobic and Facultative Anaerobic Bacteria:

Aerobic and facultative anaerobic bacteria were first characterised by the Gram stain test. Gram-positive and gram-negative bacteria were identified by evaluating their morphologic characteristics and by performing biochemical tests [8].

2) Iron-oxidizing Bacteria:

Microbial samples (0.1 mL) were inoculated in Leathen-McIntyre-Braley medium (NH_3SO_4, 0.15 g Ł$^{-1}$; $CaNO_3$, 0.01 g Ł$^{-1}$; K_2HPO_4, 0.05 g Ł$^{-1}$; $MgSO_4$, 0.5 g Ł$^{-1}$; KCl, 0.05 g Ł$^{-1}$; 10% $FeSO_4.7H_2O$, 10 mL; pH 3.5) that had been filtered through a 0.45-mm Millipore filter. The samples were incubated at 30 ℃ for 15–20 days. Subsequently, 0.2 mL of 1% K_4 [$Fe(CN)_6$] was added to each tube for detecting the presence of insoluble Fe^{2+}, where a positive reaction resulted in the development of a Prussian blue colour. Before confirmation of the presence of microorganisms, Gram staining was performed, and the cell morphology was evaluated [25].

3) Sulphate-reducing Bacteria (SRB):

A system to ensure anaerobic conditions was set up as described by Rodriguez-Cavallini and Cruz [26]. Samples (1 mL) were inoculated in the selective medium described by APHA AWWA WPCF [25]. The medium had previously been transferred to tubes with rubber lids perforated by metallic needles and autoclaved for 15 min at 120 ℃.

4) Morphological Characterization of Fungal Isolates:

Macroscopic characteristics were used to cluster fungal isolates into various morpho-types. The microorganisms were identified using the method described by Kern and Blevins [27], which permitted the analysis of fungal reproductive structures by optical microscopy [28, 29].

E. Identification of Microorganisms by Molecular Characterization

1) Sulphate-reducing Bacteria (SRB):

a) DNA Extraction:

The total genomic DNA was obtained from the corroded metallic coupons and extracted by scraping the metal surface using a MoBio Power Soil DNA isolation kit (MoBio Laboratories, Inc., Solana Beach, CA). The DNA concentration of the samples was determined using a ND-1000 UV-Vis spectrophotometer (NanoDrop Technologies, Inc., Wilmington, DE, USA), and the copy number was calculated on the basis of the measured DNA concentration (ng/mL).

b) Specific PCR Amplification:

The purified genomic DNA obtained from the metallic coupons was amplified by PCR employing primers (Table 1) specific for genes that encode 16S ribosomal RNA. These primers enabled us to identify several genera of SRB [30]. The PCR products were electrophoresed on a 1.5% (w/v) agarose gel in 1 × TRIS/acetate/EDTA and stained with ethidium bromide (2 μg/mL). DNA bands were visualised by UV illumination.

TABLE 1 16S RDNA-TARGETED PCR PRIMER SEQUENCES SPECIFIC FOR SULPHATE-REDUCING BACTERIA (SRB) SUBGROUPS (DALY *ET AL.*, 2000) EMPLOYED IN THIS WORK FOR THE DETECTION OF SRB ON METALLIC SURFACES AT THE BALBINA HYDROELECTRIC POWER STATION, PRESIDENTE FIGUEIREDO (AM), BRAZIL (2007)

Primer pair	Sequence	Product size 5′ – 3′ (bp)*	Specificity	Genera
DFM140	TAG MCY GGG ATA ACR SYK G	700	Group 1	*Desulfotomaculum* sp.
DFM842	ATA CCC SCW WCW CCT AGC AC			
DBB121†	CGC GTA GAT AAC CTG TCY TCA TG	1120	Group 2	*Desulfobulbus* sp.
DBB1237	GTA GKA CGT GTG TAG CCC TGG TC			
DBM169	CTA ATR CCG GAT RAA GTC AG	840	Group 3	*Desulfobacterium* sp.
DBM1006	ATT CTC ARG ATG TCA AGT CTG			
DSB127.	GAT AAT CTG CCT TCA AGC CTG G	1150	Group 4	*Desulfobacter* sp.
DSB1273	CYY YYY GCR RAG TCG STG CCC T			
DCC305	GAT CAG CCA CAC TGG RAC TGA CA	860	Group 5	*Desulfovibrio* sp.
DCC1165	GGG GCA GTA TCT TYA GAG TYC			*Desulfosarcina* sp. *Desulfococcus* sp., *Desulfonema* sp.
DSV230	GRG YCY GCG TYY CAT TAG C	610	Group 6	*Desulfovibrio* sp.
DSV838	SYC CGR CAY CTA GYR TYC ATC			

