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PRELIMINARY ASSESSMENT ON BACTERIAL DETERIORATION OF ASBESTOS REINFORCED CONCRETE PIPES FOR WATER DISTRIBUTION.

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ABSTRACT: Asbestos reinforced concrete (ARC) pipes were commonly used for drinking water distribution networks in North America, primarily from middle1940s to early 1980s. In the City of Regina, Canada approximately 68% of all water mains are ARC pipes, to a total length of 535 km. In this preliminary research, it was found that bacteriological activities within the internal surface coating (patina) as well as within the concrete could induce bio-deterioration, which eventually leads to pipe failures. Identification of the bacterial consortia was performed using the S43048 protocols for the chromatographic detection of the C5 to C20 fatty acids methyl esters (FAME). Using proprietary library software, high similarity indexes were statistically generated, confirming the ubiquitous nature of the bacterial consortia within the patina (a distinctively fibrous internal coating) of various pipe samples. Bacteriological activities caused deterioration to the ARC pipes was primarily related to acid producing bacteria. . These bacteria are fermentative in the reductive environments, generating sufficient fatty acids that would reduce the pH into the acidic range of 3.5 to 5.5 and could cause structural failures in the concrete.

Keywords: Asbestos reinforced concrete (ARC), Patina, Microbiologically influenced corrosion, Acid producing bacteria.

INTRODUCTION

There has been a growing body of evidence that ARC water distribution pipelines are affected by deterioration processes that are evident by the decrease of the wall thickness from the inside out. These deleterious process usually have the following common elements in ARC pipes: (1) decreased thickness of the pipe wall reduces pipe strength; (2) while the concrete becomes thinner or softer the reinforcing asbestos fibers start to expose as the concrete corrodes away; and (3) patina develops on the inner surface of pipes, generally with the exposed asbestos fibers interfacing between the patina coating and the corroding concrete wall of the pipe. This investigation examines the microbiologically-influenced mechanisms that can be associated with both the patina development and the deterioration of the internal pipe walls. The assessment involves the determination of the forms of any microbiological activity as detected by the newly developed biological activity reaction test - visual BART reader (VBR72) system. (Droycon Bioconcenpts Inc.). Subsequent identification of the bacterial species or communities utilized the Rapid Agitation and Static incubation techniques, followed by modified MIDI procedures and then referred to Sherlock® library. Additionally synthetic patina biomass was grown using a section of ARC pipe.

Patina is from Latin patina 'dish, pan'. Patina has traditionally been recognized as films on the surface of buildings, monuments, deteriorated surfaces of bronze and other metals or any such acquired change of a surface through age and exposure. Patina often refers to accumulated changes in surface texture and color that result from the normal use of an object such as the internal surface of the pipes. Numerous studies have been carried out to identify naturally occurring patinas by composition, texture and structure. Microscopic techniques have been used as an essential tool for the characterization of the microstructure of patinas[1, 2, 3, 4, and 5]. The origin of the patinas, one of the most controversies in this subject is whether it is chemical or biological[3, 6, 7, and 8]. Some authors have suggested that the formation of patinas has a biological component[9, 10, and 11]. Active populations of microorganisms, such as fungi, bacteria, lichen, and algae are seen in a variety of environments in limestone karst landscapes, and they have been found to participate in a number of patina formations on the surfaces of stone [12]. Hose et al. [13] detected 19 kinds of bacteria in a site of karst environment, in which sulfide-oxidizing bacteria oxidized sulphurated hydrogen, sulfur, and sulfides in underground waters into sulphuric acid. Moore and Sullivan [14] found *Macromonas bipunctata* in a moonmilk stone, and they projected that this species might be directly influencing the formation of the moonmilk stone. Also, patinas formation can be intensified by the effects of microbial water-keeping, acidification of organic acids secreted by microorganisms.

