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Biogenic acids produced on epoxy linings installed in sewer crown and tidal zones

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ABSTRACT

In this study the biogenic acids generated by microbes on the surface of Bisphenol A epoxy mortar coupons were investigated for up to 30 months. The epoxy coupons were installed in six sewers in three city locations, Sydney, Melbourne and Perth. Coupons were installed in both the crown and the tidal zones of the sewers to capture the effect of location within the pipe on acid production. The coupons were retrieved approximately every 6 months to provide a dynamic analysis of the biogenic acid production. Our results reveal the colonisation of epoxy mortar by the more aggressive acidophilic bacteria occurred within six months to two years of their installation in the sewer pipes. Biogenic acid generation appear to occur homogeneously from the tidal zone to the crown of the sewer pipes. The reduction in the surface pH of the epoxy lining was supported by the successive growth of microbes beginning with fungi followed be neutrophilic and heterotrophic bacteria and finally by the acidophilic bacteria and the corresponding accumulation of organic and sulphuric acids attributed to these organisms. This study also revealed the potential inhibiting effects on the microbes induced by the accumulation of metabolic products on the epoxy surface. The accumulation of organic acids and H_2S coincided with the growth and metabolism inhibition of fungi and acidophilic bacteria. These results provide insights into the microbial interaction and biogenic acids production that contribute to lining degradation and corrosion of concrete in sewer pipes.

1. Introduction

Microbiologically induced concrete corrosion or MICC is of significant concern worldwide (Okabe et al., 2007). The rehabilitation and replacement of sewer systems is expensive with estimates of the annual cost in the US alone exceeding $31 billion (O'Connell et al., 2010). An earlier estimation provided an annual cost of both water and wastewater asset concrete corrosion in the US at $36 billion (Koch et al., 2002) reflecting the growing escalation of the problem. In addition failure of wastewater collection system lead to health and environmental problems (Davis et al., 1998). MICC results from the metabolism of biogenic acids by microbes that thrive on the sewer wall and crown. The account of the involvement of sulphur oxidising bacteria (SOB), *Acidithiobacillus thiooxidans* or as formerly known as *Thiobacillus thiooxidans*, to concrete corrosion was first reported by Parker (Parker, 1945). Sulphuric acid that is generated by these microbes reacted with the alkaline components of concrete to form ettringite and gypsum, which have little or no hydraulic property and thus no structural integrity (Mehta, 1983). Since then the role of SOB and other microbes in concrete corrosion has been corroborated by other studies (Davis et al., 1998; Islander et al., 1991; Mori et al., 1991; Sand and Bock, 1991).

More recently the cooperative activity of the sewer microbes has been described as the successive growth of organism and their production of various biogenic acid and gases (Islander et al., 1991; Mori et al., 1992; Okabe et al., 2007; Valix et al., 2012). This occurs because organisms have specific pH growth range and pH growth optimum. Acidophiles, such as *A. thiooxidans* and *Acidithiobacillus ferrooxidans*, which oxidise H_2S and sulphur compounds (e.g., S_2O_3 and S) to sulphuric acid, have their growth optimum at pH 0.0 to 5.5 (Hernandez et al., 2002; Okabe et al., 2007); neutrophiles such as *Thiobacillus thioparus, Starkeya novella* (formerly *Thiobacillus novellus*), *Halothiobacillus neapolitanus* (formely *Thiobacillus neapolitanus*) and *Thiomonas intermedia* (formerly, *Thiobacillus intermedius*), which promote the conversion of thiosulphate to elemental sulphur grow optimally at pH 5.5 to 8.0 (Islander et al.,

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1991; Kelly et al., 2000; Kelly and Wood, 2000), and fungi such as *Aspergillus niger*, produces various organic acids, grow at pH 4.0 to 6.0 (Cho and Mori, 1995; Gu et al., 1997; Valix et al., 2012), although acidophilic fungi may also tolerate pH down to 1.6 (Johnson, 1998). Heterotrophic bacteria such as *Mycobacterium* sp. will also generate organic acids optimally within pH of 3.0–6.0 (Cho and Mori, 1995; Davis et al., 1998; Nica et al., 2000; Vincke et al., 2001). Fresh concrete has a pH of approximately 12.0. Each strain of organisms will need to work cooperatively by growing and generating their specific biogenic acids successively to bring down the pH of concrete. Sewer gases including H_2S, CO_2, NH_3, and SO_2 also play an important role in reducing the concrete pH particularly when it is still very alkaline and is less supportive to the growth of corrosion inducing microbes. Ammonia is oxidised to nitrite by *Nitrosomonas* and nitrite is oxidised to nitrate by *Nitrobacter* (Muller et al., 1995). When hydrolysed in humid sewer environments these sewer gases will form both weak and strong acids, specifically H_2CO_3, HNO_2, HNO_3 and H_2SO_3 (Sand, 1997). Because these hydrolysis gases and H_2S originate from the sewage, they will contribute to the corrosion of the crown and walls of concrete sewer pipes regardless of the concrete surface pH and microbe population on its surface. Controlling the corrosive effects of MICC will therefore require an understanding of this successive growth and the metabolism of these microbes. As shown this is quite a complex process and despite the advances provided by molecular methods in assessing the population distribution of MICC (Hernandez et al., 2002; Okabe et al., 2007) we still have limited understanding of the interactions of these microbes.

Various mitigation strategies have been in use around the world to control MICC, which include chemical treatment to reduce the concentration of the dissolved H_2S in wastewater with caustic soda, ferric chloride, ferrous or ferric chloride blends, ferric nitrates, hydrogen peroxides, oxygen injections and sodium nitrates (Redner et al., 2004; Scrivener et al., 1999), the use of acid resistant coatings including polymeric mortars (Redner et al., 2004) and cement lining materials (Scrivener et al., 1999) and spraying with magnesium hydroxide to raise the pH level of concrete (Sydney et al., 1996). These techniques have all showed promise in reducing corrosion. However there are significant challenges in their application, with many of these technologies proving to be costly and with inadequate control in most cases. With coatings, there are two specific technical gaps, these relates to understanding of the microbial system and their interaction with the coating materials and insufficient knowledge in predicting their performance and durability in real sewer environments. Much of the studies devoted in assessing the performance of lining materials have been based on simulated sewer environments that involve using reagent acids (e.g., sulphuric acid) and biochambers (Hewayde et al., 2007; Redner et al., 2004; Valix et al., 2011; Vipulanandan and Liu, 2005). Although these provide insights to the resistance of the coatings to acidic environment, these results are often difficult to translate to actual service life in unique and complex sewer environments. Reported field data are scarce and the results suggest great variability in coating performance and that some currently used coatings are unable to match the service conditions that continue to become more aggressive with time (Nixon, 1997; Parker, 1945).