*Ambiguities: R (G or A); Y (C or T); K (G or T); M (A or C); S (G or C); W (A or T).
†Primer DSB127 was derived from probe DSB129, which was described by Devereux et al. (1989) Daly et al. (2000).

c) rDNA Sequencing:

To confirm the genetic identity of the DNA fragments generated by PCR using primers specific for SRB, DNA sequencing was performed using the same pair of primers (Table 1) [31]. PCR was performed in a 10-μL volume of a reaction mixture containing sterile distilled water, 0.5 μL of PCR buffer (10×, Applied Biosystems), 0.5 μL of primers (50 pmol), 0.5 μL of Big Dye (Applied Biosystems), and 1 μL of the PCR products. Thirty-five cycles were performed, each consisting of 96℃ for 10 s (denaturation), 50℃ for 5 s (annealing), and 60℃ for 4 min (extension), with a 60 s initial and terminal delay. Sequencing was performed on an ABI 3130 automatic sequencer (Perkin-Elmer, Massachusetts, USA).

d) Sequence Assembly and Alignment:

Sequences were edited using BioEdit 7.0 [32]. ITS sequences were aligned on the basis of similarity using the sequence editor CLUSTAL-W 1.7 [33]. Sequence analysis was performed using the sequence alignment software BLASTn and run against the NCBI database [34], and the determined sequences were aligned using CLUSTAL-W 1.7.

2) Fungal Isolates:

a) DNA Extraction:

Fungal isolates were grown on PDA medium for 7 days at 28℃. The mycelium was ground with a mortar and pestle. Genomic DNA was obtained according to methods described by Vicente [35].

b) rDNA ITS Sequencing:

The contiguous ITS-I (DNA internal transcribed spacer), 5.8S, and ITS-II were sequenced using the conserved primers ITS1 (TCCGTAGGTGAACCTGCGG) and ITS 4 (TCCTCCGCTTATTGATATGC) [36]. PCR was performed in a 10-μL volume of a reaction mixture containing sterile distilled water, 0.5 μL of PCR buffer (10x, Applied Biosystems), 0.5 μL of primers (50 pmol), 0.5 μL of Big Dye (Applied Biosystems) and 1 μL of PCR products. Thirty-five cycles were performed that consisted of 96℃ for 10 s (denaturation), 50℃ for 5 s (annealing) and 60℃ for 4 min (extension), with a 60 s initial and terminal delay. Sequencing was performed on an ABI 3130 automatic sequencer (Perkin-Elmer, Massachusetts, USA).

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Sequences were edited using BioEdit 7.0 (Hall, 1999). ITS sequences were aligned on the basis of similarity by using the sequence editing software CLUSTAL-W 1.7 [33]. Sequence analysis was performed using the sequence alignment software BLASTn and searched against the NCBI database [34], and the determined sequences were aligned using CLUSTAL-W 1.7.

III. RESULTS AND DISCUSSION

The carbon steel coupons showed more corrosion spots than the stainless steel and copper alloy coupons evaluated under the same conditions. Iron oxide was deposited on the metallic surfaces where the biofilms were established and spots of corrosion were also observed. The stainless steel and copper alloys did not show any visible changes in the metallic surface, although biofilms were detected.

Several families of bacteria were found on the metallic surfaces. The family Pseudomonaceae was represented by *Pseudomonas cepacia*, *P. maltophilia*, *P. aeruginosa*, and *P. fluorescens*, which are known to be associated with biocorrosion [37]. Two species of Enterobacteriaceae, *Enterobacter amnigenus* and *Shigella dysenteriae*, were also found.

