Encouraging microbial activity in cementitious systems: An emerging frontier in contaminated soil treatment

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Abstract

Bioremediation is widely accepted as the most effective remediation technology for organic contamination. Nevertheless, it is ineffective for heavy metals, which cannot be degraded, and their immobilisation through pH control achieved with high-pH cementitious materials is one of very few feasible means of treatment available. However, progress is being made in encouraging microbial activity in cementitious systems, which could provide a single technology that is effective and can robustly be used for simultaneous treatment of organic and metallic contaminants in contaminated soils. This work considers efforts in this direction; the successes achieved and the challenges encountered are described. The utility of relatively low-pH magnesium phosphate cement(s) and compost in providing a favourable environment for microbes, as well as the capacity of microbes like *Saccharomyces cerevisiae* and *Rhodococcus ruber* immobilised in the cement in degrading organics is highlighted. Overall, the findings are promising and are likely to expand the frontiers of bioremediation and stabilisation/solidification technologies for sustainable treatment of contaminated soils and may be extended to other applicable hazardous waste streams.

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Abstract

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technologies for sustainable treatment of contaminated soils and may be extended to other applicable hazardous waste streams.

Keywords: bioremediation, contaminated soil, magnesium phosphate cements, mini-review, stabilisation/solidification.

INTRODUCTION

Many contaminated soils are characterised by the concomitant presence of organics and heavy metals. Furthermore, most of the established treatment technologies are contaminant-group specific. This limits the deployment of a single treatment technology for complete detoxification of contaminated soils. This may also apply to some hazardous waste streams from the mining/extractive industries and other chemical industries with similar characteristics. A few examples include petroleum sludge and chrome tanning waste from the leather industry. In the light of the above, concerted efforts have been made to develop techniques that could handle mixed contamination including organics and heavy metals. Such techniques systematically reduce the toxicity of heavy metals, while allowing for the oxidation of organic compounds. The combination of chemical oxidation, used for organics, and electrokinetic remediation, used for heavy metals, has been investigated in this regard and it yielded good results for a soil artificially contaminated with phenanthrene and nickel[1]. Moreover, progress has been made in recent times to combine bioremediation and stabilisation/solidification (S/S). These are two different technologies known to be very efficient for specific groups of contaminants – bioremediation for organics and S/S for heavy metals – and efforts aimed at combining both techniques forms the subject matter of this paper.

Biodegradation, involves transformation of organic contaminants through metabolic processes of micro-organisms;[2] while S/S involves chemical fixation and physical encapsulation of contaminants, mainly heavy metals, within the matrix of cementitious materials[3]. Individually, the technologies have limited ability for both contaminant groups. On one hand, bioremediation is not suitable for treatment of heavy metal contamination as it cannot really degrade and eliminate metals, although in some cases microorganisms have been used for the immobilisation of metals.[4] On the other hand, S/S is not suitable for organic contamination since organics are known to affect cement reactions. Hence, combining both techniques will address their individual limitations and utilise their strengths for a better treatment system. Further, since contaminants generally fall within either of the aforementioned groups, a technology that combines both techniques would provide a robust and sustainable treatment system wherein metals are immobilised within the matrix of a cementitious binder, while organics are biodegraded over time within the same system.

This work briefly discusses the findings of the few studies that have sought to actualise the robust treatment system for contaminated soils considered here. It highlights the successes achieved so far and the challenges encountered. It is aimed at improving the understanding of the scientific community on the potentials of this emerging frontier in contaminated soil treatment.

BIODEGRADATION AND CEMENTS: BRIEF HISTORICAL PERSPECTIVE

Several workers have studied biodegradation in cementitious systems, albeit with a different perspective. Most of the studies focussed on microbial influenced degradation of cements, which presents problems should microbial growth occur or be encouraged in cementitious systems.

