Antimicrobial mortar surfaces for the improvement of hygienic conditions

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Introduction

The control of micro-organisms is a major concern in a variety of environments, including domestic and industrial settings. In medicine, materials impregnated with various types of antimicrobial systems have been in use for years. More recently, surfaces that can decrease microbial loads have been introduced in the food, agricultural, waste water and building industries (Cowan et al. 2003; Navas Martin and Borralleras Mas 2005; Møretrø et al. 2006).

The active component of an antimicrobial system can either be organic or inorganic. Organic systems are generally based on small molecules that may contain a metal ion. They are incompatible with a polymer matrix and, therefore, diffuse to the surface until equilibrium is reached. Diffusion out of the polymer only occurs when the surface is wiped or washed (D’arcy 2001). Well-known examples are triclosan and benzalkonium chloride. Triclosan has been incorporated into a wide range of domestic products such as food boxes, toothbrushes and hand-washing gels (Braid and Wale 2002). In hospital settings, triclosan has been applied in fabrics, surgical gowns and gloves (Kalyon and Olgun 2001) and body washes (Russel 2002). Inorganic systems are based on metal ions, stabilized in some way, so that they are not reactive until released in association with another agent, such as moisture. The metal ions are typically formulated in a glass or inert material (D’arcy 2001). In the last two decades, the use of zeolites supporting bactericidal metal ions, such as...
silver and copper ions, has achieved considerable attention (Rivera-Garza et al. 2000; Cowan et al. 2003; Matsumura et al. 2003). The durability of the antimicrobial activity and the low toxicity for humans have resulted in an extensive use of these antimicrobial ceramics in a wide range of areas, including food preservation, dentistry, disinfection of medical supplies, and disinfection of surfaces and materials, such as toys and kitchen wares (Nikawa et al. 1997; Hotta et al. 1998; Kawahara et al. 2000; Bright et al. 2002; Matsumura et al. 2003).

For the production of antimicrobial concrete and mortar formulations, both organic and inorganic systems have been used. Antimicrobial phenol derivatives have been used as admixtures for the construction of antimicrobial floors and wall coverings made of concrete (Wayne Freed 2000; Ramirez and De Leon 2004). Navas Martin and Borraleras Mas (2005) reported the effectiveness of a concrete with polypropylene fibres treated with a combination of biocide and fungicidal agents in improving the hygienic conditions of concrete built agricultural premises. These authors noticed an inhibition of the growth of Aspergillus niger, Staphylococcus aureus and Escherichia coli when a piece of antimicrobial concrete was placed on inoculated agar plates. Do et al. (2005) investigated the antifungal effects of cement mortars containing organic antifungal agents. While a good antifungal effect was observed for the mortars containing isothiazoline and carbamate, no inhibitory effect was observed for the nitrofuran-containing mortars. The use of antimicrobial admixtures and coatings has also been investigated for the prevention of biogenic sulfuric acid (BSA) corrosion in concrete sewers (Shook and Bell 1998; Yamanaka et al. 2002; Haile et al. 2008; De Muynck et al. 2009). Depending on the type and concentration of biocides, a complete inhibition of the sulfur-oxidizing bacteria was observed. Recently, an antimicrobial concrete containing zeolites with silver and copper ions (Zeomighty, Sinanen Co., Japan) has been introduced into the Japanese market. According to the tests performed by the manufacturer, a concentration of metal zeolites of 1% on cement weight base is optimal for the inhibition of the growth of Thiobacilli, and hence, prevention of BSA corrosion. Furthermore, Zeomighty would also be effective against a variety of pathogens including E. coli, Staph. aureus and Pseudomonas aeruginosa (http://www.zeomic.co.jp/english/05_01_zeomighty.html).

This article reports on the effectiveness of antimicrobial mortars containing triclosan (Microban® B)-incorporated fibres (Fibermesh Division, Synthetic Industries Inc., Chattanooga, TN) or silver copper zeolites in inhibiting the growth of various pathogens of environmental concern, i.e. E. coli, Listeria monocytogenes, Salmonella enterica and Staph. aureus. The antimicrobial effectiveness was quantified by means of the survival of the micro-organisms after 24-h incubation on mortar prisms. Survival was determined by means of plate counting and adenosinetriphosphate (ATP) measurements.