In previous studies, the biological composition of the patina samples was analyzed for chlorophyll a and proteins and compared to patina-free surface samples [15]. However, this technique did not allow the identification of microorganisms. Aira and co-workers [16] reported that the MIDI system (Newark, DE, USA) using the fatty acid methyl esters (FAME) to identify microorganisms in patinas removed from buildings, monuments and granite outcrops throughout the Galicia region of Spain. Specifically, FAMEs were measured chromatographically in the C9–C20 range and can effectively characterize genera and species of bacteria. Gas chromatography is used for qualitative and quantitative identification of the FAMEs and the chromatographic patterns generated were then used to identify microorganisms based on their FAME profiles. Recently, this system has also been used for analysis of biofilms and patinas on Iranian monuments, where a high proportion of FAMEs came from photosynthetic organisms such as the Cyanobacteria.

In the City of Regina, Canada approximately 65% of all water mains are ARC redefine pipes, which have a total length of 535 km. In recent years, problems associated with the ARC pipes have gradually become significant, including increases in the number of pipe breaks and failures. Hu and Hubble[17] found that the predominant factors of the ARC water main breaks in Regina were related to the climate and clay soil conditions, which caused underground soil movements, and this created additional physical stresses on the ARC pipe, which was relieved by the structural failures. It was proposed that leaching of calcium from concrete cement could have significantly reduced the strength of the ARC pipe which then led to failures. Leaching from asbestos cement was also observed in the smaller diameter (100 to 150mm) ARC pipes, where the treated water had a low saturation index with low buffer capacity and a relatively long residence time. Geldreich[18] proposed that biofilms would inevitably develop on the internal surfaces of drinking water pipelines regardless of the presence of any observed disinfectant residual. Biofilms are composed of microbial cells that are deeply embedded in an exopolymeric (slime) matrix in a manner that also makes the enumeration of these microorganisms very challenging. This investigation examines the role of biofilms in the formation of patinas on the inner surfaces of ARC pipe and the subsequent bio-deterioration of the pipe walls.

MATERIALS AND METHODS

Cultural Identification of Bacterial Communities within Patina

Identification of the bacterial communities utilized the biological activity reaction tests manufactured by Droycon Bioconcepts Inc, Regina, Canada. The concepts for applying these tests for the semi-qualitative and quantitative determination of the activity levels of bacterial communities was first described and further elaborated by Cullimore[19, 20. 21, 22 and 23]. This elaboration involves the development of a please, explain coordinates system that allows for accurate positioning of the bacterial community being investigated. The system employs the oxidation-reduction potential (ORP) and the viscosity of the entrained water (in centipoise, cP) as the coordinates.

It is proposed that patinas developed on inside pipe wall would bridge the ORP interface between the oxidative stratum (in the free flowing water) and the reductive stratum (at the concrete interface underneath and protected by a patina biomass). A major challenge in characterizing biomass in the patina would therefore be the different bacterial communities in these distinctive lateral layers. This would include free floating bacteria in the flowing water (planktonic and biocolloids) and attached biofilms on the surface of the patina; integrated communities within the more oxidative (upper) patina; communities within the reductive zones under the biofilms and formed by the asbestos fibrous network left behind as the concrete corroded; and finally the reductive interface between the asbestos network and the exposed concrete surface of the pipe. Traditional water sampling in the distribution system is likely to recover only the planktonic and biocolloidal bacterial communities in flowing water.

This investigation was focused on the patina coating on concrete walls. The patina samples were taken from the porous layers from inside of ARC pipe wall. 1.5g of solide porous sample was employed per test, along with 13.5ml of sterilized distilled water to make up to15ml, which is required to develop the selective medium and ORP gradients. Standard operating practice for each test was used, but modified to allow the placement of 1.5g solid porous patina sample. For this investigation time lapse was employed for digital photography that allows the visual reader system to up to 72 tests at the same time

(VBR72, DBI, Regina, Canada). All tests were performed at an incubation temperature of 28°C, which was the lower end of the optimal growth range for mesophilic bacteria [24].

Tests selected for this study were all laboratory versions of the standard field test systems. This meant that the tests were limited to the inner test vial and should be used in a laboratory setting. Tests used included SRB- (sulfate reducing bacteria); IRB- (iron related bacteria); SLYM- (slime forming bacteria); HAB- (heterotrophic aerobic bacteria); and APB- (acid producing bacteria). All tests were performed in duplicate.