Bacillus brevis, *B. fastidiosus*, *B. licheniformis*, and *B. stearothermophilus* from Bacillaceae as well as *Acinetobacter haemolyticus* from Moraxellaceae were also identified; the latter species is known to be responsible for biofilm colonization and for spreading antibiotic resistance because it is able to conjugate with other species that produce biofilm [38]. According to Bermount-Bouis et al. [39], Enterobacteriaceae could be precursors in the colonization of metallic surfaces and may induce biofilm formation. Microorganisms from the genera and families mentioned above are known as slime producers because their EPS are abundant and contribute to biofilm formation on metallic surfaces. These exopolymers can also protect against environmental conditions when they form a protective gel-like matrix around the attached cells [3, 40]. The types of bacteria involved in corrosion at metallic surfaces coexist in biofilms and demonstrate a co-operative metabolism, thereby acting as a multicellular organism [41] that is controlled by quorum-sensing molecules [42].

Iron-oxidizing bacteria and SRB were observed by conventional methods in the carbon steel coupons only after 150 days of exposure; these microorganisms were not observed in the copper and stainless steel coupons. However, by using gene-specific PCR for the detection of SRB, we were able to detect *Desulfobulbus* sp. and *Desulfovibrio* sp. after 150 days of exposure. Other genera of SRB were observed only on carbon steel at collection Point 'A' and included *Desulfobulbus* sp., *Desulfovibrio* sp., *Desulfosarcina* sp., *Desulfococcus* sp., and *Desulfonema* sp. (Fig. 2).

Fig. 2 Band patterns obtained by PCR amplification of DNA samples obtained from collection 1 (75 days after exposure) with the pairs of primers described in Table 1. A2 ban: approximately 1120 bp corresponding to group 2 (*Desulfobulbus* sp.); A5 band: approximately 860 bp corresponding to group 5 (*Desulfovibrio* sp., *Desulfosarcina* sp., *Desulfococcus* sp., and *Desulfonema* sp.); A6 band: approximately 610 bp corresponding to group 6 (*Desulfovibrio* sp.)

Note: Molecular weight marker (L): 1-kb DNA ladder (Invitrogen). The letters and numbers represent: 'A', carbon steel at point A; 'B', carbon steel at point B; 'C', carbon steel at point C; 'D', stainless steel at point A; 'E', copper alloy at point A; and 'F', negative control reaction; the numbers correspond to the respective groups of primers in Table 1.

The Fe^{2+} deposition on the mature established biofilm demonstrates that iron-oxidizing bacteria, such as *Leptospirillum ferriphilum* and *L. ferrooxidans*, can grow in this environment and the iron deposition is influenced by SRB [43]. Iron-oxidizing bacteria can use the iron oxides from the metallic surfaces as electron donors and generate micro-environments that lack oxygen and are occupied by SRB [9, 10]. The SRB are represented by anaerobic bacteria that can reduce sulphate, sulphite, thiosulphate, and sulphur to sulphide [44].

After the establishment of biofilm by precursor bacteria, favourable conditions are generated, which permit the adhesion of other microorganisms such as fungi to the surface. Given their various metabolic pathways, fungi are especially adapted for growth on surfaces, primarily because of their absorptive nutrition mode and secretion of extracellular enzymes that are able to degrade complex molecules [45]. The production of organic acids on the surfaces leads to corrosion [14], and these substances can intensify the surface corrosion when deposited on metallic surfaces in addition to stimulating cell adhesion through electrostatic attraction. The metal/solution interface is also modified to release Fe^{2+} [46], which is the required substrate for iron-oxidizing bacteria.

Fungal isolates were obtained from all metallic coupons that showed corrosion spots after 75 days of exposure. The genera observed were *Aspergillus* sp., *Paecilomyces* sp., *Penicillium* sp., and *Trichoderma* sp. Fungal species were identified by sequencing of ITS1, 5.8S rDNA, and ITS2. All sequences obtained were deposited in GenBank according to the access numbers described in Table 2.