Materials and methods

Mortar specimens

Three series of mortar mixtures have been made, i.e. one control series and two antimicrobial series. Antimicrobial mixtures had the same composition as the reference mixtures with exception of the addition of silver copper zeolites (1%, 2%, 3%, 4% or 4.5% on cement weight base; zeolites contain 3.5% silver and 6.5% copper on weight base) or antimicrobial polypropylene fibres (1 kg m⁻³ mortar). The active component of these fibres is 2,4,4’-trichloro-2’-hydroxydiphenylether (triclosan/Microban® B). In order to investigate the influence of the porosity on the efficiency of the antimicrobial mortar, for each series of mortar mixtures, mixtures with two different water to cement ratios (w/c) were produced, i.e. w/c 0.5 and w/c 0.65. The control mixtures consisted of 450 g CEM I 52.5 N, 1350 g normalized sand and 225 (w/c 0.5) or 292.5 g (w/c 0.65) tap water. Standardized mortar prisms (40 x 40 x 160 mm³), prepared according to European Standard EN196-1:1995 (1995), were cut into small prisms with dimensions 40 x 20 x 0.8 mm³. Prior to sawing of the specimens, prisms were cured for 28 days under humid atmosphere (95% relative humidity, 20°C).

As the surface pH of the 28-day-old mortar specimens was too high (pH 12-13) to allow bacterial growth, specimens used for the viability assay (see further) were subjected to a commonly used aging method, i.e. accelerated carbonation, prior to the assay (Do et al. 2005; Escadeillas et al. 2007). Carbonation results in the conversion of calcium hydroxide to calcium carbonate and concomitantly in a decrease in the pH level. Accelerated carbonation was achieved by exposing the specimens for 3 weeks in a conditioning chamber to CO₂ levels of about 10% (at 70 ± 10% relative humidity and 20°C). On a weekly basis, the pH of the mortar specimens was verified by placing a drop of demineralized water on the surface (ACI Committee-302 2006; ASTM, 2008). After 5 min of contact time, the pH of the water drop was measured by means of a pH indicator strip (pH 9-13; Vel N.V., Belgium). Once the pH of the surface amounted to about nine, the specimens were removed from the conditioning chamber. Subsequently, in order to remove some remaining basic compounds from the mortar matrix, specimens were immersed in demineralized water for 1 week (20 specimens in 500 ml), with daily replenishment of the water (Escadeillas et al. 2007). As all mortar specimens
were subjected to the same ageing procedure, differences in pH level were negligible, as could be observed from pH measurements with indicator strips. Hence, differences in survival of the micro-organisms because of the differences in pH of the mortar specimens should be negligible. Following this immersion procedure, specimens were dried in an oven at 80°C until a constant weight was achieved. Finally, prior to the viability assay, the mortar specimens were sterilized by means of exposure to an UV lamp under laminar flow for 12 h. Mortar specimens were not autoclaved in order to avoid substantial alterations of the mortar matrix.

Micro-organisms

Bacterial strains and growth media used for this study are presented in Table 1. *Escherichia coli* (Gram-negative, G−), *L. monocytogenes* (Gram-positive, G+) *Salm. enterica* (G+) and *Staph. aureus* (G+) were chosen as indicator organisms representing G− and G+ foodborne pathogens and hygiene indicator organisms of environmental concern (Ten Broeke 1975; Schlundt 2002). Furthermore, these species have been frequently used for the evaluation of biocides (Bright et al. 2002; Devere and Purchase 2007; Ruparelia et al. 2008), disinfection agents (Midelet and Carpenter 2004; Deza et al. 2005) and survival on the surfaces of different materials (Fatíndez et al. 2004; Oulalhal et al. 2008). All strains were obtained from the Belgian coordinated collection of micro-organisms (BCCM/LMG, Ghent, Belgium). Cultures used for the agar plate and viability assays were obtained after two subsequent cultures in Tryptic Soy Broth (TSB; Difco, France) starting from a –80°C stock culture.