Efforts to design protective systems for wastewater collection facilities based on lining materials will be ineffective without adequate fundamental understanding of microbial colonisation of coatings. Although significant studies have been carried out on microbial communities in sewers, much of these have focused on microbes that grow only on the surface of concrete (Cho and Mori, 1995; Davis et al., 1998; Gu et al., 1998; Hernandez et al., 2002; Islander et al., 1991; Milde et al., 1983). While the study of microbes colonising lining materials remains scarce (Gu, 2003; Pagaling et al., 2014). In addition, although the microbial analysis may detect the presence and concentration of acid generating microbes, accurate assessment of their corrosive ability will require an analysis of the metabolic acidic products generated. The aim of this study was to examine the microflora and biogenic acidic products that are generated, which contribute to the degradation of protective polymeric lining in sewers. Of specific interest are the effect of environmental conditions and period of service on the nature and concentrations of these acids.

2. Material and methods

2.1. Polymeric coating

Commercially available diglycidyl ether of Bisphenol A epoxy resin cured with cycloaliphatic amine curing agent was used for this study. The epoxy mortar was prepared and cured according to the manufacturer's instruction and coupons of 50 mm × 50 mm x 10 mm were prepared by casting them in molds. The XRF analysis of the composition of the filler and LOI or loss on ignition of the polymeric lining is shown in Table 1.

2.2. Coupon installation and sewer site environmental conditions

Field testing involved the installation of the epoxy coupons in sewers in Sydney, Melbourne and Perth. Two sewers were chosen in each city to reflect the variable corrosive conditions. The sites and their environmental conditions are summarised in Table 2. The Sydney and Melbourne sites represent low H_2S environment and moderate temperatures, whilst Perth sewers represent highly elevated H_2S concentrations with slightly higher temperatures. All humidities were high (>90% RH) with the exception of Perth MS. Coupons were installed both in the crown and tidal zone of each sewer (see Fig. 1) to reflect the potential variability in microbial activity at these two locations. Coupons on the crown reflect largely the microbial activity in the gas phase of the sewer with little or no contact with the sewage. Whilst coupons in the tidal zone, as the term implies, would experience intermittent contact with the sewage flow. The coupons that were installed in the tidal zones would have been subjected to cyclic flooding. Under regular operations, the coupons would be flooded during heavier sewage flow in the morning until midnight. However, with heavy rainfall, the tidal zone would have been constantly flooded. Removal of solid build on the bottom of the pipe as they are cleaned may lower the tidal zone leaving the installed samples above the sewage flow. Based on the flow of sewage through the pipes, the coupons on the tidal zone would have been submerged at 30–40% of the period they were installed. As such it would be anticipated the microbiological activity on the walls and coupons at this location would have been disrupted by removal of microbes and neutralisation of biogenic acids by the alkaline sewage (~pH 7.0–8.0), whilst the supply of potential nutrients were replenished. The effect of cyclic flooding cannot be decoupled, however the installed coupons should capture the cumulative effect on microbiological activity imposed on the sewer wall in the tidal zone. The coupons installed on the roof would have also closely reflected the actual environmental conditions and moisture content experienced by the sewer concrete on the crown. The air flowrates though these sewer are, high from 4 to 6 kL/s and would have maintained a high convective heat transfer through the pipe. The high relative humidity within the sewer environment meant air and dew point temperatures were similar with some sewers experiencing about 0.5–2 °C difference. Condensation on the walls and on the coupons installed in the crown were observed at retrieval supporting the fact that the walls and samples reached temperatures below the dew point of

Table 1
XRF analysis of the epoxy A mortar.

Filler components	Composition (wt%)	Filler components	Composition (wt%)
Al_2O_3	0.17	MnO	<0.01
SiO_2	67	Fe_2O_3	0.46
K_2O	0.02	NiO	0.227
CaO	5.6	V_2O_5	<0.01
TiO_2	0.38	ZrO_2	0.01
Cr_2O_3	0.1		
Total- Filler	73.967		
LOI	26.34		

Table 2
Sewer environmental conditions.

City	Sewer	H_2S (ppm)	T (°C)	Humidity (RH%)
Sydney	SWOOS[a], South Barrel	1.5–4.6	18.8–20.9	90.0–96.6
	SWOOS[a], North Barrel	1.4–4.6	17.0–22.3	91.6–95.1
Melbourne	Western Trunk Sewer (WTS)	4.5–5.5	18.9–20.1	99.0
	Southern Eastern Trunk Sewer (SET)	0.79–3.6	17.1–18.5	99.0
Perth	Perth MS (Main Sewer)	68–95	26.6–27.7	85.5–93.3
	Bibra Lakes	580–654	28.5–30.1	94.5–99.1

[a] SWSOOS —Southern and Western Suburbs Ocean Outfall Sewer.

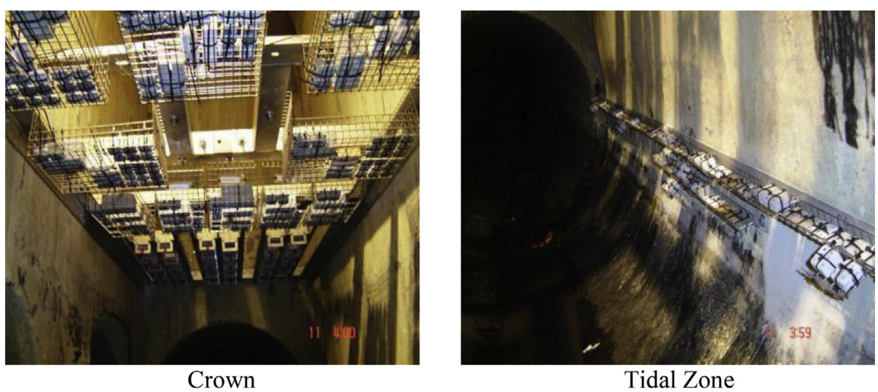


Fig. 1. Photographs of coupons in field installations in the crown and tidal zone of sewer pipe.

humid air in these sewers. About 10 coupons were installed in the crown and another 10 coupons in the tidal zone. Two of the coupons at each location were retrieved within approximately 6 months intervals and subjected to destructive analysis.

