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## **Bacteriological challenges to asbestos cement water distribution pipelines**

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### **Abstract**

Asbestos cement (AC) pipes were commonly installed in the drinking water distribution systems from the mid 1920s to the late 1980s. In recent years, an increase in the number of water main breaks has occurred in the AC portions of some pipe networks, which can be partially attributed to the corrosion of the aged pipes. This study evaluated the potential role that microorganisms may have played in the degeneration and failure of AC pipes. In this study, a fresh AC pipe section was collected from the distribution network of the City of Regina, Canada and examined for microbiological activities and growth on inside surfaces of pipe sample. Black slime bacterial growths were found to be attached to inner pipe surfaces and a distinctively fibrous internal coating (patina) with iron oxides was formed over the time. The microbial populations inside the patina and the black slime were tested with BART™ testers. Heterotrophic aerobic bacteria (HAB) and slime forming bacteria (SLYM) dominated in both the black growths and inside the patina. Iron related bacteria, denitrification bacteria and sulfate reducing bacteria were also commonly present. Microbial challenge assays were conducted by submerging the cut segments of the AC pipe into selected bacterial cultures for a period of 10 days under both aerobic and anaerobic environments. Weight changes were determined and the surface morphology was examined for each of the assayed pipe segments. Results indicated that acid producing bacteria, SLYM and HAB could facilitate the pipe weight loss under anaerobic environments.

Key words: asbestos cement pipes; drinking water distribution network; bacteriological challenges; biofilms; patina

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### **Introduction**

Asbestos cement (AC) pipe is a concrete pipe reinforced by asbestos fibers, containing approximately 20% asbestos fibers and 80% of portland cement, with or without silica. Free lime, calcium hydroxide,  $\text{Ca}(\text{OH})_2$ , in the AC pipe can reach up to 15.5% of the total weight. These pipes were commonly used for drinking water distribution networks in North America, Australia and European countries primarily from the mid 1920s to late 1980s. AC pipes often form the major portion of the drinking water distribution network. For example, about 35% of the total length of drinking water distribution pipes in the Netherlands consists of AC pipes (Slaats et al., 2004). In the City of Regina, Canada, approximately 65% of all water mains are AC pipes, which have a total length of 535 km (City of Regina, 2000).

AC pipes are susceptible to attack by acidic, soft or sulphate-bearing conveyed water due to the cementitious nature of the pipes. An aggressive drinking water could cause the leaching of free lime and result in a significant loss of calcium (Toft et al., 1984; Millette *et al.*, 1984; Webber *et al.*, 1989) and therefore loss of strength of AC pipes. Severely deteriorated AC pipes also released asbestos fiber into the drinking water and could pose a hazard of malignant tumors of the gastrointestinal tract and other organs in consumers (WHO, 1991; Andersen et al., 1993; Cherubini et al., 1998; Browne et al., 2005). These AC pipes were laid down before the potential environmental, social and health impacts were recognized and evaluated. In recent years, problems associated with the AC pipes have gradually become significant including increases in the number of pipe breaks and failure. Hu and Hubble (2007) found that the causes of the AC water main breaks in Regina were primarily related to the underground soils movements, which were extremely active due to the local soil type and the climates. Loss of calcium from cement is a key factor that can reduce the strength of the AC pipe, leading eventually to increased failures (Slaats et al., 2004). Leaching loss of calcium was observed in the smaller diameter (100--150 mm) AC pipes, where the treated water had a low Saturation Index with low buffer capacity and a relatively long residence time.