Agar plate assay

Micro-organisms from an overnight grown culture (third culture starting from –80°C stock) were homogenously distributed over Tryptic Soy Agar (TSA; Difco, France) plates by means of a sterile cotton swab stick (Biolab Zrt., Romania). After inoculation, Petri dishes were left open in a laminar flow for 5 min. Subsequently, a 10 μl drop from a 0.01 g ml−1 zeolites suspension or a stack of antimicrobial fibres was placed on the TSA plates of the respective micro-organisms. The Petri dishes were then closed and incubated at 37°C. After 24 h, the plates were checked for zones of inhibition of bacterial growth.

Metal ions release

Mortar specimens were submerged in sterile centrifuge tubes (TPP AG, Trasadingen; Switzerland) containing 20 ml of sterile TSB or demineralized water for 3 h (one specimen per tube). Subsequently, 10 ml of this solution was taken for the determination of the concentration of silver and copper ions. The sample was prepared by adding 2 ml HNO3 and two times 0.5 ml H2O2, followed by subsequent heating and transfer to 50 ml vessels. Analyses were performed using a Varian Vista-MPX Inductively Coupled Plasma (ICP) optical emission spectrometer (Varian, Belgium) and a Perkin Elmer ICP-mass spectrometer (Perkin Elmer, USA).

Viability assay

Preparation

All manipulations for this assay were performed under a laminar flow. UV-sterilized mortar prisms were completely immersed for 30 min in a Petri dish (10 cm diameter) containing sterile saline solution (four to five specimens per Petri dish). Then, samples were transferred to a new Petri dish and left to equilibrate for 10 min. During these 10 min, the Petri dishes remained closed to prevent drying of the surface. As such, moist prisms were obtained without any excess of water on the surface. Next, for each bacterial strain and type of mortar, five replicates were transferred to a large Petri dish (15 cm diameter). This large Petri dish contained a small Petri dish (5 cm diameter) filled with water to sustain a high humidity. Subsequently, 100 μl of TSB was homogeneously

<p>| Table 1 Overview of the different bacterial strains and growth media* used in this study |
|-----------------------------------------------|-----------------------------------------------|-----------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Species</th>
<th>Accession number‡</th>
<th>Selective growth media</th>
<th>Supplement</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>LMG 8195</td>
<td>Baird-Parker (63 g l−1) (Oxoid, UK)</td>
<td>Egg-yolk (50 ml l−1) (Oxoid, UK)</td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>LMG 8063</td>
<td>MacConkey Agar (51-5 g l−1) (Oxoid, UK)</td>
<td>Technical Agar n.3 (3 g l−1) (Oxoid, UK)</td>
</tr>
<tr>
<td><em>Salmonella enterica</em></td>
<td>LMG 10 396</td>
<td>Chrom Agar (34-9 g l−1) (Chromagar™)</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Salmonella, France</td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>LMG 13 305</td>
<td>Aloa Listeria Agar (70-6 g l−1) (Biolife, Italy)</td>
<td>Aloa Enrichment Selective Supplement (Biolife, Italy)</td>
</tr>
</tbody>
</table>

*Used for agar plates.
‡Strains were obtained from the BCCM/LMG culture collection (Ghent, Belgium).
distributed over the moist surface of each specimen with a Transferpette (Brand GmbH & Co, Wertheim, Germany), in order to promote bacterial growth and biofilm formation. The Petri dish then remained closed for 10 min, in order to allow the superficial absorption of the TSB. Subsequently, 100 μl of an overnight grown bacterial culture (approx. 7–8 log cells ml⁻¹) was distributed over the surface with a Transferpette (Brand GmbH & Co). Afterwards, the Petri dishes were closed and stored under laboratory conditions (20 ± 3°C) or in a refrigerator (4 ± 1°C). In order to prevent the surfaces from becoming dry, the Petri dishes were sealed with parafilm and aluminium foil. After 24 h of incubation, the specimens still had moist appearance.