2.3. Surface pH measurement

The surface pH of the epoxy specimens was estimated using a Metrohm LL Flat Membrane Electrode surface pH probe. The initial pH of the coupon was measured before their installation in the sewer. Because of the low conductivity of clean distilled water that can challenge accurate measurements of the surface pH, initial surface pH was conducted with the aid of an ionic strength modifier. $(NH_4)_2SO_4$ was dissolved in distilled water to make up 0.1M solution. A small droplet of this solution was used in measuring the initial surface pH of the coupons in at least four locations. The surface pH of the coupons after they are retrieved from the sewers were measured after organic matter build up was removed using a brush followed by sterilisation in the UV fume hood. A small droplet of deionised water was placed on at least four locations on the coupon surface and was allowed to equilibrate for about 2–3 min. The surface pH probe was then used to measure the pH of the water droplets. The pH electrode measurements that are less than 1.0 would be subject to an acid error. A pH higher than the real pH would be measured because of the lower activity of acids with increasing acid concentration. The corresponding bioacid analysis by ion chromatography should provide a more accurate representation of the acid concentrations on the surface of the coupons (section 2.5).

2.4. Microflora analysis

Differentiation and detection of microorganisms that were present in the coupons after their internment in the sewer followed the procedures based on the use of selective media as outlined by Cho and Mori (Cho and Mori, 1995). After inoculation the selective media plates were incubated for two weeks at 28 °C. The abundance of the microbes was based on the rate of spreading on the plates.

2.5. Biogenic acid analysis

The concentrations of inorganic and organic acid anions, including sulphate, citrate, malate, ketoglutarate, pyruvate, lactate, succinate, on the epoxy coupons were determined using Dionex ICS-2000 Ion Chromatography. To extract the biogenic acid, about 1 mm thickness of the coupon was sliced using a Leco V50 diamond saw. The sliced epoxy was milled using a high speed Retsch (PM 100

CM) planetary ball mill at 500 rpm for 5 min. The particle size of the milled epoxy was less than 120 μm. One gram of the milled epoxy was immersed in 10 mL of Millipore® H_2O to prepare approximately 10w/w% pulp density of slurry. The pHs of the solutions were then measured as a function of time until the change in pH was within ±0.2. The slurries were filtered using 0.2 μm membrane filter prior to anion analysis by ion chromatography.

3. Results and discussion

3.1. Reduction in surface pH of epoxy mortar with time

The reduction in surface pH of the epoxy coupons at each sewer with time reflects the colonisation and generation of biogenic acids on the surface of the epoxy mortar (Okabe et al., 2007). The surface pHs of the epoxy A mortar were plotted as a function of installation period in Fig. 2. Superimposed on these graph is a line for surface pH 4.0. This pH was used to represent the threshold; below which greater acidic attack of the lining would be anticipated resulting from proliferation of acidophilic bacteria (Valix et al., 2012). The initial pH of the epoxy lining is 7.5, which could promote the growth of neutrophiles and fungi (Cho and Mori, 1995; Gu et al., 1997; Islander et al., 1991; Valix et al., 2012). As shown the epoxy linings achieved surface pH below 4.0 within approximately 6–20 months. Within 24–30 months, the pH dropped further to −0.03 to 2.13. These low pHs reflect the growth of acidophilic sulphur oxidising bacteria. It is evident from these results that epoxy linings can be rapidly colonised by microbes and that aggressive acidic conditions can be attained within two years of lining installation.