Biofilms inevitably develop on the internal surfaces of drinking water pipelines, regardless of the presence of disinfectant residual (Geldreich, 1996). Biofilms are composed of microbial cells that are embedded in an exopolymeric matrix in a manner that makes the enumeration of these microorganisms difficult (Lazarova and Manem, 1995). It has therefore been very challenging to determine the community structure of microorganisms in the biofilms. As a consequence of these limitations, evaluation of microbiologically influenced pipe corrosion rates has been scarce. Previous studies of biofilm formation in distribution pipes have been focused on its effect on bulk water quality, including the bacteria detached from the biofilms, which was shown to cause increased bacterial counts in the drinking water (Van der Kooij, 1992). In some cases, it was believed that the formation of biofilms on corroded pipes might have provided protection to the pipe materials by restricting further oxidation (LeChevallier et al., 1993; Dubiel et al., 2002). In a study of the effect of various pipe materials on fixed bacterial biomass on pipe surfaces, Niquette et al. (2000) determined that the density of bacterial biomass on AC pipe was around  $0.1 \mu\text{g C/cm}^2$ , which was much higher than polyethylene, PVC, cemented steel, cemented cast iron and tarred steel pipes. The microbiological effects on the degeneration of the drinking water distribution network, particularly the AC pipes, still remains largely unknown.

The objective of this study was to evaluate the potential role that microorganisms may have played in the degeneration of AC pipes. This was accomplished using the Biological Activity Reaction Tests (BART™ testers), which were developed by Droycon Bioconcepts Inc, and commercialized by the Hack Company (USA). The BART methods use selective media that are specifically engineered to allow only the bacterial group of interest to grow and the all other bacteria to be suppressed. The activity of the different bacterial communities of interest can be reestablished and examined by the application of a reduction-oxidation gradient (Cullimore, 1999). Detection of bacteriological activity is determined by the time lapse between starting up a test and positive reactions recognized. Time lapse has been shown to link to the total population using the colony forming units generated by streaking agar plates using pure cultures of bacteria.

## 1 Methodology

### 1.1 AC pipe section and morphological examination

In August 2006, a section of AC water distribution main failed on Dutton Crescent in the east end of the City of Regina, Canada. The 150 mm diameter AC pipe was installed in 1971 and had been in service for over 38 years. A pipe sample of 120 cm in length was obtained directly from the site. The pipe sample was removed from the broken pipe section and immediately wrapped with protection plastics and transported to the laboratory. The pipe was handled carefully during sampling by keeping the pipe section horizontal to prevent possible bacteriological contamination and during transportation to avoid getting any materials inside the pipe.

The pipe section was examined for the presence of biofilms and associated microorganisms and visible clusters of biomass that could contain evidence of microbial activities on the internal pipe surface. Black bulbous slime biomasses were recovered from the inside of the pipe, and tested for the presence of iron related bacteria with IRB-BART testers (Fig. 1). Within the pipe it was noted that patina (distinctive surface appearance acquired over time) was consisted of a 3--4 mm thick layer of microbial biomass along with interwoven fibrous materials (Fig. 2).

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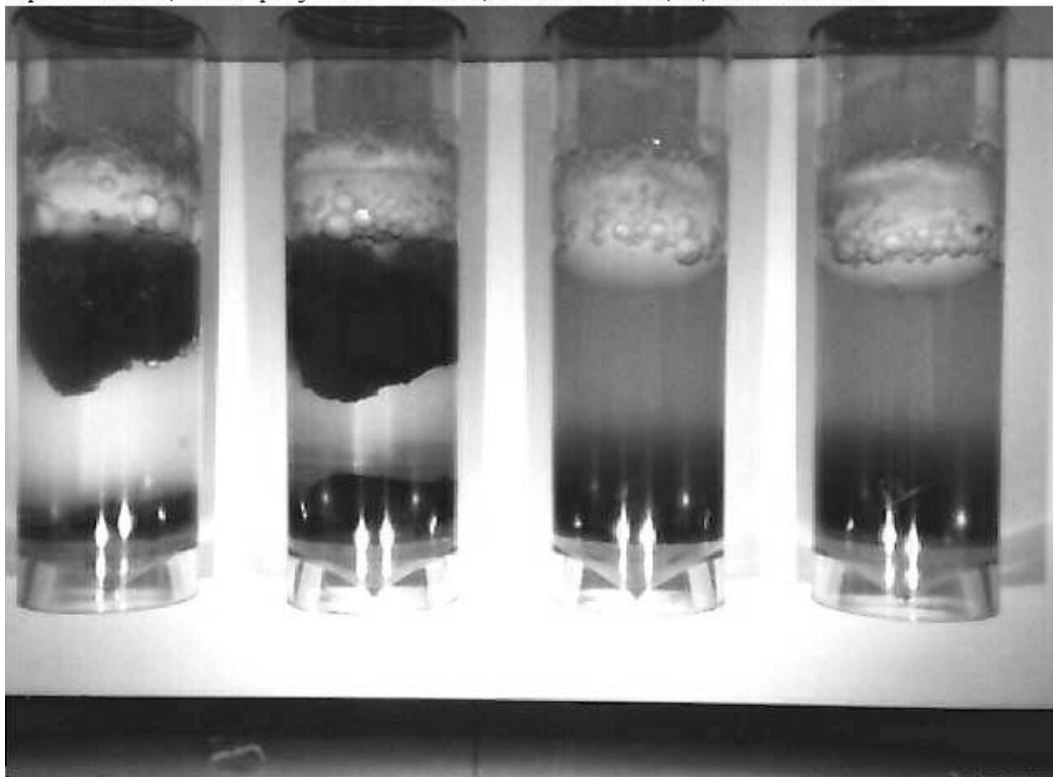