Biochemical Identification of Bacterial Communities in Selected Patinas

The protocol [25] employed two stages in the generation of the bacterial community from the patina sample. The first stage was to enrich the bacterial activity within the patina sample by a combination of rapid agitation followed by static incubation. The second stage utilizes the determination of the FAME profile and comparison with the standard library. To conduct the S43048 protocol then the following steps have to be taken: (1) agitate the sample within SLYM-BART tester to create very oxidative conditions. Transfer 15 mL of the sample into a SLYM-BART tester following standard aseptic practices. Rotate the tester with a LABQUAKE® shaker at a speed of 8 rpm for 4 hrs at the room temperature of 22±2°C. This produces radical turbulent oxidative agitation inside the SLYM-BART tester due primarily to the BART ball moving up and down; (2) at the end of the 4 hrs agitation period then the SLYM-BART tester is removed from the shaker and incubated for 20 hrs at 30±1°C in a static vertical mode. It should be noted that during the first 4 hrs of agitation it is common for the patina sample to develop foam when there is a very active bacterial population. This foam usually subsides completely during the secondary phase of static incubation. This two-staged RASI preparation primarily creates selective cultures of aerobic bacteria (agitated stage), followed by the differential culture of all surviving bacteria.

Recovery of the bacterial cellular growth from the cultured test using protocol S43048 is conducted by using the standard Millipore filtration apparatus with a sterile 0.45µm pore size membrane. First, moisten the membrane filter by adding 10ml of sterile water and then apply vacuum of 20 psi to let water passing through membrane. Next step is to loosen the tester cap off but do not remove completely and carefully tilt the tester to pour the entire contents of the incubated SLYM-BART tester (15ml) into the filter membrane while using the cap position to prevent the tester ball from landing on the filter membrane. In the event of the tester has generated a dense and gelatinous basal mass the tester is gently shaken to disperse the incubated content and then only the first two thirds of the volume (approximately 10ml) is filtered. Allow time for the entire contents to pass through the membrane filter. The surface of the membrane filter now contains the filtered cellular material from the cultured sample.

Identification of the bacterial communities generated by the S43048 enrichment protocol is performed using the Sherlock® Microbial Identification System version 6.0 published as the MIS Whole Cell Fatty Acid Analysis by Gas Chromatography (MIDI, Newark, DE). Sherlock® uses the Instant FAME sample preparation and Agilent GC 6850 series gas chromatograph to yield precise and reproducible FAME profiles. In the final preparation of the sample for the standard rapid MIDI bacteriological identification which involves recovery of the filtered cultured material from the membrane filter; a sterile plastic disposable 1µl loop should be used to scrape the surface of the membrane to recover the entrapped cellular biomass. Generally it is recommended that this procedure should be done using a series of three slow strokes with the loop flat down on the surface of the membrane, followed by three more slow strokes at right angles to the first strokes. Repeat these sequences of strokes until there is a clear

collection of cellular biomass rising out of the center of the loop. Two to five sets of strokes across the membrane will normally be necessary to collect required cellular materials. This means that 2.5-3.0 mg of biomass material has been collected, adequate for MIDI testing. To start the MIDI analysis, place the loop tip loaded with the cultured biomass into the bottom into the standard 1ml glass vial. To maximize the movement of cellular material into the vial, the loop can be flicked inside the vial to improve the transfer sample material until there is no visible cellular material left in the loop. From this point on, the MIDI analysis follows the standard protocol generating a FAME chromatograph where they have been detected. Comparison of the "unknown" cultured biomass sample with a standard library identification system commonly uses "probability" percentages to express the closeness of any given match with the library.

In the Sherlock system, the comparison employs a similarity index (SI) which is a numerical value expressing how closely the chromatograph of the unknown sample compares with the mean fatty acid compositions of the recognized members of the defined library. Samples with a SI of at least 0.50 are considered to be a good match provided the next closest identification is separated by greater than 0.10 unit of SI. The SI generation assumes that the species of microorganisms have a normal Gaussian distribution (bell shaped curve). The bacterial population is recognized by the series of traits created by the chromatograph. An SI of 1.00 would indicate a perfect match while 0.70 would indicate there remains a statistical difference potential of three standard deviates in the generated values. In practice, the Sherlock system would recognize as a close match comparisons between the unknown and the generated library where the SI is greater than 0.85 with the closeness improving as the SI approaches 1.00.