A comparison of the surface pH reduction in epoxy installed in

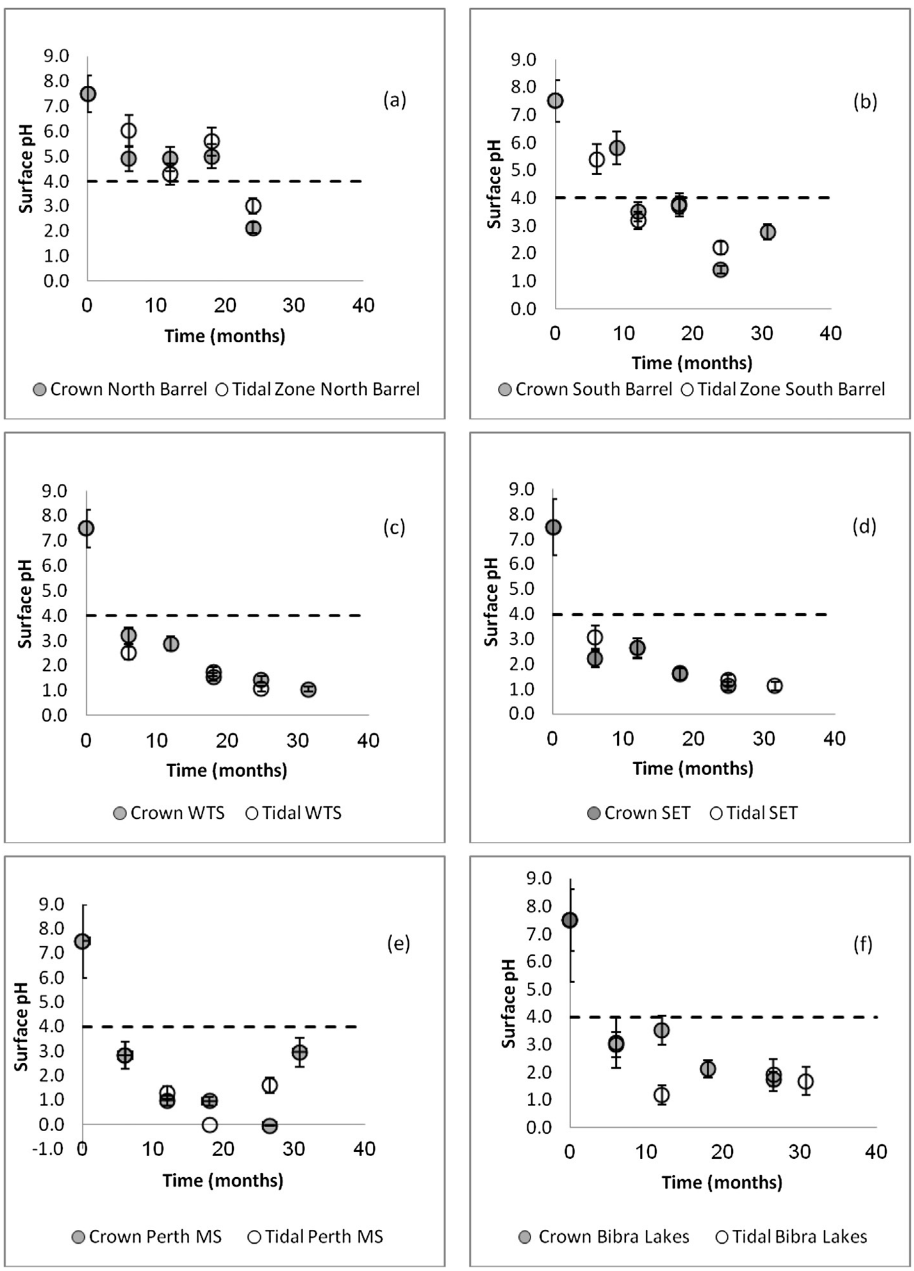


Fig. 2. Reduction in surface pH with time of epoxy A mortar installed in the crown and tidal zones of: a) SWSOOS North barrel, b) SWSOOS South barrel, c) WTS and d) SET, e) Perth MS and f) Bibra Lakes sewers.

the crown and tidal zone show there is relatively little difference between the surface pH reduction achieved in these sites. This suggests that in these sewers the extent of corrosion and lining degradation occurs homogeneously from the tidal zone to the crown.

The reduction in surface pH in epoxy installed in the six sewers in this study show the effect of H_2S concentrations on the amount of acids produced is quite variable. Both Sydney barrels have similar environmental conditions (1.5–4.6 ppm of H_2S). However as shown in Fig. 2a and b, the different surface pH of the epoxy installed in these sewers reflects dissimilar acid production. In 24 months the pH achieved in the Sydney South barrel of 1.4 is lower compared to North barrel (pH 2.13). In Melbourne sewers, WTS has a slightly higher H_2S concentration (4.5–5.56 ppm of H_2S) than SET (0.79–3.6 ppm of H_2S). As shown in Fig. 2c, the pH achieved in 24 months in Melbourne WTS of 1.44 is slightly higher compared to pH 1.13 attained in SET (see Fig. 2d). Increasing the H_2S environments as in Perth MS (68–95 ppm of H_2S) resulted in lower surface pH of −0.05 in 24 months (Fig. 2e). However further increase in H_2S concentration to 580–654 ppm of H_2S in Bibra Lakes did not support this trend. In fact the opposite was observed with attainment of pH of 1.75 in 12 months (Fig. 2f). The surface pH results presented here demonstrate the complexity of acid generation in sewers. It is clear that the production of biogenic acids in the sewer is dependent on many other factors in addition to H_2S concentration. Sand (Sand, 1997) referred to the abiotic factors including heavy metals, dissolved organic matter, pH, salinity and biotic factors that can affect both the microbe population and their metabolism.

3.2. Effect of environmental conditions on microflora and acids generated

3.2.1. Effect of time

The acids produced on epoxy A mortar as a function of installation period are plotted in Fig. 3. The organic acid anions that make up the total organic acid, the sulphuric acid anion and their concentrations are reported in Table 3. Table 4 shows the corresponding identified fungi and bacteria isolates from the epoxy coupons classified according to their nutrient needs.

Fig. 3 shows the acid generation involved two stages consistent with the sequential growth of fungi, heterotrophic bacteria and the acidophilic bacteria (Islander et al., 1991; Okabe et al., 2007). The occurrence of these two stages is more apparent in Sydney barrels and Melbourne WTS (Fig. 3a–c). The first stage involved the generation of organic acids in the early phase of installation (within 12–18 months). As shown in Fig. 3a and b, the peak acid generated in the early phase of epoxy installation in the Sydney barrel was primarily organic acids (14–22.4 g/m^2). This behaviour was also observed in the epoxy installed in the Melbourne WTS (organic acid of 37 g/m^2) in Fig. 3c. The second stage involved a decline in organic acid generation (<1 g/m^2) accompanied by a rise in sulphuric acid generation from 0 to 5.8 g/m^2 in Sydney and to 4.7 g/m^2 in Melbourne in 20–24 months. The microflora analysis in Table 4, which suggests the presence of heterotrophic fungi and bacteria in the early phase of installation followed by their decline and proliferation of acidophilic bacteria at the later phase, supports the sequential growth of the organisms on the epoxy. The lack of correlation in the surface pH observed in Melbourne WTS and Sydney barrels to the H_2S concentration could be attributed to the preferential growth of fungi and heterotrophic bacteria at the early phase. Fungi and heterotrophic bacteria rely on organic substrates for growth.

Two aspects of these results were examined further in this paper. The first is the relative roles of fungi and heterotrophic bacteria in the generation of organic acids and the second the mechanism for the two stage acid production. Table 4 shows that both fungi and heterotrophic bacteria, that are able to generate organic acids, were isolated from the coupons installed in all sewers. Heterotrophic bacteria, however, were only isolated on the crown and none or negligible quantities were observed near the tidal zone. This is consistent with similar observation made by Cho and Mori on the absence of heterotrophic bacteria in samples located in the tidal zone (Cho and Mori, 1995). The abundance of heterotrophic bacteria in the crown however was limited to surfaces with pH above 2.88 and even then they only exhibited trace and light growth. Whereas fungi growth was quite heavy in both tidal and crown samples at surface pH above 2.0 and noted to grow at pH as low as 1.44. Acidophilic heterotrophic bacteria that have been isolated from various environments including acid mine drainage, sewage, sewer pipes and soils, have been noted to grow only within pH 3.0–6.0 (Coupland and Johnson, 2008; Kishimoto and Tano, 1987; Vincke et al., 2001). The observed limited growth of heterotrophic bacteria in the crown, particularly in acidic surfaces, is consistent with this pH limitation. It can be implied from our results that both fungi and heterotrophic bacteria are involved in the generation of organic acids on the crown samples at the early phase, whilst fungi are solely responsible for organic generation near the tidal zone. Based on the greater relative abundance of fungi particularly as the pH decreases, it appears fungi may also be the primary producer of the organic acids in the crown as the surface achieves pH below 3.0. The nature of the organic acids generated suggests too that fungi may have been responsible for their production. Table 3 shows the primary organic acid generated was malic acid with minor quantities of lactic, succinic, pyruvic and ketoglutaric acids. Previous comparisons of organic acids generated by a number of heterotrophic bacteria and fungi isolates showed fungi preferentially generated malic acid (Palmer et al., 1991).