Fig.1 IRB-BART test indicated that the black growths on the inside wall of the AC pipe contained iron related bacteria. The two samples used in the testers on the left came from black growths attached to the inside of AC pipe. The two samples on the right were controls.

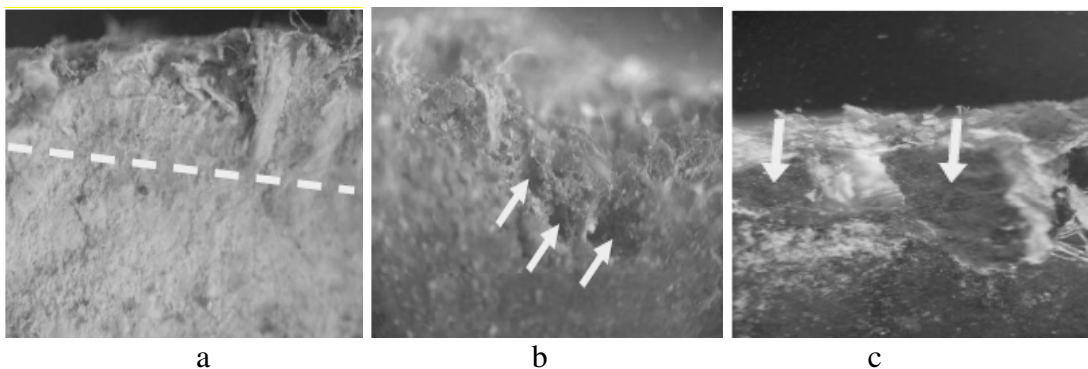


Fig. 2 Photos of AC pipe: (a) inside wall of AC pipe is coated with a layer of patina 3-4 mm depth; (b) patina is thick fibrous films that are composed of high fibrous asbestos and some biomass forming gelatinous like materials; (c) patina tends to have yellow to brown colorations implying the presence of some iron..

This investigation was focused on the bacterial community structure of black growths and the patina coating on concrete walls. Surface morphology of patina developed on cultured pipe segments were also examined with an optical microscope equipped with digital camera.

## 1.2 Water treatment and drinking water quality

Drinking water for Regina is provided by the Buffalo Pond Water Treatment Plant, which is located about 70 km west of the city boundary. This plant treated about 100 million liters of water daily and providing clean drinking water to City of Regina, City of Moose Jaw (approximately 30 km southwest) and several other small communities nearby. The treatment process consists of six stages: chlorination, cascade de-gasification, coagulation/flocculation, clarification, filtration and carbon adsorption. In general, the treated water has a pH from 7.3 to 7.5, turbidity less than 0.1 NTU, and consistently free from indicator bacteria. Alkalinity ranges from 105 mg/L in summer to 160 mg/L in winter; hardness varies from 165 to 260 mg/L and total dissolved solids range from 320 mg/L during summer to approximately 500 mg/L during winter. Concentration of sulphate ranges from 120 to 200 mg/L, which is not considered to be aggressive to the AC pipeline. Several water samples were collected from the fire hydrants connected to AC mains in Moose Jaw and Regina and tested with the BART tester for the presence of iron related (IRB), sulfate reducing (SRB), heterotrophic (HAB), denitrifying (DN), slime forming (SLYM) and acid producing (APB) bacteria.