Such problems could include corrosion of cement by sulphuric acid produced by sulphur-oxidising bacteria, as well as other organic and mineral acids. Others are formation of active biofilm on the surface of cement-solidified wastes, and gas formation within cementitious systems leading to cracks and loss of homogeneity.[5-9]

One study in similar direction with the object of this paper focussed on the biological clean up of concrete surfaces contaminated by n-hexadecane and naphthalene.[10] It was observed in the said study that bacteria removing n-hexadecane from concrete did not exhibit any significant growth within a 30 day incubation period due to slow diffusion of the contaminant, which was also the carbon/energy source, in concrete. Biodegradation of the contaminant absorbed in concrete by strains of *Pseudomonas aeruginosa* and two unidentified bacteria was reduced 7 times compared to biodegradation of the 'neat' contaminant not absorbed in concrete. Biological removal of naphthalene was not straightforward due to abiotic losses arising from its higher volatility compared to n-hexadecane. All the same, about 20 – 55% of the initial contaminant concentration was biodegraded compared to 70 – 80% for hexadecane. It was concluded that hydrocarbon diffusion within the pores of concrete was the rate-limiting step for biodegradation in such media.[10] However, it is noteworthy that this work dealt with decontamination of concrete surfaces by microbial biomass rather than contaminants fixed in a cementitious system.

Furthermore, there is fair amount of work on microbial influenced degradation of concrete employed in the immobilisation of radioactive wastes.[5,11] Three different genera of bacteria involved in cement degradation have been identified. These include sulphur-oxidising bacteria (e.g. *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*), nitrifying bacteria (e.g.

Nitrosomonas and *Nitrobacter*) and organic-acid producing heterotrophic bacteria (e.g. *Pseudomonas cepacia*).[11] Scanning electron microscopy of degraded cement/concrete samples used for immobilisation of strontium and caesium indicated cracks associated with formation of low-density corrosion products in the interior of the cement. The leaching of the major constituents of cement matrix, Ca^{2+} and Si^{2+}, were also shown to increase significantly during microbial degradation of the cement. This was attributed to the reaction between the biogenic sulphuric acid produced by sulphur-oxidising bacteria like *Halothiobacillus neapolitanus* and *Thiomonas intermedia* and free lime in cement.[8] Evidence from the active colonisation of low-level radioactive waste-form surface and the resulting formation of biofilm showed that bacteria involved in cement degradation were not adversely affected by cement chemistry. In fact, heterotrophic bacteria can grow in alkaline conditions (pH around 11) common on concrete surfaces and promote acidic conditions with $pH < 3$.[11] The result of the above is the eventual accelerated release of radionuclides due to microbial action.

Overall, it is the perspective of the above works not to encourage microbial activity in cementitious systems due to the likely disintegration of such systems and eventual migration of contaminants contained within them. In contrast, the following section deals with published works which considered the benefits of encouraging microbial activity in cements.

INCORPORATING BIODEGRADATION IN CEMENTITIOUS SYSTEMS

In the light of the above perspective, very few studies have considered incorporating biodegradation within cementitious systems for bioremediation applications. The findings of five studies which dealt with biodegradation within cementitious systems such as Portland cement,

magnesium oxide cements and magnesium phosphate cements, are considered here.[12-16] These are discussed in terms of microbial survivability within cementitious systems, biodegradation and immobilisation of contaminants, and the structural integrity of the cementitious system.

Microbial survivability within cementitious systems

Initial attempts to encourage microbial growth in cementitious systems entailed using a range of additives to Portland cement-stabilised/solidified contaminated soil with a view to taking advantage of microbial survivability for a long period to degrade organic contaminants. Such additives included compost as source of microbes and nutrients, other nutrient sources and perhaps air entraining and water retaining agents. It was learnt that the effect of compost was more significant than the other additives.[12,13] This is because of the numerous and diverse microbial population associated with composts. Further, the high levels of substrate in compost can lead to cometabolism of organic contaminants.[17]

Subsequent efforts considered the utilisation of cement that might be much more benign to microbes in terms of providing a relatively lower pH closer to the optimum for bacterial survival (5.5 – 8.5) as opposed to using highly alkaline Portland cement (pH 12 – 13). Magnesium phosphate cements, which are formed at room temperature by rapid acid-base reaction between dead burned (or unreactive) magnesia (MgO) and an acid phosphate source,[18-20] and has the potential to form mixes with different pH ranges, depending on the constituents, was considered an obvious choice for the subject matter. Magnesium oxide cement, which is composed of reactive MgO have also been considered owing to its relatively lower pH of approximately 10. It is well known that conventional cement stabilisation of hazardous materials takes advantage of

the high pH of cement for immobilisation of metals since metals generally precipitate as insoluble hydroxides with minimum solubility within the pH range, 8 – 11. Thus, there are concerns on the capability of the afore-mentioned lower-pH cements to immobilise metals due to the potential for leaching of stabilised metals owing to increased metal solubility. However, it is documented that the phosphate reactions of magnesium phosphate cements convert hazardous contaminants into non-leachable phosphate reaction products and the cement encapsulates these insoluble reaction products into a dense and durable matrix.[21] Further, the phosphates of the contaminants have a much lower solubility than their oxides or other salts.[19] Hence, magnesium phosphate cements provide a very effective chemical immobilisation of contaminants.[13]