The two stage acid production observed suggests that while the organic acid concentration is high (>5 g/m^2), the generation of sulphuric acid is suppressed. This is also evident in the acid generation in Melbourne SET and Perth sewers (see Fig. 3d–f). In these sewers both sulphuric and organic acids were generated simultaneously at the early phase of the installation. However the level of organic acids observed in these sites were already significantly lower at the early phase (<0.1 g/m^2). The microflora analysis in Table 4 shows the growth of acidophilic and neutrophilic autotrophs and heterotrophic bacteria are either zero or light particularly in coupons installed in the Sydney and Melbourne sewers, where greater quantities of organic acids were generated. Our results suggest that organic acids appear to be inhibiting bacterial growth and the generation of sulphuric acid on epoxy installed in sewers. Organic acids including acetic acid, citric acid, benzoic, fumaric, lactic, and malic acids are effective inhibitors of bacterial growth (Carpenter and Broadbent, 2009; Johnson, 1998). The antibacterial property of organic acids stems from the fact that protonated organic acids are membrane soluble and can therefore enter the cytoplasm by simple diffusion (Lambert and Stratford, 1999). Once in the cell, the near-neutral intracellular pH favoured by most bacteria induces acid dissociation and the release of protons and anions inside the cell. The accumulation of acids within the cell will then inhibit cellular functions (Ricke, 2003; Roe et al., 2002). The minimum inhibitory concentrations, or the lowest acid concentration that will inhibit growth has been reported to be from 0.05 to 50 g/L (Johnson, 1998). The inhibitory effect of organic acids has been associated with both the dissociated and un-dissociated state of the acids, however the inhibitor effect is greater with acids in their un-dissociated state (Hewayde et al., 2007; Johnson, 1998; Kishimoto and Tano, 1987; Palmer et al., 1991; Valix et al., 2011; Vipulanandan and Liu, 2005). The inhibitory effect of

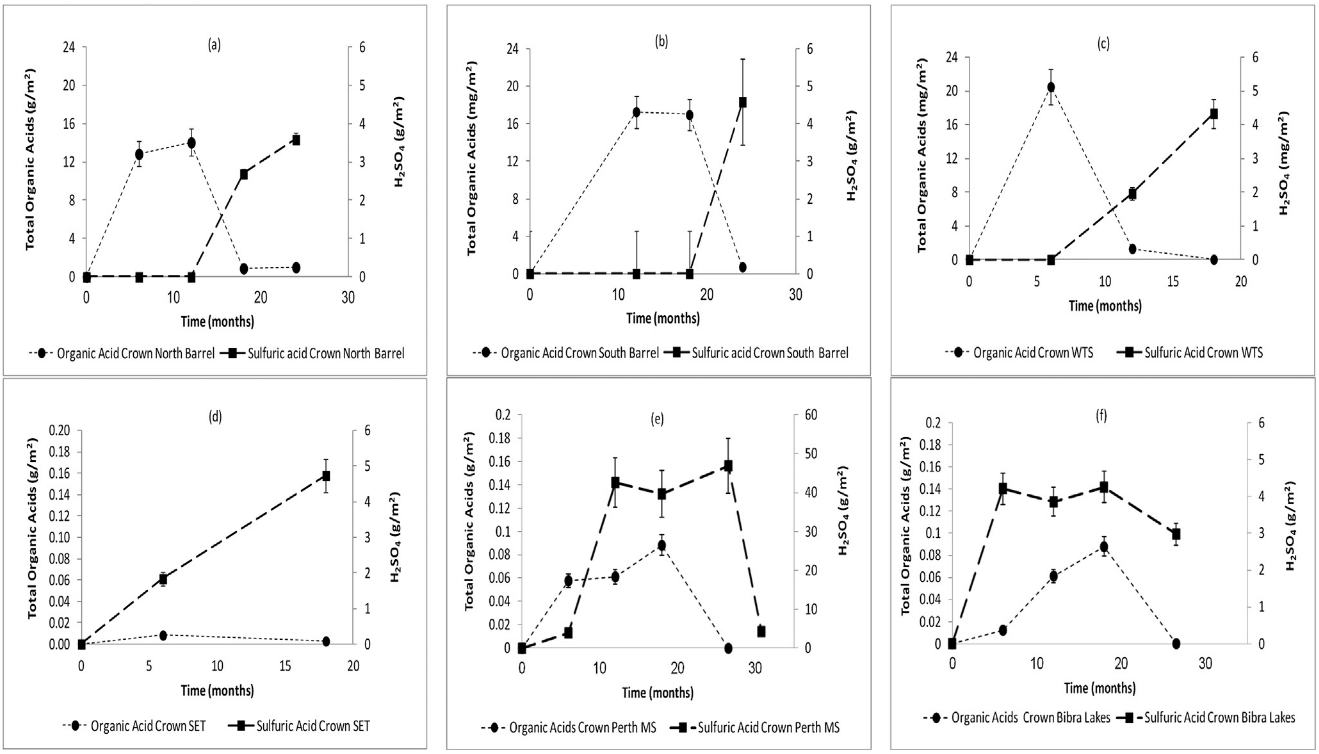


Fig. 3. Metabolised biogenic acid on epoxy A mortar installed in the crown of: a) SWSOOS North barrel, b) SWSOOS South barrel, c) WTS and d) SET, e) Perth MS and f) Bibra Lakes sewers.

Table 3
Acid anion concentrations in epoxy A coatings after their interment in sewers.