## 1.3 Identification of bacterial groups

Identification of the bacterial communities utilized the BART manufactured by Droycon Bioconcepts Inc, Regina, Canada. BART tests work on the concept that specific bacteria within the sample would be able to generate activities or reactions in which the time lapse reflects the active population of those bacteria. Application of these tests for the semi-qualitative and quantitative determination of the activity levels of bacterial communities was first described by Cullimore (1993). In estimating the active population of bacteria, replicate testing was taken on many samples of known population to determine time lapses. On a sample when duplicate tests are performed, the time lags commonly vary by less than 5% and the same sequence of reactions is observed. The time lapse from setting up the BART test to

producing a complete reaction indicates the intensity of biological activity in the sample and this time lapse was closely related to the original population of microbes in the sample. A BART tester offers a variety of environments within which all types of bacteria in the group of interest can become active. These environments are created primarily along oxidation-reduction potential (ORP) gradient and selective nutrient culture medium diffusion slope.

The black growths and the solid porous patina were sampled for microbiological testing. The black growths were carefully removed from the inside surface of the pipe, mixed 0.5 g sample with 15 mL sterilized distilled water (30× dilution). For HAB test, 0.05 g sample was mixed with 15 mL of sterilized distilled water (300× dilution). This 15 mL of the homogenized sample was poured to the BART testers. The patina samples were taken from the porous layers inside of AC pipe wall. A 1.5 g of solid porous sample was used for each test, with 15 mL of sterilized distilled water, which provides enough water to develop the selective medium and ORP gradients. Both types of samples were cultured in the BART testers with IRB, SRB, DN, SLYM, HAB and APB selective medium at room temperature. When the time lapse is determined to be within 2 days, the bacteria were active, normally with high population, whereas a time lapse of greater than 6 days means the bacteria population and activity levels were low. The BART testers were used with an automatic digital reader, which could accurately record the time lapse; therefore, the bacterial group of interest in samples can be qualitatively determined with high precisions based upon digital readings for reactions every fifteen minutes instead of once a day.

#### **1.4 Evaluation of bacterial challenges on pipe segment**

The AC pipe was cut into arc segments of approximately 55 mm by 130 mm. The segments incorporated a patina layer that was (3±1) mm thick and was raised above the surface of the concrete. The pipe segments were 19 mm in thickness and weighed an average of 225.3 g, with a standard deviation of 25.5. The coupons were placed in pairs in one liter containers which were filled with dense cultures of IRB, SRB, DN, SLYM, HAB and APB. The bacteria in each dense culture were previously purified from the water network environment and cultured with the corresponding selective media. One container was sealed tight with the lid on to create anaerobic conditions while the parallel container was left open to the air to create aerobic conditions. Incubation continued for 10 days at 28±1 °C. After incubation the segments were removed separately, drained and air-dried for 24 hours in a humid atmosphere. Each segment was then weighed to obtain the wet weight. There were total four segments applied to each type of bacterial community, with two exposed under anaerobic and other two under aerobic conditions.

## **2 Results**

### **2.1 Examination and microbial composition of inside walls of AC pipe**

The pipe section was examined and black bulbous masses were recovered from the inside surface of AC pipe. These black growths were found to be dominated by HAB, followed by SLYM, and then, DN, IRB and SRB, but no APB was found (Table 1). The presence of IRB was indicated by the BART test that generated a special reaction pattern in which a black slime formed, incorporating 40%--60% of the water and floated under the ball (Fig. 1). Within the pipe it was noted that a patina layer was formed on the pipe surface (Fig. 2). This patina was a 2-4 mm layer of interwoven fibrous materials and mixed with some biomass, forming a gelatinous-like mat tending to have yellow to brown coloration, which



implied the presence of iron-containing materials and iron related bacteria. Test result showed that inside patina, three groups presented in the bacterial community, with HAB dominated, followed by IRB and SLYM (Table 2).