Majority of the studies in which microbial activity was encouraged in cementitious systems involved contaminated soil. In fact, four of the five studies considered here dealt with contaminated soils.[12-15] As mentioned earlier, compost was used as the source of microorganisms in these studies. It has to be pointed out that the addition of compost to the cementitious systems is expected to provide suitable refuges for microbes within the soil-cement matrix, and hence, protect them from the harsh cement environment.[13] To facilitate the release and activity of microorganisms within the cementitious systems, contaminated soil is amended with compost prior to mixing with the cements. Microbial activity testing carried out mostly between 14 – 28 days after the cement solidification process indicated that microbes were already acclimated and active in the cement environment within the above time frame.[12,14,15]

In contrast, the work of Soltmann et al[16], which did not involve contaminated soil, had bacteria directly mixed with magnesium phosphate powder and ammonium phosphate solution and the

resulting cement paste was allowed to solidify. Microbes were found to be active in the cementitious system within 24 hours after solidification. Electron micrographs of the cements showed that bacterial cells were even visible within the cement's macropores and fluorescence microscopic evaluations showed biofilms of tightly adhered bacteria on the surface of the cements.[16] Microbial colonies were also observed on the surface of the cement-solidified contaminated soils mentioned earlier[14] These suggest that the pore structure of magnesium phosphate cements allows for the transfer of nutrients and oxygen within the cements to keep immobilised microbes alive and active. Further, it also suggests preferential growth of microbes on the surface of the cements probably due to more benign conditions.

One major challenge with research in this area has been microbial activity testing. Different test methods normally employed for analysis of soil microbiological properties have been used for evaluating microbial growth and survivability in cementitious systems. These include the 2,3,5-Triphenyltetrazolium chloride (TTC) dehydrogenase assay, Ninhydrin assay, Glucose consumption assay and plate counts.[22] The most probable number technique was used in a related work that studied bacterial survival in concrete albeit with a view to apply bacteria as self-healing agent for development of sustainable concrete.[23] Interestingly, we learn from the said study that alkali-resistant spore-forming bacteria embedded within hard concrete remained viable for up to 4 months.

The TTC dehydrogenase assay,[24] which is normally used as a proxy for microbial activity featured in most of the few studies in this area. The test showed good results depending on the cement type and specifically the mix formulation of the cement. With Portland cement, there are

no problems using the test.[12-14] However, in magnesium phosphate cement formulations, the test could yield good results depending on the mix formulation. In other words, the ratio of unreactive MgO to an acid phosphate source or more specifically the type of acid phosphate used, determines the outcome of using TTC dehydrogenase activity in the cementitious systems. Potassium dihydrogen phosphate (KH_2PO_4) was used as acid phosphate source in the works of Harbottle and Al-Tabbaa[12,14] and the test had good results. However, with calcium dihydrogen phosphate as acid phosphate source (from triple super phosphate, TSP – a common fertiliser), the outcome was different. It was observed that unreactive MgO apparently stimulated dehydrogenase activity, while TSP apparently inhibited or suppressed dehydrogenase activity. In a special case, dehydrogenase activity was not observed for the first 84 days as it was suppressed by TSP. However, dehydrogenase activity was observed after 140 days, indicating that its suppression by TSP subsides over time. Prior to these findings, a number of substances that could cause similar stimulatory and inhibitory effects on dehydrogenase assay results have been identified in the literature[22] but none of the cement's constituents were listed. Furthermore, the dehydrogenase assay has been used with bacterial and fungal counts in several studies,[22] as well as in previous related studies[12,14] without any problems. Hence, the results of the assay could be used with confidence. However, the observation that magnesium phosphate cement's constituents interfere with the results of the dehydrogenase assay questions the suitability of the assay for evaluating microbial activity in the cementious system.