Laboratory Investigation of the Deterioration of Concrete by Cultured Patina

Cultures generated using test enrichment culturing and subjected to the chromatographic identification system were primarily involved to investigate the deterioration of concretes. . Modify this sentences Coupons (arcs or ring sections) were cut approximately 55 mm wide and 130 mm tip to tip from collected ARC pipe samples. All coupons had 3±1mm growths of a patina that was in some cases raised about 2±1 mm above the surface of the pipe. The coupons were 19 mm thick and weight average of 225.3g 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. One container was sealed 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^{o}C. After incubation the segments were removed separately, drained 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.

An additional experiment was developed to evaluate the potential to culture patina *in situ* in a closed continuous loop system in which a section of new ARC pipe was used. This mesocosm (Figure 1) was subjected to continuous aerated inoculation with mixed cultures of bacteria recovered from the patenas of ARC pipes and a selective cultural medium. For this mesocosm a 228 mm section of pipes with an outer diameter of 195 mm, internal diameter of 150 mm and a wall thickness of 22.5 mm was used. The pipe section was placed between two 8 mm thick clear polycarbonate plates which were silicon sealed to the cement pipe. Four 6mm stainless steel bolts were used to hold these end plates in position. The upstream (inlet) plate for the mesocosm had a single 8mm port setting 5 mm above the lowest point of the inside cement wall and formed the entry point into the mesocosm. A similar sized exit port was placed in a similar position on the downstream plate but towards the top of the mesocosm. Flow through

the mesocosm was therefore from the bottom to the top and then returned to the reservoir chamber. The mesocosm had a filled volume of 4,030 ml with a concrete surface area of 1,017 cm^2. Immediately above the mesocosm was a reservoir in which the liquid culturing medium was maintained at a height of 200 mm above the base in a cylindrical 36mm (internal diameter) vertical polycarbonate tube. This reservoir held 815 ml of culturing fluids. For the operation of this 4,030 ml mesocosm there was a reserve head of 815 ml which was constantly aerated. Aeration was achieved using a domestic aquarium aerator that generated 1,500 ml of air per minute using a 1.75 psi pump. Air was fed into the base of the reservoir with the return culturing medium from the mesocosm. The air then bubble up the reservoir while the return line to the entry port for the mesocosm was set at 150 mm above the base. In operation without significant biofouling, there would be a recycling of the medium with 17% fluid flow in the reservoir and the remaining 83% within the mesocosm. Flow rates would be dependent upon the level of biofouling occurring in the lines between the mesocosm and the reservoir.

Figure 1 The Mesocosm Developed for Accelerating Patina Culturing

The culturing fluids is obtained using the concentrated APB selective medium which was prepared by a tenfold dilution using sterilized distilled water to normal concentrations and the pH adjusted to 7 using sterile normal caustic soda. At pH 7, the liquid culture medium was purple in color.

RESULTS AND DISCUSSION

Microscopic Investigation of the Patina from Deteriorated ARC pipe

Patina forms on the inside surface of the ARC pipe as a distinctive coating commonly 1 to 3mm in thickness. The surface is commonly continuous with rippling which possibly reflects the water flows through the pipe at the patina-water boundary. Generally the patina is pigmented within the range of yellows to oranges, browns and blacks depending on the metallic cations that have become bioaccumulated as the patina has grown. From initial microscopic investigations using reflective light

microscopy the patina can be seen as involving four layers between the ARC cement wall and the flowing water inside the pipe. The position, form and function of these layers are summarized in Table 1.