Table 1 Composition of microbes of the black growths attached to the inside AC pipe

Sample	Test	Time lapse	Reaction	Population calculated* ( $\times 10^6$ cells/g)
Replicate 1	IRB	2 days	CL-FO-BB	110.7
	SRB	3 days	BB	41.9
	HAB	33,083 sec	UP	4,374,000
	SLYM	2 days	CL-BB	362.7
	DN	2 days	FO	143.1
	APB	ND		ND
Replicate 2	IRB	2 days	CL-FO-BB	110.7
	SRB	3 days	BB	41.9
	HAB	29,687 sec	UP	4,995,000
	SLYM	2 days	CL-BB	362.7
	DN	2 days	FO	143.1
	APB	ND		ND

CL: clouded; FO: foam around ball; BB: black based; UP: Upward reaction begins in the base and moves up the tester; ND: None detectable.

\* Calculated population is expressed in predicted active cells per gram: 0.5g sample is diluted in 15 mL water (30 $\times$  dilution) except for the HAB tests in which 0.05g samples were used (300 $\times$  dilution). Tests were performed using BART tester under 28 and the time lapse was measured in day except for the HAB tests that used a reader and bacterial activity was recorded every second during the test.

Table 2 Composition of microbes inside the patina from the AC pipe

Test	Time lapse	Reaction	Population calculated* ( $\times 10^6$ cells/g)
IRB	2 days	FO-BB	11.0
SRB	ND		ND
HAB	266,546 sec	DO	80.7
SLYM	2 days	CL-SR	6.6
DN	ND		ND
APB	ND		ND

\* Calculated population is expressed in predicted active cells per gram: 1.5 g of sample is diluted in 15 mL of water (10 $\times$  dilution).

DO: downward reaction begin just below the ball to move slowly downward.

## 2.2 Bacteriological testing on water samples

Initial testing of the drinking water samples collected from fire hydrants connected to AC pipes did not show a large number of active bacteria. These results were expected for the water treated under regular operational conditions as controlled by standard quality management procedures. Such treatment includes the use of chlorine, which is well known to suppress the growth of all kinds of bacteria. Water quality, however, may be affected in events of sudden changes of hydraulic conditions, such as abrupt decrease of pressure, as detachment of the black growths and release of microorganisms from the patina may occur and cause the microbes discharging into the flowing water. At the testing time, there was no

evidence that the bacteria associated with the patina had moved in a significant manner into the water stream, nor was there evidence that the attached black growths on the wall of the pipe were unstable and would deteriorate the water quality.

### 2.3 Microbiological challenges to the AC pipe segment

Change of wet weight for the paired AC segments submerged in different bacterial cultures is shown in Fig. 3. The changes were resulted from interactions between the bacterial cultures and the pipe segment during the 10 days of culture. The X-axis gives the six bacterial cultures applied in the order (from left to right) of IRB, SRB, DN, HAB, SLYM and APB. This order is selected to show the progression from weight gains (iron related and sulfate reducing in both aerobic and anaerobic conditions) to weight losses under anaerobic conditions particularly in the slime forming and acid producing. Weight gains have been associated with the buildup of iron oxides, and to a less extent, sulfides, under a reduced environment. Weight losses were experienced with four of the bacterial communities particularly under anaerobic conditions in a progressive manner from denitrification to heterotrophic, to slime forming and to acid producing bacterial cultures.