Figure 1 summarises selected dehydrogenase assay results (representative of the general findings) for cementitious systems, from previous studies in which microbial activity was encouraged through incorporation of compost (10% of mixture, w/w).[14,15] Similar binder dosages

of magnesium phosphate cements (33%, w/w) were used in both types of magnesium phosphate cements (i.e. the potassium and calcium phosphate-based cements in Figures 1a and 1b, respectively); while 25% dosage (w/w) was used for Portland cement. However, different contamination levels were used; 700 mg/kg of an organic, 2-chlorobenzoic acid (2CBA) was used in all cases in Figure 1a and 3,000 mg/kg each of 2CBA, lead nitrate and zinc chloride in Figure 1b. The results show that the level of soil contamination affects the extent of microbial activity in the cementitious systems (Figure 1). Potassium phosphate-based magnesium phosphate cement with pH ~8.5 was also found to sustain microbial activity over a longer period than Portland cement with a more alkaline pH (~12) even though Portland cement showed much higher initial response (Figure 1a). Microbial survival in the very alkaline Portland cement matrix corroborates the earlier mentioned observation of Rogers et al[11] on bacterial growth in alkaline conditions common in concrete used for immobilisation of radionuclides. Furthermore, use of higher magnesia content in magnesium phosphate cement formulations, effectively immobilised heavy metals after the standard curing age of 28 days and thus counteracted their effect in suppressing microbial activity (Figure 1b).

The above dehydrogenase activity results have also been corroborated by bacterial and fungal counts. This points to the fact that even in unoptimised systems, microbes could still survive in such environments for up to five months while degrading available organics.[14,23] Table 1 gives an overview of biological tests carried out in studies aimed at encouraging microbial activity in cementitious systems. Majority of the studies dealt with contaminated soils amended with compost, with only the work of Soltmann et al[16] considering degradation of an organic contaminant, phenol, by fungal and bacteria cells (*Saccharomyces cerevisiae* and *Rhodococcus*

ruber) embedded in the cement. In nearly all cases, the incubation temperature of the cement samples was elevated to at least 30°C in order to encourage biodegradation. All the same, there was still evidence of microbial activity in the work of Harbottle and Al-Tabbaa[12] in which cement samples were incubated at 23°C. It is clear from Table 1 (Harbottle and Al-Tabbaa[12,14]) that a lower cement pH favoured fungal growth, just as the higher pH of Portland cement led to considerable detrimental impact on microbial activity.

Most of the studies listed in Table 1 were carried out in unoptimised systems as there were no concerted efforts to first determine the optimal mix formulations, microbial source and/or population.[12-16] Hence, in some cases the same tests did not yield the same response for even very similar cementitious systems. Overall, it can be deduced from the studies in Table 1 that microbial survivability and activity is possible for long periods in optimised cementitious systems whose pH are well above neutral but less than the highly alkaline pH of Portland cement. Specifically, a cementitious system with pH between 8.5 and 10 might be ideal especially in a mixed contamination scenario. This is because effective immobilisation of heavy metals has to be considered such that biodegradation of organic contaminants is not impeded by heavy metal stress. The above position is important notwithstanding that magnesium phosphate cements have certain advantages in this regard since the higher the pH that can still support effective biodegradation, the better the metal immobilisation achieved.

Biodegradation and immobilisation of contaminants

The extent of contaminant losses in cementitious systems considered in the few studies in this area are summarised in Table 2 and comments made on how they compare with the microbial activity results. Details of the cementitious systems have been in provided in Table 1, hence, only relevant details are repeated in Table 2. Degradation of the organic contaminant (2CBA) corroborated fungal count results as magnesium oxide cement, which showed fungal presence, had higher percentage reduction of the contaminant compared to the other cements that had no fungal presence (Harbottle and Al-Tabbaa,[12] Table 2). Generally, contaminant losses did not corroborate dehydrogenase activity results which suggest abiotic pathways of contaminant loss[14,15] (Table 2). All the same, it was observed that higher compost amendment to soil-Portland cement system led to a higher percentage reduction in contaminant loss and caused increased microbial activity[13] (Tables 1 and 2). It can be deduced from the findings in Tables 1 and 2 that loss of organic contaminants in the cementitious systems is due to biodegradation and to some extent other abiotic factors. This is supported by the fact that such cementitious systems on their own also have the potential for direct immobilisation of organics through interaction with the cement's hydration products.[3] The results of the only two organic contaminants considered so far (2CBA and phenol) suggest that with optimum amounts of the right microbial consortium, increased biodegradation of organics can occur effectively in cementitious systems.