TABLE 2 FUNGAL ISOLATES FROM METALLIC COUPONS IMMERSED IN THE WATER RESERVOIR OF THE BALBINA HYDROELECTRIC POWER STATION AT PRESIDENTE FIGUEIREDO/AM, BRAZIL (2007) WERE IDENTIFIED BY EVALUATING THEIR MORPHOLOGICAL CHARACTERISTICS AND BY COMPARISON WITH ITS1, 5.8S RDNA AND ITS2 SEQUENCES DEPOSITED IN GENBANK (NCBI)

Isolate*	**Source**	**ID morphology**	**Related organism**	**GenBank Accession No.**	**E-Value**	**Identity%**	**GenBank Accession No. of isolates†**
ACBF 002-3	*Carbon Steel*	*Penicillium* sp.	*Penicillium dipodomyicola* strain NRRL 35582 18S	DQ339550	0.0	99%	GQ161752
ACBF 003-1	*Carbon Steel*	*Paecilomyces nivea*	*Byssochlamys nivea* strain CBS 373.70	DQ322220	0.0	97%	GQ229084
ACBF 003-2	*Carbon Steel*	*Penicillium chrysogenum*	*Penicillium chrysogenum*	AF034449	0.0	99%	GQ241341
ACBF 004-1	*Carbon Steel*	*Trichoderma* sp.	*Trichoderma koningiopsis* strain CCF3813	FJ430784	0.0	99%	GQ229070
ACBF 005-2	*Carbon Steel*	*Aspergillus niger*	*Aspergillus niger* strain MPVCT 158	EU440768	0.0	98%	GQ229071
AIBF 001-3	*Stainless Steel*	*Paecilomyces lilacinus*	*Paecilomyces lilacinus*	AB103380	0.0	99%	GQ229072
AIBF 002-1	*Stainless Steel*	*Trichoderma* sp.	*Trichoderma viride* isolate NW537	EU622261	0.0	99%	GQ229073
AIBF 003-3	*Stainless Steel*	*Paecilomyces lilacinus*	*Paecilomyces lilacinus* strain BCC 2012	EU828665	0.0	97%	GQ229074
AIBF 005-1	*Stainless Steel*	*Fusarium solani*	*Fusarium solani* voucher NJM 0271	AY633746	0.0	99%	GQ229075
AIBF 007-2	*Stainless Steel*	*Paecilomyces nivea*	*Byssochlamys nivea* strain BCC 14366	AY753338	0.0	98%	GQ241340
AIF 013-1	*Stainless Steel*	*Aspergillus niger*	*Aspergillus niger* contig An03c0110	AM270052	0.0	99%	GQ229076
LT3 003-2	*Cooper Alloy*	*Aspergillus niger*	*Aspergillus niger* contig An03c0100	AM270051	0.0	99%	GQ229077

LTBF 001-2	*Cooper Alloy*	*Aspergillus sp.*	*Aspergillus sydowii* strain VKM F-968	AM883158	0.0	99%	GQ229078
LTBF 006 B 1	*Cooper Alloy*	*Paecilomyces lilacinus*	*Paecilomyces lilacinus* strain UWFP 674	AY213667	0.0	99%	GQ229079
LTBF 007 1	*Cooper Alloy*	*Paecilomyces lilacinus*	*Paecilomyces lilacinus*	AB103380	0.0	99%	GQ229080
LTBF 008 1	*Cooper Alloy*	*Paecilomyces spectabilis*	*Talaromyces spectabilis* strain CBS 121583	EU037060	0.0	99%	GQ229081
LTBF 011-1	*Cooper Alloy*	*Aspergillus versicolor*	*Aspergillus versicolor* strain NHRC-FE080	AM883156	0.0	98%	GQ229082
LTF 006 A-1	*Cooper Alloy*	*Paecilomyces lilacinus*	*Paecilomyces lilacinus* strain UWFP 674	AY213667	0.0	99%	GQ229083

*All strains were deposited in the fungal collection of LabMicro, Laboratório de Microbiologia e Biologia Molecular, DPAT, UFPR.
†All DNA sequences from isolates obtained in this paper were deposited in GenBank.

ID morphology: identification based on morphological characteristics; Related organism: organisms with ITS1, 5.8S rDNA, and ITS2 sequences similar to the sequence of the fungal isolates obtained in the present study; GenBank Accession No.: reference number of the sequence deposited in GenBank; E-value: parameter that describes the number of hits that can be 'expected' by chance when searching a database of a particular size. This value decreases exponentially with the score (S) that is assigned to a match between 2 sequences. Essentially, the E-value describes the random background noise that exists for matches between sequences; Identity: percentage similarity between 2 DNA sequences; GenBank Accession No. of isolates: reference number of the sequences of the isolates obtained in this study.