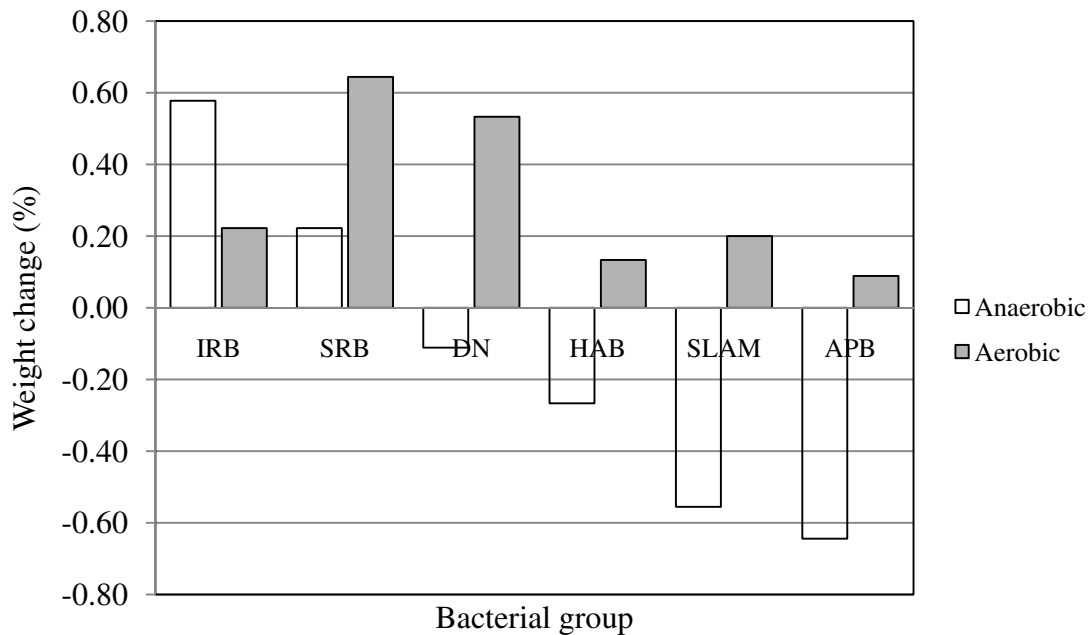


Fig. 3 Percentage of wet weight change of AC segments after 10 days immersion in previously prepared bacterial cultures.

### 3 Discussion

From these microbiological investigations, it became evident that the greater risk was to the water distribution infrastructure related to the sustainability of AC pipes rather than to the water quality within the pipes. In this experiment, there was no evidence that the bacteria associated with the patina moved in a significant number into the bulk water; nor was there evidence that the attached black slime growth on the walls became unstable and contributed to the deterioration of the drinking water quality. It appears that concerns should centre on

the potential challenges created through microbial activity deteriorating the integrity of the AC pipe wall. Microbial activities associated with the patina formation may have been associated with the corrosion of any underlying concrete in a manner that could either cause thinning of the wall and a decline in the structural strength of the concrete, or generate pitting and fracturing. These latter events could then develop a porous layer on the internal wall that could then harbor additional microbial activities. The black slime growths on the surface of the pipe wall can generate a significant effect for the concrete-patina interface to become more reductive. Under that environment, there could be accelerated levels of Ca leaching from the cement when heterotrophic anaerobic and acid-producing bacteria are present and active.

The dominant microorganisms in the black growths accumulated inside the AC pipe was the heterotrophic bacteria with populations measured around  $4.7 \times 10^9$  cells/g while all other bacteria were less than 1% of that population size. Of the bacterial types tested, only the APB were not detected in the black slime, however, they were detected in low numbers in the patina samples, indicating that APB are not common bacteria in AC pipe distribution system but does exist in particular spots such as inside of the patina, particularly the corrosion pits.

Heterotrophic bacteria in the black slime accumulates are predominantly aerobic given that upward reactions (UP) were observed. In UP reaction, reductive conditions rise up from the base of the tester. However, unlike in the black slime, heterotrophic bacteria in patina seemed to be predominantly anaerobic as the reactions were DO type with reactive conditions beginning just below the ball to move slowly downwards. This is not surprising given that the black slime accumulates on the pipe walls would be in contact with oxygenated flowing water while the patina would be in a more oxygen stressed situation.