A major issue to contend with in this area is the isolation of abiotic losses from biodegradation in the cementitious systems. Moreover, the fact that there are not many non-volatile organic compounds that are easily biodegradable probably confined the researchers to 2CBA, which is non-volatile and easily biodegradable, and phenol, due to its low volatility. The major pathways

for abiotic losses in the cementitious systems considered here include irreversible sorption of contaminants to cement matrices, volatilisation and reductive dechlorination of chlorinated organics in the presence of Fe(II).[25] It is likely that one of these mechanisms may have contributed to organic contaminant losses in the studies considered here. However, the difficulty of distinguishing between biotic and abiotic losses in the cementitious systems even with the use of autoclaved controls[12,15] probably explains why there was no focus on isolating abiotic losses from the organic contaminant loss recorded in any of the studies. Future studies in this direction are required to give credence to claims of effective biodegradation in the cementitious systems.

Furthermore, in a mixed contamination scenario, there is evidence that biological activity in the cements is not detrimental to immobilisation of heavy metals as well as the structural integrity of the stabilised/solidified material. The leachability of metals like lead and zinc in cement matrices containing biological life, especially magnesium phosphate cements, was well below the most stringent (inert) landfill waste acceptance criteria.[15] The cements also showed good compressive strengths, elastic stiffness and hydraulic conductivity similar to those of solidified systems without biological activity.[15,16] Furthermore, the cements were found to show other important advantages like high chemical and thermal stability. The fast setting time and high early strength of the cement matrix made its use as a ‘biocatalyst’ possible shortly after preparation and embedded bacterial cells were not prone to drying in consequence of the preparation process.[16]

CONCLUSION

Cementitious systems have established capabilities for immobilisation of heavy metals, owing to their high pH regimes, which corresponds to the solubility minima of most heavy metals. Incorporation of a biodegradative mechanism in cementitious systems through a rich microbial matrix like compost, and direct immobilisation of microbial cells in the cements, have been investigated in recent times with a view to developing a robust sustainable treatment technology that can deal with a wide range of contamination in soils and other hazardous waste streams. This work has highlighted the efforts in this direction and the challenges encountered.

It has been shown that with proper optimisation, relatively lower-pH magnesium phosphate cements can facilitate effective heavy metal immobilisation while providing a more benign environment for microbes to degrade organics. Construction of the cements at pHs lower than those of conventional cementitious systems does not pose problems for metal immobilisation as the cement's phosphate reactions provide very effective chemical immobilisation mechanisms. Biodegradation of organic contaminants like 2CBA and phenol within the cementitious system have been demonstrated alongside effective immobilisation of heavy metals like lead and zinc. The utility of compost and microbes like *Saccharomyces cerevisiae* and *Rhodococcus ruber* in facilitating the biodegradation has also been highlighted. Further research into the optimisation of the 'bio-cementitious systems' as well as development of effective biological testing methods are necessary to fully harness the potentials of this emerging frontier in contaminated soil treatment and may be extended to other applicable hazardous waste streams.