**Sampling**

After 24 h, the surface was sampled with a moist sterile cotton swab stick (Biolab Zrt.). The swab stick was moistened with sterile saline solution (glass tubes containing 9 ml of 8.5 g l⁻¹ sodium chloride). After sampling, the stick was placed again in the glass tube. Subsequently, the glass tube was vortexed (Labinco BV, Netherlands) for 30 s. The amount of cells and ATP presence in the saline solution were determined with the aid of the plate count technique on selective growth media (Table 1) and bioluminescence measurements (see further) respectively. The effectiveness of the sampling procedure has been evaluated by means of two series of experiments in control specimens that had been incubated with *Salm. enterica* for 24 h at 20°C. In the first series of experiments, the surfaces of six mortar specimens were subjected to two subsequent swab-sampling steps. The amount of bacteria determined with the second sampling procedure was always at least 1 log unit lower compared to the first sampling procedure. Similarly, the total ATP content differed by a factor of 10 between the two subsequent sampling procedures. In the second series of experiments, after one swab sampling, six mortar specimens were pushed against the respective selective agar plates. The specimens were removed from the plates after 5 min. Subsequently, the agar plates were incubated at 37°C. The amount of bacteria found on these plates after 24 h of incubation was below 200 colony-forming units (CFU). From the above, the swab procedure was considered to be a reliable method for the sampling of the surfaces.

**ATP measurements**

In addition to plate counting, ATP measurements were taken to obtain a rapid estimation of the survival of the micro-organisms. Determination of total ATP was performed by means of a Bac Titer Glo™ ATP kit (Promega, USA). A volume of 100 μl of liquid obtained from the tubes containing the swab sticks was mixed with 100 μl of Bac Titer Glo™ reagent and left to equilibrate at room temperature. After 5 min, the luminescence of the sample was measured by means of a Lumac Biocounter (Lumac, Netherlands). In the case of signal overload, measurements were taken on the first dilution that was used for the plate counting. For each mortar specimen, two ATP measurements were taken after the preparation of the dilution series used for plate counting. ATP values were corrected for background levels as a result of the sampling procedure. In this case, sterile swabs were immersed in sterile saline, and determination of the ATP content was performed as described earlier, and ATP levels of about 0.04 ± 0.01 pmol could be observed. A calibration curve with dilutions of pure ATP (Promega) was constructed to obtain a correlation between the relative light units as measured with the Lumac Biocounter and the amount of ATP in solution.

**Scanning electron microscope (SEM) analysis**

SEM analysis was performed in order to investigate the distribution of the polypropylene fibres in the mortar specimens. By means of a hammer and a chisel, chunks of mortar were obtained that contained fibres at the surface. The sample for SEM analysis was coated with gold with a Baltec SCD005 Sputter Coater (Bal-Tec AG, Principality of Liechtenstein). The samples were subsequently studied by means of a FEI XL30 scanning electron microscope (FEI, Netherlands).

**Data analysis**

Metal ions release experiments and viability assays were performed in triplicate and quintuplicate (*n* = 5) respectively. The reported values of the plate count are the mean ± standard deviation. Group means were compared using independent group *t*-tests. Differences between group means were considered significant when the level of confidence was higher than 95%. Significant differences between the antimicrobial mortar specimens and control series are marked with an asterix (*) in the tables.

**Results**

**Agar plate assay**

The presence of antimicrobial compounds on agar plates resulted in an inhibition of the growth of all micro-organisms under investigation (Fig. 1). The presence of polypropylene fibres incorporated with Microban® B (triclosan) resulted in a large zone of inhibition of bacterial growth around the fibres (Fig. 1a–d). The distance from the circumference of the inhibition zone to the antimicro-
bial fibres amounted to about 10, 11, 12 and 13 mm for *L. monocytogenes*, *Salm. enterica*, *E. coli* and *Staph. aureus* respectively. This is in contrast with the inorganic system consisting of zeolites, for which the zone of inhibition almost completely coincided with the zone on which the drop of zeolites was put (Fig. 1e–h).

**Metal ions release**

No differences were observed between the leaching of silver and copper ions from the carbonated and untreated mortar specimens, as could be observed for w/c 0·65 specimens in TSB (Table 2). The composition of the leaching solution had a significant influence on the extent of leaching, as could be observed from the w/c 0·5 specimens. The extent of leaching was much higher for specimens that had been immersed in TSB compared to specimens that had been immersed in demineralized water. Additionally, no differences could be observed between the amounts of ions leached for specimens with different water to cement ratios, as seen from the specimens that had been subjected to carbonation. Finally, a higher amount of zeolites in the mortar mixture resulted in an increased leaching of ions. The amount of copper and silver ions found for the specimens with 4·65% of zeolites was 4·22 and 8·66 times higher, respectively, compared to the specimens with 1% of zeolites.