Isolates ACBF003-1 and AIBF007-2 (Table 2) were characterised morphologically as *Paecilomyces nivea* (anamorph). However, in GenBank, the DNA sequences were related to strains CBS 373.70 and BCC 14366 of *Byssochlamys nivea*, showing 97% and 98% sequence identity, respectively (Table 1). *B. nivea* is known as the teleomorph of *P. nivea*, and thus, these two species share the same genetic background.

Similar findings were obtained for isolate LTBF008-1 (Table 2), which was identified as *P. spectabilis* (anamorph) and demonstrated 99% sequence homology with its teleomorph *Talaromyces spectabilis*, strain CBS 121583 (Table 2).

Isolates from copper coupons demonstrated a high diversity of fungal isolates, and *P. lilacinus* was isolated from all metallic coupons assayed, whereas *Penicillium chrysogenum* was only isolated from carbon steel 1045 (Table 2). Elvers *et al.* [47] identified *Fusarium solani*, *F. oxysporum*, and *Paecilomyces variotii* in flowing water in photo-processing tanks. Lugauskas et al. [48] isolated several fungal genera such as *Aspergillus* sp. and *Paecilomyces* sp. from polymeric materials. These fungi were described to be attached to polymeric surfaces showing deterioration and thought to be associated with acid production [14] and consequent biocorrosion on metallic surfaces in aquatic, atmospheric, or soil environments [49].

Filamentous fungal biofilms have been described for *Aspergillus niger* cultures grown on a polyester support [50], and phenotypic changes, including increased enzyme production and secretion, were also observed in cultures of *Aspergillus* and *Trichoderma* when they were grown on hard surfaces [51-53].

The adhesion of *Aspergillus niger* spores may cause surface deterioration on different substrates [54], leading to the production of several organic acids. Different enzymes, such as glucose oxidase, inulinase, amylase, and cellulases, have also been described in *Aspergillus* biofilm systems [55-58]. *Aspergillus foetidus* biofilms are able to degrade some plastics under favourable growth conditions [59], and *Aspergillus versicolor* was observed to form biofilms on perlite particles in a packed column reactor where it degraded *n*-alkanes, aromatic hydrocarbons, and carbazoles of petroleum samples [60]. *A. niger* and *A. terreus* were described to remove heavy metals as copper and iron in biofilms established on polyurethane [61].

As described by Marshall et al. [7], Surman et al. [6], Coetser and Cloete [4], and Simões et al. [5], the first stage of biofilm development is the adsorption of the organic material on the metallic surface. In the present study, such adsorption was observed at the corrosion station placed at Point 'A', which received water near the bottom of the reservoir, in contrast to what was observed at the corrosion station at Point 'B', where the water table is relatively higher. This difference probably occurred because the bottom of the reservoir was deeper at Point 'B' than at Point 'A'. Therefore, as expected, higher microbial diversity was observed in coupons placed at Point A, which had abundant organic material because of its low depth. Similar results were described by Roske et al. [62] and Roske et al. [63], i.e. the number of cells increased according to the depth of the man-made reservoir and with the accumulation of sediments and organic compounds.

The transport of cells and nutrients to sites where bacterial adhesion had already initiated represents the next stage related to biofilm establishment. Bacterial strains were isolated from metallic coupons collected after 75 days of exposure. Aerobic and facultative anaerobic bacteria were isolated to obtain a qualitative profile of these microorganisms on metallic surfaces exposed to continuous water circulation.

Additionally, the complexity of mature biofilms and the interactions among different bacterial and fungal species create micro-environments that permit chemical reactions culminating with biocorrosion. Our study shows that different microorganisms contribute to the various steps of biofilm establishment and corrosion in the metal alloys assayed. The

Brazilian Amazon forest is well-known for its high biodiversity, and our findings contribute to the understanding of the microbial diversity in this region.

IV. CONCLUSIONS

Our study reveals the presence of important groups of bacteria that are known precursors for biofilm establishment and also involved in biocorrosion, as well as many fungal species that are associated with biofilms and consequent metal corrosion, on metal surfaces at the Balbina Hydroelectric Power Station in Presidente Figueiredo (AM), Brazil. The data presented provide important information about the high microbial diversity in the Amazon region of Brazil that, perhaps, contributes to the elucidation of the biocorrosion process as well as the determination of the cost required to prevent damage to metallic structures.

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