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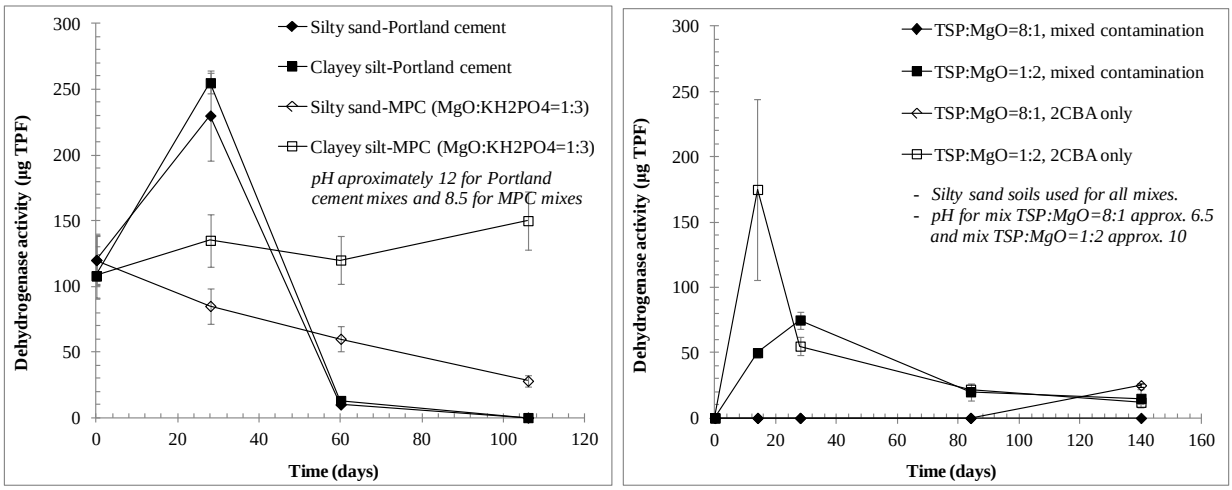

(a) **(b)**

MPC: magnesium phosphate cements, KH_2PO_4: potassium dihydrogen phosphate, TSP: triple super phosphate, MgO: dead burned magnesia, 2CBA: 2-chlorobenzoic acid
10% compost (w/w) added to all mixes; binder dosage (w/w): 33% for MPC and 25% for Portland cement mixes
All mixes had: 700 mg/kg 2CBA in Fig. 1a, and 3,000 mg/kg each of 2CBA, lead nitrate & zinc chloride in Fig. 1b

Figure 1. Selected dehydrogenase assay results for (a) Portland cement and potassium phosphate-based magnesium phosphate cement[14] (b) calcium-phosphate based magnesium phosphate cement[15]

Table 1. Overview of testing biological activity in cementitious systems

Literature	Soils / cements & contaminants	Time (days)	System pH	Biological test details	Outcome / Comments
Harbottle and Al-Tabbaa[12]	- Silty sand and clayey silt soils mixed with green waste compost. - Portland cement (PC), magnesium oxide cement (MOC) and magnesium phosphate cements (MPC) compared. - 2-chlorobenzoic acid (2CBA) used as organic contaminant (500 mg/kg).	- 59 for Portland cement samples. - 150 for magnesia-based cement samples.	- 12.5 for PC. - 11.0 for MPC. - 10.0 for MOC.	- Plate counts for bacteria and fungi done at 37°C.* - Dehydrogenase assay carried out at 30°C. - Ninhydrin assay. - Aerobic incubation of cement samples at 23ºC.	- No trends visible in bacterial counts over time, great variability between replicates. MOC samples showed fungal growths, but others did not. - Dehydrogenase and ninhydrin assays showed no positive results.
Al-Tabbaa et al[13]	- Silty sand with 2 levels of compost. - Spiked with 2CBA (amount not stated). - PC used for stabilisation treatment.	106	Varied from 12.5 – 10.0 over 106 days.	- Dehydrogenase assay carried out at 37°C. - Aerobic incubation of cement samples at 37°C and 95% RH.	- Moderate activity visible and sustained over 106 days decreasing by a factor of 100 during the period. - Higher compost level led to increased microbial activity.
Harbottle and Al-Tabbaa[14]	- Silty sand and clayey silt soils with and without green waste compost. - PC and MPC used for stabilisation. - 2CBA used as organic contaminant (700 mg/kg).	106	- Varied from 12.5 to 10.0 for PC systems. - Fluctuated around 8.5 for MPC systems.	- Plate counts for bacteria and fungi done at 21°C.* - Dehydrogenase assay carried out at 30°C. - Aerobic incubation of cement samples at 32°C and 95% RH.	- Compost amendment led to considerable increases in dehydrogenase activity and microbial counts. - PC had considerable impact on dehydrogenase activity and culturable microbial numbers.
Kogbara et al[15]	- Silty sand mixed with eco-compost. - Spiked with 2CBA, and lead nitrate and zinc chloride as heavy metals. - 2 MPC formulations used for treatment (see Fig. 1b).	140	Around 6.5 for higher phosphate mix and 10 for higher MgO mix.	- Dehydrogenase assay carried out at 37°C. - Aerobic incubation of cement samples at 32°C and 95% RH.	- MgO exhibited stimulatory effect on dehydrogenase activity while TSP exhibited inhibitory effect. - Dehydrogenase activity evident in mixes with heavy metals.
Soltmann et al[16]	- No soil involved, microbes mixed with cement. - MPC used as cement, made by mixing magnesium phosphate powder with ammonium phosphate solution. - Phenol used as organic contaminant. - *S. cerevisiae* (yeast) cells and *R. ruber* bacteria mixed with cement.	19	Neutral pH conditions	- Glucose consumption carried out at 30°C. - Aerobic incubation of cement samples at 30°C.	- Embedded yeast cells showed reasonable glucose consumption although it took over 10 times the time taken by non-embedded yeast cells to consume same amount of glucose. - embedded yeast and *R. ruber* cells survived and remained active within the cement during test period.