**Viability assay**

Table 3 shows the importance of the preconditioning of mortar specimens, more specifically, the availability of

Table 2  Effects of the mortar composition, preconditioning of the specimens and composition of the leachate on the leaching of copper and silver ions from mortar specimens containing antimicrobial zeolites

<table>
<thead>
<tr>
<th>Zeolites conc. (%)</th>
<th>w/c</th>
<th>Preconditioning</th>
<th>Leaching solution</th>
<th>Cu (mg l⁻¹)*</th>
<th>Ag (mg l⁻¹)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0·5</td>
<td>Carbonation</td>
<td>Aq. Dem.</td>
<td>0·028 ± 0·008</td>
<td>0·004 ± 0·002</td>
</tr>
<tr>
<td>1</td>
<td>0·5</td>
<td>Carbonation</td>
<td>Tryptic Soy Broth (TSB)</td>
<td>0·441 ± 0·046</td>
<td>0·020 ± 0·006</td>
</tr>
<tr>
<td>1</td>
<td>0·65</td>
<td>None</td>
<td>TSB</td>
<td>0·439 ± 0·051</td>
<td>0·047 ± 0·025</td>
</tr>
<tr>
<td>1</td>
<td>0·65</td>
<td>Carbonation</td>
<td>TSB</td>
<td>0·465 ± 0·087</td>
<td>0·015 ± 0·004</td>
</tr>
<tr>
<td>4·65</td>
<td>0·65</td>
<td>None</td>
<td>TSB</td>
<td>1·853 ± 0·091</td>
<td>0·407 ± 0·059</td>
</tr>
</tbody>
</table>

*Concentrations found in the leaching solution.
water and nutrients on the survival of *E. coli* on control mortar surfaces. A high survival of *E. coli* after 24 h could only be observed on specimens that had been moistened with saline and nutrients. When nutrients had been applied on dry specimens, a decrease in bacterial cell numbers by 4 log units could be observed. From these preliminary experiments, it was decided to use both preconditioning steps in all further experiments.

The effect of the antimicrobial additives on the survival of the different micro-organisms can be observed from Tables 4 and 5 for the experiments performed at 20°C and Table 6 for the experiments performed at 4°C. For the various mortar mixtures, no significant differences could be observed between the survival of the micro-organisms at 20°C for w/c 0.5 and w/c 0.65 specimens, as determined from plate counting and ATP measurements. For the mortar specimens containing antimicrobial fibres (Fig. 2), a significant lower amount of cells compared to the control specimens could only be observed for *E. coli* on w/c 0.5 specimens. The decrease, however, was less than 1 log unit. For all micro-organisms investigated, w/c 0.5 specimens containing 1% of antimicrobial zeolites showed a decreased number of cells compared to the control specimens after 24 h of incubation. For the w/c 0.65 specimens containing 1% of zeolites, a significant decrease in cell numbers compared to the control series could only be observed for *E. coli*. From Table 5, it can be observed that a significant decrease in *Salmo enterica* cell numbers could already be observed for concentrations of zeolites of 1%. For concentrations of 3% and 4% of zeolites, a decrease in cell numbers of about 3 and 5 log units, respectively, could be observed. For w/c 0.65 specimens containing 4.65% of zeolites, no cells could be detected for *E. coli* and *Salmo enterica* (Tables 4 and 5). Furthermore, compared to the control series, a decrease in cells of about 3 and 5 log units could be observed for *L. monocytogenes* and *Staph. aureus*, respectively, for the w/c 0.65 specimens containing 4.65% of zeolites.

At 4°C, a significant decrease in cell numbers compared to the control series could be observed for all
Table 5 Effects of the mortar composition on the survival of *Salmonella enterica* after 24-h incubation at 20°C

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Control series CFU</th>
<th>Zeolites 1% CFU</th>
<th>Zeolites 2% CFU</th>
<th>Zeolites 3% CFU</th>
<th>Zeolites 4% CFU</th>
<th>Zeolites 4:65% CFU</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFU</td>
<td>1.47 × 10⁸</td>
<td>1.28 ± 0.44</td>
<td>9.35 ± 1.43</td>
<td>2.95 ± 2.03</td>
<td>3.51 ± 2.15</td>
<td>2.1 ± 2.82</td>
</tr>
<tr>
<td>Adenosinetriphosphate</td>
<td>4.50 ± 0.5</td>
<td>7.68 ± 3.69</td>
<td>10.62 ± 2.61</td>
<td>6.64 ± 4.09</td>
<td>6.41 ± 2.22</td>
<td>0.27 ± 0.44</td>
</tr>
</tbody>
</table>

N.D., Not detectable (below 10² cells).
*Significant different from to the control series.