Table 1 Position, form and function of layers within observed natural patina in ARC pipe within water distribution systems

Layer	Patina layer position	Form	Function
A	Intimate with ARC wall	Pitted cavities extending 0.1 to 2mm into concrete wall	Deterioration of concrete but with recalcitrant asbestos fibers extending outwards
B	Between wall and patina	Void zone 0.1 to 3mm deep with patina supported by asbestos fibers	Reductive void zone which acts as an anchor to the patina layer
C	Inside patina	Interwoven asbestos fibers which form the substrate for the bio-accumulative biomass growing in the patina	Supportive biomass growing within the fibrous mat causing bioaccumulation
D	Patina surface	Interface with the flowing water	Site of biofilms as the primary sites for exchange between water and biomass

Each of the four layers (A, B, C, and D) performs different functions within the patina influenced environment. Layer A is in intimate contact with the pipe wall exhibits clear evidence of deterioration of the concrete. This is evidenced by the pitted cavitation of the concrete and the exposure of asbestos fibers that extend outwards through layer B and into layer C where these fibers become intertwined into a mat within the patina itself (layer C). Layer D is formed by the biofilms that act as the living interface between the flowing water in the pipe and layer C patina. Water flow would be high over layer D which is composed mainly of biofilms but then becomes restricted by the permeability of the layer C patina.

Using patina layer C removed from five City of Regina ARC pipe sections, it was found by using the descending head permeometric methodology reference that these patina layers had relatively low permeabilities ranging 0.0042 and 0.0058 mm×cm^2/sec. This would indicate a relatively low flow of water between layer C and into layer B which was an open (porous) zone through which asbestos fibers exposed by the deteriorating activities occurring in layer A.

Diagram (Figure 2) shows in boxes (left to right) a vertical section through concrete from: A, non-deteriorated; to B, initial deterioration and the start of a tight patina; C, formation of the four layers associated with patina influenced deterioration; and D, advanced deterioration with lateral deterioration as well as vertical cavitation attack. These four stages of patina-influenced deterioration are depicted. In stage A, the original concrete-water interface is shown as a dotted line (1). Stage B involves the formation of a thin tight patina within which the asbestos fibers become entangled (2). In stage C there is acid induced collapsing of the concrete (3) with the fibers (4) becoming entangled within the patina (5). In stage D, the patina moves with the attacked interface between the concrete and the water from the original interface (6). Since the asbestos fibers (7) are not degraded by the biodeterioration process, they

remain exposed in the deteriorated concrete (8) that is primarily vertical but can become lateral (9) causing structural weakening and failure of the tensile strength of the concrete.

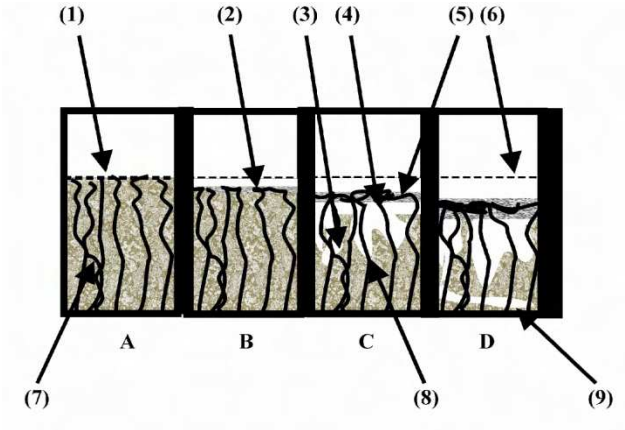

Figure 2 Asbestos reinforced concrete attack with the formation of an interfacial patina

Rates of deterioration are dependent upon the ability of the growing patina to utilize nutrients (primarily organic carbon and phosphorus) flowing in the water and becoming entrapped in the patina. Secondarily nutrients may also be assimilated from the concrete itself. Here the primary bio-accumulative interface with the water is layer D, the biofilms. Beneath that, the layer C patina supports much of the microbiological activity retained with the tangled asbestos fibers exposed by the reductive acidic corrosion of the concrete. In a fast growing deterioration process, the fibers may form a support for the patinas (layer B) while the actual concrete interface with considerable microbiological activity (layer A) will also be occurring. Deterioration will primarily occur as vertical pitting and later in aggressive stage lateral deterioration of the concrete. The rates of dterioration would be controlled to a significant extent by the through put of bio-assimilatable organic and phosphatic nutrients. Here it would be the organic carbon substrates that would be of most concern since this reductive degradation would generate the shorter chained fatty acids that would cause localized drops in pH into the acid range, triggering attack to the concrete.