2CBA: 2-chlorobenzoic acid; PC: Portland cement; MPC: magnesium phosphate cement; MOC: magnesium oxide cement; MgO: dead burned magnesia; TSP: triple super phosphate; RH: relative humidity; S. cerevisiae: Saccharomyces cerevisiae; *R. ruber*: Rhodococcus ruber. *Dehydrogenase assay was carried out for 24 hours in all cases.*

**Plate counts were taken after 1 and 2 days, for bacteria and fungi, respectively, incubated at 37°C, and 5 and 6 days for bacteria and fungi, respectively, incubated at 21°C.*[12,14]

Table 2. Contaminant losses in cementitious systems with microbial activity

Literature	Contaminants studied	Initial contaminant concentration	Final contaminant concentration	Time (days)	Percentage reduction (%)	Comments
Harbottle and Al-Tabbaa[12]	2CBA	500 mg/kg	~ 480 mg/kg lowest for PC & MPC samples. ~150 mg/kg lowest for MOC samples.	- 59 for PC samples. - 150 for MPC and MOC samples.	- 4 for PC and MPC samples. - 70 for MOC samples.	- It corroborated the fungal count results as only MOC samples showed fungal growth in the microbial tests. - Extraction of contaminant was performed using sulphuric acid digestion followed by ethanol extraction.
Al-Tabbaa et al[13]	2CBA	Not stated, percentage recovery used	Not stated, percentage recovery used	106	98 60	0.2g compost/g soil in silty sand-PC system led to 98% contaminant reduction compared to 60% for 0.025g compost/g soil.
Harbottle and Al-Tabbaa[14]	2CBA	700 mg/kg	~ 20 mg/kg lowest for PC samples. ~ 400 mg/kg lowest for MPC samples.	106	- 97 for PC samples. - 43 for MPC samples.	Generally, the contaminant losses did not corroborate dehydrogenase activity although within the first 28 days, the activity in PC samples was twice that in MPC samples.
Kogbara et al[15]	2CBA Lead Zinc	3,000 mg/kg 1,877 mg/kg 1,439 mg/kg	- 1,688 mg/kg of organic was lowest achieved. - 0.0045 and 0.34 mg/kg leachable concentrations of Pb and Zn, respectively.	140	- 44 for organic contaminant.	- Contaminant loss did not corroborate the dehydrogenase activity test results as samples in which dehydrogenase activity was inhibited exhibited similar contaminant loss. - Abiotic sources of contaminant loss like interaction between cement's hydration product(s) and contaminant suggested. - Biological activity was not detrimental to heavy metal immobilisation. - Metal leaching done with water at neutral pH.
Soltmann et al[16]	Phenol	500 mg/l	0 mg/kg	- 1.04 (25 hrs) - 1.67 (40 hrs)	- 100 - 100	- 224 mg of immobilised *R. ruber* cells used. - 1,118 mg of immobilised *R. ruber* cells used. - Compared to the above, non-immobilised *R. ruber* cells degraded 80% of available phenol in 0.625 days (i.e. 15 hours). - dye formation with 4-aminoantipyrine and $K_3Fe(CN)_6$ used to obtain phenol concentration.

2CBA: 2-chlorobenzoic acid; PC: Portland cement; MPC: magnesium phosphate cement; MOC: magnesium oxide cement; $K_3Fe(CN)_6$: *potassium hexacyanoferrate(III)*