Table 6 Effects of the mortar composition on the survival of *Escherichia coli*, *Listeria monocytogenes*, *Salmonella enterica*, and *Staphylococcus aureus* after 24-h incubation at 4°C

<table>
<thead>
<tr>
<th>Micro-organisms</th>
<th>Inoculum</th>
<th>Control series</th>
<th>Zeolites 4%</th>
<th>Zeolites 4:65%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>CFU</td>
<td>6.11 ± 10⁷</td>
<td>1.21 ± 1.79</td>
<td>0.54 ± 1.21</td>
</tr>
<tr>
<td></td>
<td>Adenosinetriphosphate (ATP)</td>
<td>10.79</td>
<td>5.28 ± 3.03</td>
<td>0.07 ± 0.03*</td>
</tr>
<tr>
<td><em>L. monocytogenes</em></td>
<td>CFU</td>
<td>4.37 ± 10⁷</td>
<td>3.27 ± 1.89</td>
<td>5.38 ± 2.41*</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>13.85</td>
<td>5.28 ± 3.03</td>
<td>0.07 ± 0.03*</td>
</tr>
<tr>
<td><em>Salm. enterica</em></td>
<td>CFU</td>
<td>1.08 × 10⁸</td>
<td>7.70 ± 7.64</td>
<td>1.63 ± 3.47*</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>9.01</td>
<td>7.68 ± 3.69</td>
<td>0.09 ± 0.08*</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>CFU</td>
<td>8.01 × 10⁵</td>
<td>4.58 ± 1.88</td>
<td>1.80 ± 4.02*</td>
</tr>
<tr>
<td></td>
<td>ATP</td>
<td>22.07</td>
<td>2.99 ± 0.97</td>
<td>0.16 ± 0.04*</td>
</tr>
</tbody>
</table>

N.E., Not evaluated.
*Significant different from the control series.

Figure 2 Scanning Electron Micrograph of an antimicrobial polypropylene fibre in the mortar matrix.

Micro-organisms on the w/c 0.5 specimens containing 4% of zeolites (Table 6). The largest decrease in cell numbers (four log units) was observed for *E. coli* and *Staph. aureus*. For *Salm. enterica* and *L. monocytogenes*, a higher survival of cells was observed at 4°C (Table 6) compared to 20°C (Tables 4 and 5). The decrease in cell numbers of *Salm. enterica* amounted to about 2 and 4 log units at 4 and 20°C, respectively, for specimens containing 4% of zeolites. While at 20°C a decrease in *L. monocytogenes* cell numbers of about 3 log units was observed (Table 4), no significant inhibition was obtained at 4°C for the specimens containing 4:65% of zeolites (Table 6).

Discussion

Metal ions release

The observed increased leaching of silver and copper ions in TSB compared to demineralized water (Table 2) is in agreement with several findings in literature. Metal ions are released from the zeolites by ionic exchange with other cations. The amount of silver and copper released is, thus, dependent on the concentration of cations in the solution. Therefore, ions are minimally released in deionized water or solutions with low ionic strength (Kawahara et al. 2000; Matsumura et al. 2003). Release of silver ions, however, can be enhanced by the presence of sulfur-containing amino acids. Kawahara et al. (2000) noticed
that more than 75% of Ag⁺ (up to 40 μg ml⁻¹ Ag⁺) was released from silver zeolites powders in 9 ml of brain heart infusion (BHI) broth after 30 min, with no additional release up to 24 h. On the contrary, after 24 h, no and only limited release of Ag⁺ was observed in distilled water and phosphate-buffered saline, respectively (Kawahara et al. 2000).