Prime factors that are identified as possibly linking to any microbiological activities within the patina are related to HAB and SLYM, which play different roles within the patina. Heterotrophic bacteria appear to dominate in terms of cell populations and can create a reductive environment that may then stimulate the acid-producing bacteria to become active and grow, which may cause the local pH to drop within the patina to an acidic range. When this happens there is a greater risk that the concrete will begin an accelerated degradation and cause the patina layer to thicken.

When microorganisms challenge the concrete in AC pipe, it is clear that some of the bacterial groups can be very aggressive (Fig. 3). Under anaerobic conditions, the AC pipe segment immersed in APB and the SLYM bacteria cultures showed substantial losses in weight (range from 0.48% to 0.70%) during the 10 days period or a total loss of up to 1.5 g. A significant loss (0.25%) was also observed for the segment in HAB bacteria under anaerobic conditions. There was variance amongst the bacteriologically challenged AC segments and some conditions did not result in a significant loss in weight. Under aerobic environments, the concrete weights did not change much, or in some cases, gained in weight. For example, aerobic IRB immersion resulted in over a 0.2% weight gain that might have been the result of the deposition of a ferric form of iron and, for the SRB (0.65% gain), the depositions of the visible black sulfides on the concrete surface.



After the AC pipe segments were immersed in the pure bacterial cultures for 10 days, there were visible evidences that AC segments had compromised through changes in color, weight losses, and lateral layering or spotting. There were distinct differences among the various bacterial communities used in the experiments. For example, with IRB exposure, a layering of the surface on the concrete was observed with ferric forms of iron deposits (Fig. 4 a). With the APB, discolored patches were observed within the concrete, as was a discolored layer immediately behind the patina that extended 4-6 mm deep into the concrete (Fig. 4 b). In the SLYM solution, the concrete main body had uneven darkened spots under the anaerobic conditions (Fig. 4 c), while under aerobic conditions, the concrete generated three layers which consisted of (1) a bleached inner zone close to the patina, (2) a cortical layer in the midpoint of the concrete which had been further darkened and (3) a bleached outer layer (Fig. 4 d). When exposed to HAB under anaerobic conditions, Methylene blue was found on patina, a thin layer immediately behind the patina and other staining sites (arrow) indicating the weathering spots on the segment (Fig. 4.e). Under aerobic conditions, the SRB generated a similar type of reaction to anaerobic environment with black sulfide being deposited back into the main body of concrete but a sulfide-free zone appeared behind the patina (Fig. 4 f).

In the event that AC pipe is being challenged by microbial activities then it can be conjectured that some of the effects would come from the fatty acids that microbes produce during their growth, particularly under anaerobic conditions. These fatty acids tend to decrease local pH and weaken the concrete but leave the asbestos fibers in place as “growing” extensions from the degrading concrete. From patina, it can be assumed that the asbestos fibers left after the concrete structure being deteriorated would tangle and form into a woven matrix on the inside wall of the pipe. Such a woven pad would then form a natural harbor for the growth of biomass. The occurrence of APB in some patina samples tested and the presence of HAB and SLYM would indicate that there is a potential for microbiological influenced localized acidulation activities that could contribute to the concrete degradation and leave asbestos fibers unaffected. Eventually, these asbestos fibers, along with the biomass associated with them, will be released into the drinking water stream and degrade drinking water quality.

#### **4 Conclusions**

In summary, the study results suggest that microbial activities associated with patina formation may cause corrosion in the underlying concrete in a manner that could either result in wall thickness to decrease, the structural strength of the concrete to fall, and/or extensive pitting and fractures to develop. The attached black growths dominated by the heterotrophic bacteria on the inside walls of AC pipe generate a “footprint” of secondary effects at the concrete-patina interface causing it to become more reductive which, under some conditions, could result in accelerated levels of acidolytic corrosion of the concrete. Through the accelerated microbial challenge experiment, it appears that degeneration of the AC pipe segment could have been resulted from the fatty acids produced during bacterial growth. Acidic conditions tend to leach the free limes from concrete but leave asbestos fibers intact. These asbestos fibers would then tangle to form woven matrices on the inside surface of the pipe wall that could then provide natural harbors for further growth of biomass.

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