The recalcitrant asbestos fibers within the deteriorated concrete will cause collapsing concrete (layer B) and then become interwoven through hydraulic and biological forces into patina layer C. With this being a common occurrence in ARC water distribution pipelines, any process that would either stabilize the patina preferably for desired life span of the pipe, or prevent the initial formation of the patina would be an economic and environmental advantage to the distribution system.

One example of this kind of deterioration was an asbestos cement pipe installed in 1969 and recently replaced due to severe leakage. In the forty years of service a patina had grown over the inner surface of the pipe to a depth of 2,200 microns as layer B. The patina remained tightly bound to the concrete interface and the exposed asbestos fibers were entwined within the patina. Also there was now vertical cavitational attack beneath the patina which means that layer A appeared closely associated with the patina layer C. However there was evidence of lateral deterioration (see arrow 9, Figure 2) that extended through the concrete wall and presumable was an important factor in the collapse of the pipe. Patina deterioration influenced the concrete would appear to have minimally 55 microns per year, but the major attack appears to be arc-like lateral cavitational also formed inside. The bio-deterioration of the concrete became evident when the stresses imposed on the pipe by external forces exceeded the weakened strength of the pipe, then, caused pipe failure.

Cultural Identification of Bacterial Communities within Patina

For the total population recovered within all four layers of the patina (Table 2) it was found that HAB bacteria formed 61.8%, APB, 20.6%; SLYM, 18.7%; and IRB, 16.2%. Examination of the bulk data for each of the four patina environment layers it was found that most of the bacterial population (53.7%) was active in layer C. Layer A also had a high population at the interface between the concrete and the voided fibrous layer with 32.4% of the population. Layer B within the void zone where the asbestos fibers were still intact then the bacterial population was 13.3%. Only 0.6% of the total patina bacterial population was found in layer D interfacing with the flowing water in the pipeline. It would appear that the bulk of the bacterial activities in the patina environment were divided between layer C biomass and the cement interface forming layer A.

Table 2 Percentage of detected bacteria in selected patina layers

	A1	**B1**	**B2**	**C1**	**D1**	**D2**	**D3**
Cumulative population ($\times 10^3$)	55,800	29,700	16,300	92,600	1,050	897	1,400
IRB	0.5%	0.8%	1.9%	0.7%	44.9%	39.1%	25.4%
SLYM	99.5%	99.0%	88.1%	87.6%	23.4%	14.4%	20.6%
HAB	N/D*	N/D	10.0%	11.6%	4.5%	13.4%	53.9%
APB	N/D	0.1%	N/D	N/D	28.5%	33.2%	N/D
SRB	N/D	N/D	N/D	N/D	N/D	N/D	N/D

*N/D: Not Detected; Cumulative bacterial populations were calculated from time lapse data as predicted active cells per g (pac/g) time lapse digital photographic software.

The nature of the low impermeability of the main patina layer C would indicate that there would be a low probability of layers A and B being oxidative, since there would be a restricted movement of oxygen through layer C to layer B. The best construct would be that layers A and B are reductive and dominated by bacteriologically influenced fermentative activities. Under these conditions with a negative oxidation-reduction potential these fermentative activities are likely to result in the local infested environment receiving acidic products (primarily short chain fatty acids). The outcome of this activity would be that the pH in the sub-patina (layer A and B) environment is most likely to decline into the pH range from 3.5 to 5.0. Under these conditions the activity of the HAB and APB is most likely to be acid generating which could then trigger structural weakening of the concrete but not the asbestos fibers which would be recalcitrant.

Five replica analyses were performed on layer C patina recovered from unchallenged section of ARC pipe that had been removed because of localized failures. Quantitative enumeration of the population sizes in the patina were undertaken using time lapse digital photography set every 15 minutes while being incubated in constant light at 28±1^{o}C. Four patina samples were subjected to this analysis (Table 3).