City	Sewer	Location	Time (days)	Surface pH	Acid anion concentration (g/m^2)							
					Lac	Suc	Pyr	Ket	Mal	Fum	Cit	Sul
Sydney	SWSOOS South	Crown	364	3.5 ± 0.18	n	n	n	n	17.3 ± 0.86	n	n	n
	Barrel		553	3.8 ± 0.19	n	n	n	n	16.7 ± 0.90	n	n	n
			727	1.4 ± 0.07	n	n	0.7 ± 0.03	n	n	n	n	4.6 ± 0.23
	SWSOOS South	Tidal	268	5.4 ± 0.20	n	n	n	n	22.4 ± 1.10	n	n	n
	Barrel		364	3.2 ± 0.16	n	n	n	n	16.6 ± 0.86	n	n	n
			553	3.7 ± 0.19	n	n	0.9 ± 0.04	n	13.6 ± 0.62	n	n	n
			727	2.2 ± 0.11	n	n	n	n	1.0 ± 0.05	n	n	3.6 ± 0.18
	SWSOOS North	Crown	268	4.9 ± 0.25	n	n	0.6 ± 0.10	n	12.3 ± 0.60	n	n	n
	Barrel		364	4.9 ± 0.21	n	0.8 ± 0.04	n	13.3 ± 0.21	n	n	n	n
			553	4.2 ± 0.10	0.2 ± 0.01	n	n	n	22.0 ± 0.80	n	n	n
			924	2.13 ± 0.3	n	1.0 ± 0.05	n	n	n	n	n	3.6 ± 0.69
	SWSOOS North	Tidal	553	5.6 ± 0.28	n	n	n	n	18.1 ± 0.91	n	n	n
	Barrel		727	3.4 ± 0.17	n	n	n	n	n	n	n	5.8 ± 0.12
Melbourne	WTS	Crown	231	3.2 ± 0.48	n	n	n	n	20.5 ± 2.53	n	n	n
			382	2.88 ± 0.29	n	n	1.3 ± 0.19	n	n	n	n	2.0 ± 2.3
			563	1.53 ± 0.34	n	n	n	n	n	n	n	4.3 ± 0.65
	WTS	Tidal	231	4.8 ± 0.53	n	n	0.8 ± 0.2	n	22.2 ± 3.3	n	n	n
			563	3.8 ± 0.57	n	n	n	n	37.0 ± 3.7	n	n	n
	SET	Crown	231	2.21 ± 0.35	n	n	n	n	n	n	n	1.8 ± 0.36
			563	1.63 ± 0.33	n	n	n	n	n	n	n	4.7 ± 0.50
	SET	Tidal	382	2.65 ± 0.27	n	n	n	n	n	n	n	3.0 ± 0.48
			563	1.6 ± 0.07	n	n	n	n	n	n	n	4.0 ± 0.62
Perth	Perth MS	Crown	215	2.82 ± 0.56	n	n	n	n	n	n	n	3.8 ± 1.22
			418	0.98 ± 0.15	n	n	n	n	n	n	n	42.6 ± 8.51
			796	−0.05 ± 0.02	n	n	n	n	n	n	n	46.9 ± 13.6
			924	2.95 ± 0.59	n	n	n	n	n	n	n	4.2 ± 1.15
	Perth MS	Tidal	418	1.30 ± 0.26	n	n	n	n	n	n	n	3.1 ± 0.30
	Bibra Lakes	Crown	215	2.99 ± 0.89	n	n	n	n	n	n	n	0.4 ± 0.10
			418	3.53 ± 0.35	n	n	n	n	n	n	n	0.4 ± 0.08
			579	2.12 ± 0.64	n	n	n	n	n	n	n	0.4 ± 0.06
			796	1.75 ± 0.35	n	n	n	n	n	n	n	0.3 ± 0.09
	Bibra Lakes	Tidal	215	3.07 ± 0.89	n	n	n	n	n	n	n	2.1 ± 0.42
			418	1.18 ± 0.29	n	n	0.1 ± 0.03	n	n	n	n	1.9 ± 0.38
			796	1.90 ± 0.19	n	n	n	n	n	n	n	2.1 ± 0.63
			924	1.68 ± 0.50	n	n	n	n	n	n	n	3.5 ± 0.35

Lac: Lactic acid; Suc: Succinic acid; Pyr: pyruvic acid; Ket: Ketoglutaric acid; Mal: malic acid; Fum: fumaric acid; Cit: citric acid; Sul: Sulphuric acid; n: negligible or < 0.1 g/m^2.

Table 4
Identification and abundance of microbes in epoxy A coatings after their interment in sewers.

City	Sewer	Location	Time (days)	pH	Fungi	Heterotrophic bacteria	Neutrophilic autotrophs	Acidophilic autotrophs
Sydney	SWSOOS South Barrel	Crown	364	3.5	++++	+	+	+
			553	3.8	++++	+	+	+
			727	1.4	++	0	+	++
	SWSOOS South Barrel	Tidal	268	5.4	++++	0	0	0
			364	3.2	++++	0	+	+
			553	3.7	++++	0	+	+
			727	2.2	++	0	++	++
	SWSOOS North Barrel	Crown	268	4.9	++++	++	+	0
			364	4.9	++++	++	+	0
			553	4.2	++++	++	+	0
			924	2.13	++	0	++	++
	SWSOOS North Barrel	Tidal	553	5.6	++++	0	0	0
			727	3.4	+	0	+++	++
Melbourne	WTS	Crown	231	3.2	++++	+	+	0
			382	2.88	+	+	++	++
			563	1.53	+	0	+	+++
	WTS	Tidal	231	4.8	++++	0	0	0
			563	3.8	++++	0	0	0
	SET	Crown	231	2.21	0	0	++	++
			563	1.63	0	0	+	+++
	SET	Tidal	382	2.65	+	0	++	++
			563	1.6	+	0	+	++
Perth	Perth MS	Crown	215	2.82	+	+	++	++
			418	0.98	0	0	0	+++
			796	−0.05	0	0	0	++++
			924	2.95	0	+	+	+++
	Perth MS	Tidal	418	1.3	+	0	+	++
	Bibra Lakes	Crown	215	2.99	+	++	++++	+
			418	3.53	+	++	++++	+
			579	2.12	+	0	+	+
			796	1.75	0	0	+	+
	Bibra Lakes	Tidal	215	3.07	+	0	++++	+
			418	1.18	+	0	+	+++
			796	1.9	+	0	+	+++
			924	1.68	0	0	+	+++

Growth scale: ++++ (>60% spread or heavy growth); +++ (30–60% spread or medium growth); ++ (10–30% or light growth); + (<10% spread or trace growth); 0 (no growth).

organic acids will depend on the type of acid, pKa and solution pH which determines the acid dissociation. Organic acids are mostly un-dissociated at pH below 3.0 (Koenigsberger et al., 2000). Thus the inhibitory effect of organic acids to bacteria will increase with reduction in pH or as the organic acid concentrations increases. For the sequential growth process to proceed to allow the acidophilic bacteria to proliferate, however, the organic acid production must decline. This aspect was examined further below.