As could be expected, an increased concentration of zeolites in the mortar mixture resulted in a release of metal ions almost proportional to this increase (Table 2). From the weight of the mortar prisms (10–12 g), the amount of zeolites can be estimated to about 0.02–0.03 g of zeolites for w/c 0.65 specimens containing 1% of zeolites. Therefore, the amount of silver and copper leached from the specimens amounted to about 0.1% and 1.4%, respectively, after 3 h of immersion of untreated mortar specimens in TSB. The fact that leaching of the ions occurs has important consequences on the biocidal activity of the mortar in the long term. Successive leaching of ions could result in the loss of the biocidal activity. In practice, the life span during which the mortar shows bactericidal activity will depend on environmental parameters such as the type and amount of soiling on the surface and the time during which the surface is kept moist. Therefore, it can be expected that antimicrobial cementitious surfaces containing zeolites will have a longer lasting activity in areas such as hospitals, kitchens and athletic locker rooms compared to applications where heavy soiling and wetting of the surface occur almost continuously (i.e. floors in agricultural premises). Further research is warranted to investigate the long-term biocidal activity by means of successive leaching tests coupled with viability assays. More specifically, the effect of current cleaning methods on the leaching of metal ions should be investigated.

Viability assays on mortar surfaces

Because water is an essential factor for microbial growth and biofilm formation, the moisture content of the mortar specimens has an important influence on the survival of the micro-organisms. This could be clearly observed from the decreased survival of E. coli on dry mortar specimens (Table 3). The influence of moisture on the survival of E. coli on mortar specimens confirms the findings from literature. Williams et al. (2005) observed an enhanced survival of E. coli O157 on wood and steel surfaces, soiled with cattle faeces, when the surfaces were kept moist.

The fact that no differences could be observed in the survival of the micro-organisms on mortar specimens with a different w/c ratio (Table 4) is in agreement with the fact that no differences were observed in the leaching of silver and copper ions between w/c 0.5 and w/c 0.65 specimens. In accordance with Navas Martin and Borralleras Mas (2005), it was decided to include w/c 0.65 mixtures, with the aim of accentuating the porosity of the mortar specimens and making the test more restrictive. A higher porosity facilitates the adhesion and survival of micro-organisms (Pereira et al. 2000). On the other hand, a higher porosity results in a larger surface area, and hence, biocidal contact surface.

While a clear inhibitory effect towards all micro-organisms was noticed for the antimicrobial fibres in the agar plate assay (Fig. 1), no antimicrobial activity was observed for the mortar specimens containing these fibres (Table 4). The latter could be attributed to the fact that the concentration or diffusion of the biocides in the mortar mixture was insufficient to exert any biocidal effect. Navas Martin and Borralleras Mas (2005) observed large differences in biocidal activity for mortar mixtures to which different amounts and types of biocide-incorporated fibres have been added. Kalyon and Olgun (2001) did not observe an antimicrobial effect when liquid cultures of E. coli and Bacillus thuringiensis were in contact with triclosan-incorporated polystyrene disks, even if the bulk concentration of triclosan in the polymer was much higher than the minimum inhibitory concentrations (MIC) for the respective micro-organism. The authors attributed this to a too limited diffusion of triclosan from the fibres. Møretrø et al. (2006) reported a very low antibacterial effect of a triclosan-containing floor as a result of too limited concentrations of the biocidal component.

The decrease in biocidal activity at lower temperatures (Table 6) is in agreement with findings from literature (Faúndez et al. 2004; Mehtar et al. 2008). Abushelaibi (2005) reported on the biocidal activity of concrete with polyurethane coatings containing copper oxide or copper sulfate, towards L. monocytogenes. While all copper oxide concentrations decreased L. monocytogenes counts to nondetectable levels (7 log decrease) after 4 days at 25°C, the decrease in cell counts at 4°C was limited to 2.5–4.5 log units after 8 days. The differences observed in the inhibitory effect with temperature could be attributed to an increased release of copper and silver ions, or to an increased uptake of these ions by the bacterial cells at higher temperatures (Faúndez et al. 2004). Furthermore, the growth rate of bacteria is also lower at lower temperatures, thus rendering lower susceptibility to agents acting on cell membrane synthesis (Braid and Wale 2002).