Table 3 Populations of APB and HAB within layer c patina recovered from broken arc pipe

	Population (million pac/gr)	
	APB	**HAB**
Patina 39	16.9 ±11*	1,920 ±2,720
Patina 51	39 ±12.3	2,390 ±2,130
Patina 81	71.8 ± 0	4,580 ±470
Patina 86	61±28.9	5,820 ±3,970

* Standard deviation for five replicates.

The populations for HAB bacteria ranged from one billion to six billion while the APB populations were found to be less than 10% of the HAB populations. While the HAB bacteria are more numerous under reductive conditions, these can be fermentative and generate acidic conditions. It was therefore projected that the acidulolytic deterioration of the cement may be influenced by the joint fermentative activities of the APB and HAB bacteria.

FAME Identification of Bacterial Communities Within patina

Patina samples as continuous coatings on the inner walls of the ARC pipes were recovered and tested with the S43048 protocol (Table 4). The objective was to determine whether the patina involved a single stable bacterial community that had a consistent chromatograph that would be recognizable by a unique library tentatively called "new patina". This library was limited to patina entries and did not include reference to other libraries.

Table 4 Similarity indexes (si) for the chromatographic analysis using the new patina library

Source	**Replication**	**Average SI**	**SD***	**% variation**
In-line filter[1]	2	0.846	0.024	2.90%
Medium[2]	4	0.786	0.057	7.30%
Surface patina[3]	8	0.917	0.057	6.20%
Patina 39[4]	3	0.876	0.03	3.50%
Patina 51[4]	3	0.662	0.125	18.80%
Patina 81[4]	3	0.646	0.072	11.10%
Patina 86[4]	3	0.762	0.033	4.40%
Patina ARC pipe[5]	5	0.429	0.3	69.90%

* Standard Deviation; [1] Filter refers to the entrapment of particles in an in-line filter on the recycling concrete mesocosm system; [2] medium refers to the nutrient medium employed in the recycling system and was commonly very turbid with bacterial activity; [3] surface patina was the layer C patina newly generated in the mesocosm; [4] Patina 39, 51, 81, and 86 were layer C patina from various assembled ARC pipes; [5] Patina ARC pipe was from patina freshly collected (less than five days old) from the City of Regina water distribution system

For the ARC mesocosm in which medium was recycled under an oxidative regime the medium contained a pH indicator bromo-cresol purple which moves to dirty yellow under acidic (pH < 4.8) and to purple in neutral condition (pH, > 5.5). To encourage fermentative activities in the mesocosm by bacteria, the pH was adjusted downwards using 1N acetic acid whenever the bacterial activity buffered

the pH above 5.5 (to more neutral conditions). When a surface patina formed as a light orange pustular film primarily on the inside walls of the mesocosm, samples were taken and the S43048 protocol applied. For the replicate SI analyses from the eight samples, there was a close similarity (SI=0.917±0.057), showing a 6.2% variation with the bacterial community being recognized by the newly generated patina library (Table 4). When four samples from the 4 micron in-line filter were tested these also gave SI values of 0.846±0.024 with a variation of 2.5%. This analysis would indicate that the bacterial community forming the surface patina and being entrapped within the filter were identifiable. When the liquid medium being recycled through the mesocosm system was analyzed (four replicates) there was a greater variation in the SI (0.786±0.057) with a variance of 7.3%.

Of the patina subjected to the S43048 protocol, high SI values were found for patina 39 (0.876±0.03, 3.5%). Patina 86 showed marginal similarities for the three replicates (0.762±0.033, 4.4%) while the other three patinas showed significant variation (11 to 70%) and mean SI values that were below 0.7. By using the RASI-MIDI method, five of the eight patina sample groups were identified as new patina while three were rejected as not significantly identifiable by that library.