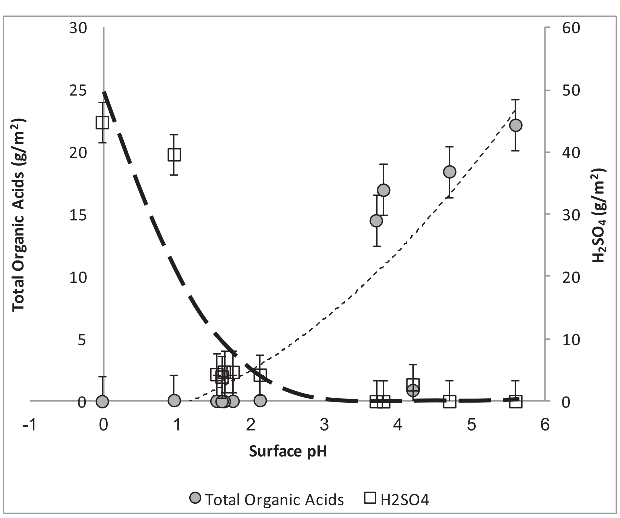


Fig. 4. The effect of surface pH on the metabolised acid epoxy A at 18 months of internment.

3.2.2. Effect of surface pH

As shown above, the growth of fungi and heterotrophic bacteria and the corresponding generation of organic acids coincided with suppressed growth and metabolism of SOB. Eventually the activity of fungi, as shown in Fig. 3, declined with time paving for the growth of the acidophilic bacteria. A correlation of the biogenic acids generated plotted as a function of the epoxy surface pH in Fig. 4 may offer some explanation for the decline in organic acids.

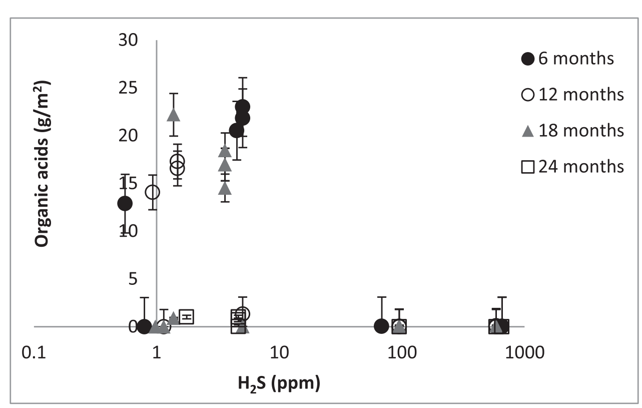


Fig. 5. The effect of H_2S concentration on the metabolism of organic acids on epoxy A mortar installed in the crown and tidal zone with time.

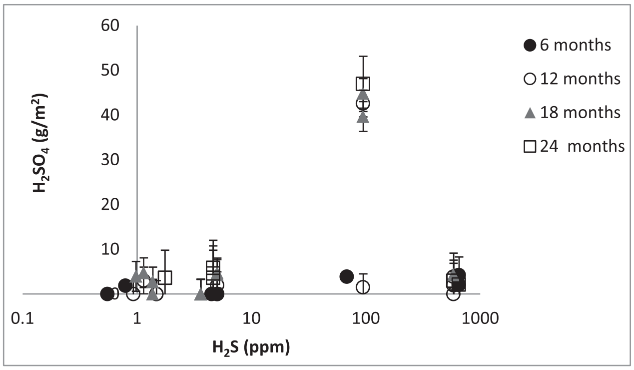


Fig. 6. The effect of H_2S concentration on the metabolism of sulphuric acid on epoxy A mortar installed in the crown and tidal zone with time.

As shown, at pH above 3.0, there is a preferential generation of organic acids, whilst very little sulphuric acid is generated. As the pH approaches 2.0, there is a significant reduction in organic acid generation accompanied by an increase in sulphuric acid generation. As Table 4 shows, the growth of fungi and heterotrophic bacteria decreased as pH declined below 3.0. Most fungi and heterotrophic bacteria can tolerate acidic conditions through an intracellular detoxification strategy whereby the cells establishes a low proton membrane permeability (Nica et al., 2000). Despite this most acidophilic fungi, for example, are only able to tolerate pH down to 2.0–3.0, with very few species tolerating pH below 1.6 (Johnson, 1998). We propose the significant reduction in organic acid generation as surfaces pH achieves pH < 3.0 is associated with the acid inhibition of the fungi and heterotrophic bacteria.

3.2.3. *Effect of H_2S concentration*

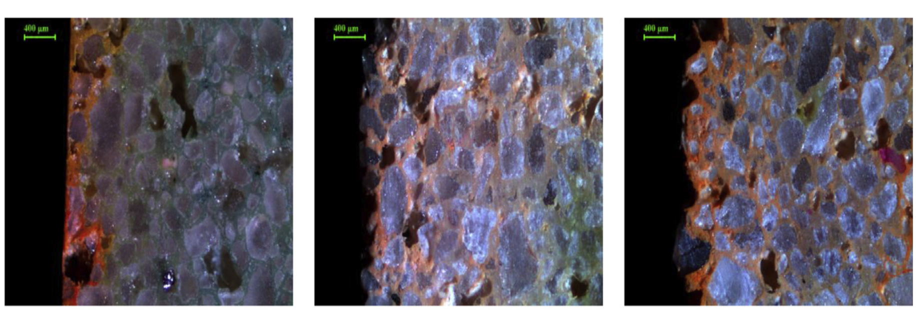
The H_2S concentration has been indicative of the corrosivity of sewer environment (Gu and Gu, 2005; Islander et al., 1991; Mehta et al., 1979; Parker, 1945). This is attributed to the fact that sulphide compounds including H_2S is used as nutrient and energy source by the sulphur oxidising bacteria to generate sulphuric acid. However our results show that acid metabolism does not correlate consistently with H_2S concentration.