A clear decrease in the total ATP content was observed for the mortar specimens for which a decrease of the cell numbers was reported (Tables 4–6). However, no linear correlation could be observed between the actual decrease in the amount of ATP and CFU. The amount of ATP per cell observed for the different bacteria was in the same
range as found in literature, i.e. between 0:1 and 16 fg ATP per cell (Jakubczak and Leclerc 1980; Leach and Webster 1986; Dostálek and Brányik 2005). However, for w/c 0:65 mortar specimens containing 4:65% of zeolites, the amount of ATP determined was much higher than would be expected based upon the number of surviving cells. Similarly, Hammes et al. (2008) observed unexpected high values of total ATP in drinking water after an ozonization treatment. The authors attributed this to high levels of extracellular ATP. The latter probably accounts for the observations in this research. In practice, ATP measurements could be used for a rapid indication of the effectiveness of antimicrobial surfaces. As seen in this research, a decrease in total ATP gives a clear indication of the decrease in the total cell numbers. However, total ATP may give an overestimation of the number of surviving cells.

Future research recommendations and remarks

The antimicrobial effect of the antimicrobial zeolites can be attributed to the presence of the silver and copper ions, rather than the presence of the zeolites itself. Kawahara et al. (2000) did not observe any inhibition of oral bacteria at concentrations of 16 g l⁻¹ type A zeolites, while minimum inhibitory concentrations for most bacteria where in the range of 0:2–2 g l⁻¹ for type A zeolites containing silver. Similarly, Bright et al. (2002) did not observe inhibition of Staphylococcus aureus in the presence of regular zeolites, while in the presence of silver- and zinc-containing zeolites, a 5-log decrease could be observed.

In order to prevent large variations in the survival of micro-organisms because of drying, the following measures are proposed for the evaluation of antimicrobial cementitious formulations in future research: (i) extension of the immersion period in the saline solution up to 24 h and (ii) keeping the mortar specimens in direct contact with water or water vapour during the incubation period. The latter could be obtained by means of placing the mortar specimens on saturated vermiculite and by placing the Petri dishes in a humidity cabinet as proposed by Dubosc (2000) and Midelet and Carpentier (2004) respectively.

For the highest concentrations of zeolites (4:65%), a small decrease in compressive strength of the mortar cubes was observed, i.e. 41:1 ± 0:8 MPa compared to 49:0 ± 3:4 MPa for the control series. This should be taken into account when designing concrete structures for applications in situ.

Although in this research a good inhibitory effect towards a variety of pathogens was obtained at concentrations of zeolites greater than 3%, some concerns may exist about the long-term performance of such antimicrobial mortar formulations. There exists some concern about the potential selection for silver resistance as a result of the wide use of silver products as biocides (Silver 2003). Meyer (2006), however, indicated that because of the unspecific mode of action of biocides, the development of resistance at the level of bactericidal concentrations is highly unlikely. On the other hand, Gilbert and Mcbain (2001) indicated that contact of micro-organisms with sublethal biocide concentrations may support the selection of strains with resistance at the MIC level. This is especially the case for triclosan. Although the occurrence of cross-resistance to triclosan and antibiotics is still a matter of debate, there are concerns about the widespread use of triclosan and the selection for antibiotics resistance (Levy 2002; Russel 2002, 2003). Therefore, as no demonstrable antibacterial effect was obtained for the triclosan-containing mortars, these fibres should not be used for applications in practice. Furthermore, as the presence of soil may lead to sub-bactericidal concentrations, frequent cleaning is crucial to avoid prolonged contact between biocides and microbes under soiled conditions. Navas Martin and Borralleras Mas (2005) previously indicated that antimicrobial concrete or mortar formulations should be used as an extension of currently used decontamination strategies, rather than being a substitute.

In order to evaluate the long-term performance of the antimicrobial mortars, testing the antimicrobial effect of several series of mortar specimens subjected to a different number of leaching steps is recommended. Furthermore, the cumulative leaching of the metal ions during the different leaching steps should be evaluated. The latter not only provides an idea about the percentage of metals that have been leached, but also supplies information on the rate of diffusion of the metal ions through the mortar matrix. In addition to laboratory experiments, the performance of the antimicrobial composition should be evaluated in situ (i.e. floors in agricultural premises and the food industry). The latter can be performed by means of swabs and plate counting or more rapidly, with ATP measurements as indicated in this research.

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