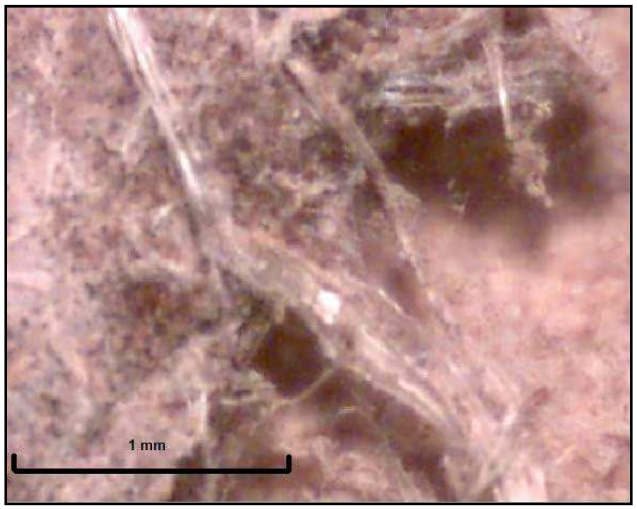

Figure 3 Vertical Section at the Upper Side of Layer B Patina Showing the Tangled Weave of Asbestos Which Is Directly Supporting the Growing Layer C Patina must be located at the end of the text.

Close up microscopy of the layer B patina reveals that there is an open series of columnar structures formed by the asbestos fibers that become tangled and provide physical support for the layer C patina.

From the various investigations of the patina growing in ARC water distribution pipelines, attentions should be drawn to the following points: (1) there is a significant bacteriological influence in the formation, growth and positioning of the patina; (2) the patina sits across an oxidative-reductive interface in which two distinct forms of growth occurs; (3) the major bacterial communities identified using the customized tester systems with time lapse photography of the reactions are the heterotrophic aerobic (HAB) bacteria on the oxidative side and the acid producing bacteria (APB) on the fermentative, reductive side; (4) concrete, as an essentially alkaline material, is particularly vulnerable to the acidic products of fermentation which can cause pitting attack and cavitation; (5) the net effect of the APB influenced acidic attack by pitting and cavitation is that the concrete pipe walls become thinner and more prone to catastrophic failure; (6) patina layer C generates two thirds of the biomass with the

remaining third associated with layer B and particularly layer A; (7) the patina in layer C forms a natural barrier for the movement of water (low permeability), nutrients and oxygen into layer B and A; (8) the durability of ARC pipe is significantly dependent upon minimizing the activity of the layer A and B by the APB bacterial communities; (9) sustainability for ARC water distribution pipelines will be achieved only when there is a management strategy to restrict the fermentative activities of bacteria associated with patina. This investigation examined the patina as being a primary factor in controlling the rates of deterioration in the ARC water pipelines. It was determined that the patina was, in part, formed (it is not clear) the deterioration of the concrete which released recalcitrant asbestos fibers into the layer B and C layers where they formed the substrate for the growth of the layer C. It is in this layer (C) that the bulk of the bacteriological activity occurs within the patina. Control management to restrict the deterioration of the ARC pipe, where it is primarily caused by the activities of APB bacterial communities, has to involve not the destruction of the patina (since it would grow back) but the manipulation of the patina to achieve two objectives. They are: (1) reducing the permeability of the patina layer C to restrict the movement of nutrients, oxygen and water down into layers B and A, which would then encourage the deteriorating activities of the APB in those reductive layers; and (2) incorporate elements into the patina that would retard the deterioration activities of the APB by such means as generating a more oxidative environment to reduce fermentative function that are generating acidic daughter products.

CONCLUSIONS

Patina grows on the inside walls of ARC pipe used for water distribution. These patina commonly have four distinct layers (A, B, C and D) with A and C being the most bacteriologically active. Layer A formed by the patina-cement interface appears to be dominated by fermentative bacteria which present under the reductive environment, causing the generation of acidic products that gradually deteriorates the cement by a process of pitting attack and cavitation. The deterioration processes encourage the development the layer B from the recalcitrant asbestos fibers that are exposed by the bio-dissolution of the concrete. Through hydraulic action and the effects of the perched biomass forming layer C, then these fibers become intertwined to provide a stable substrate for the perched patina layer C. As the pitting attack and cavitation on the concrete cement continues then the walls become thinner and less able to bear the various physical and hydraulic forces. This situation now leads to the ARC pipe failure.

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