To explore the role of H_2S on microbial growth and metabolism, the organic and sulphuric acid generated on epoxy A mortar are plotted as a function of H_2S concentration in Figs. 5 and 6 respectively. In sewer environments containing less than 10 ppm of H_2S, organic acids were preferentially generated (Fig. 5). As shown in Fig. 6, while the organic acid concentrations were high sulphuric acid generation was suppressed, which lends further support to the notion of the inhibitive effect of organic acids to SOB (see Fig. 6). At higher H_2S concentration (>10 ppm), organic acid generation was suppressed and is consistent with previously observed inhibitive effect of H_2S on the heterotrophs growth, primarily that of fungi (Koch et al., 2002). Furthermore the lower growth of fungi in coupons installed in Perth sewers, where H_2S concentrations are greater than 68 ppm (see Table 4), provides further evidence on the inhibitive effect of H_2S on fungi.

The sulphuric acid generation increased significantly from 4.7 g/m^2 to 46.9 g/m^2 as H_2S was raised from 5.5 to around 95 ppm. This was however followed by a significant decline in sulphuric acid generation (4.2 g/m^2) as H_2S increased to 500–650 ppm. These results and the lower growth of acidophilic bacteria in Bibra Lakes (see Table 4) suggest the toxicity of H_2S has a similar inhibiting effect on bacterial microbes, as it does with fungi in the sewer. However the tolerance of bacteria appears higher. H_2S has been reported to fully inhibit the growth of sulphur reducing bacteria (SRB) at concentrations of 500–600 ppm (Gu, 2003; Reis et al., 1991). Sulphur reducing bacteria is responsible for the initiation of the corrosion process by converting sulphide compounds to bisulphide but predominantly to H_2S in the sewage (Davis et al., 1998). H_2S that partitions into the gas phase is oxidised by sulphur oxidising bacteria to H_2SO_4. The inhibition of sulphur reducing bacteria interrupts the sulphur cycle and eventually hampers the sulphuric acid production as observed in Fig. 6. Although the direct inhibition of sulphur oxidising bacteria by H_2S alone has not been noted, the reaction of SO_4^{2-} oxidised from H_2S with another malodorous sewer gas, NH_3, to form $(NH_4)_2SO_4$ has been shown to inhibit both SOB (Nixon, 1997) and SRB (Pikaar et al., 2014). Ammonia gas was not monitored in this study, however this gas with H_2S and volatile organic compounds make up typical constituents of sewer gases (Hernandez et al., 2012; Kim et al., 2013; Upadhyay et al., 2013) and may have also impacted the growth of both SRB and SOB. Our results show the potential roles played by the organic acid and H_2S metabolites in the growth and metabolism of fungi and acidophilic bacteria.

3.3. *Relative acid permeation of organic and sulphuric acid*

The acid permeation of the epoxy at the early phase (after 12 months) of internment were compared for coupons installed Sydney South barrel, Melbourne SET and Perth MS in Fig. 7. The choice of these coupons was based on the preferential generation of organic acids in early phase (6 months) in the coupons at the South barrel (17 g/m^2), while sulphuric acid were preferentially generated in Melbourne SET (1.8 g/m^2) and Perth MS (3.8 g/m^2) (see Table 3). The photographs in Fig. 7 show acid permeation examined by



Sydney South Barrel — Permeation: 560 ± 67 μm
Melbourne SET — Permeation: 2604 ± 390 μm
Perth MS — Permeation: 3242 ± 648 μm

Fig. 7. Acid Permeation through the epoxy coatings after approximately 12 months of internment in the crown.

microscopy using a Universal indicator to highlight the pH profile across the specimens. The orange tinge from the left of the samples reflects the acid ingress. As shown, the acid permeation in South barrel (94 μm) is significantly lower compared to Melbourne SET (560 μm) and Perth MS (1800 μm). This shows the relative migration of sulphuric acid is greater than organic acids emphasising the greater impact of acidophilic bacteria on the service life of epoxy coatings.

4. Conclusions

This study showed that microbial colonisation of epoxy mortar and reduction of its surface pH to within highly acidic conditions (<pH 2.0) can be achieved in less than two years after its installation in the sewer. Recognition of this is quite important as coatings, such as epoxy mortar, appear to show the least resistance to sulphuric acid permeation. This study demonstrated the role of sequential growth of organism in reducing the pH of epoxy mortar in sewers through the analysis of microflora and the corresponding metabolic acidic products generated in the lining installed in various sewer environments. The process followed two distinct phases; the first involving the generation of organic acids by heterotrophic fungi and bacteria and the second phase involving the decline in organic acid and simultaneous generation of sulphuric acid, as they are generated by acidophilic sulphur oxidising bacteria.

This study also showed the accumulation of microbial metabolites; principally organic acids and H_2S, coincided with inhibition of both fungi and acidophilic bacteria. As the surface pH of the epoxy lining declined, it was proposed that the organic acids become more toxic to the fungi. The inhibitory effect of organic acids has been noted to be greater in their un-dissociated state, which would occur at the lower pH (<2.0). The decline in organic acid concentration paved for the growth of the sulphur oxidising bacteria, which could not otherwise grow because of organic acid inhibiting effects on bacteria. Generation of organic acid to concentrations exceeding 5 g/m^2 appear to suppress bacterial growth and sulphuric acid generation, whilst fungi inhibition required a higher organic acid concentration of >16 g/m^2. This was attributed to diffusion and accumulation of organic acids within the cell cytoplasm inhibiting cellular functions. The inhibiting effect of H_2S above 10 ppm was noted for fungi and organic acid generation and above 500 ppm for sulphur oxidising bacteria. Elevated concentrations of H_2S was noted to be toxic to SRB, whilst the combination of H_2S and NH_3 was proposed to inhibit both SRB and SOB.

These results provide insights into the microbe growth and metabolism in epoxy linings. It revealed the speed of microbe colonisation and metabolism of biogenic acids on the surface of the epoxy linings. The nature and concentration of the biogenic acids and the microflora that contributes to the degradation and eventual corrosion of concrete sewer pipes were established. The role of successive growth and the potential role of microbe metabolites in controlling the growth and metabolism of the fungi and acidophilic bacteria have been demonstrated. These conclusions were derived partially based on culturing methods to analyse the microflora. Because of the limitations of culture based methods, as used in this study, further tests involving molecular methods to accurately enumerate the microbe population of coating exposed to sewers will be required to confirm these results. Nonetheless the insights in this paper provide a basis by which further studies could be performed into coating and microbe interactions and mitigation strategies could be designed and implemented that would fit the environmental conditions and to further the life of both the lining materials and concrete.

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