Development and experimental validation of an overlay mortar with biocide activity

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Abstract

Biodeterioration of concrete by microorganism colonisation may be a problem in several structures, especially in irrigation and hydroelectric canals. The main problem in such structures is the proliferation of algae and cyanobacteria that affect the performance of the structure, increase the maintenance costs and affects its durability. A research was conducted to develop a novel cement-based material with biocide activity that can be used as an overlay mortar in existing structures, such as canals and pipes. With this aim, ten commercial biocides were evaluated in a laboratory campaign to assess the effectiveness of the compounds against the microbial colonization of concrete. Both mono- and multicomponent formulations were designed from the commercial products, to increase their antimicrobial effect obtaining a set

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of biocide formulations. The formulations were submitted to a flowchart process to determine their influence on the physical properties of the concrete, evaluate the release of the actives, and their antimicrobial efficiency both before and after accelerated aging processes. During the campaign, some formulations were observed to diminish the strength of the concrete. Such behaviour was normally due to the interaction of the active with the cement hydration process. Other formulations showed a high release of active from the concrete in water, compromising the durability of the treatment. In general, monocomponent formulations did not succeed to fulfill all the requirements, thus multicomponent formulations were analysed. One studied multicomponent formulation presented particularly good results in all properties analysed. This product did not significantly change the properties of concrete and the release of active in water from the concrete was low, while the antimicrobial effects were long lasting.

Keywords

Biocide; Mortar; Cement; Bioreceptivity; Colonisation; Algae; Bacteria; Fungi

1. Introduction

Concrete is one of the most used construction materials because it has high structural performance, long durability, and a relatively low cost. The surface of concrete is resistant to corrosion and provides a physically and chemically stable environment that can be exposed to potable water or wastewater; for this reason, it is commonly used in pipes and canals (Alum et al., 2008). In these structures, microorganisms can easily colonise concrete and cause biodeterioration of the material. The main problem in irrigation and hydroelectric canals is the growth of algae and cyanobacteria among other microorganisms on the canal walls. This biological growth creates many problems that are reflected in increased maintenance costs.

The effective section of the canal is decreased and the roughness coefficient of the canal wall is increased, which diminishes the water flow (Lancar and Krake, 2002). Some studies reflect a decrease of 10% in the hydroelectric generation capacity because of algal growth (Andrewartha et al., 2007; Perkins et al., 2009). Furthermore, it is worth noting that presence of some types of fungi can have detrimental effects in concrete walls as well as can affect production of certain cereals, as Fusarium (Giannantonio at al., 2009). Moreover, the detachment of filamentous organisms such as green algae carries the risk of clogging and plugging filters. Both the growth of these organisms on concrete and the required cleaning activities to remove them from the walls cause erosion to the surface of the concrete. This intensifies the porosity and roughness of the material and makes it more vulnerable to re-colonisation.

Algal growth is usually preceded by concrete biofouling. This phenomenon is often grouped in the literature into several stages that include an initial accumulation of adsorbed organics, the settlement and growth of pioneering bacteria creating a biofilm matrix and the subsequent succession of micro and macrofoulers (Chambers et al., 2006). Usually, bacteria play a dominant role in the formation of this biofilm and further colonisation of larger microorganisms (Wei et al., 2013).

Several antimicrobial treatments have been used in concrete and cement-based materials. Conventional studied procedures involve the usage of water repellents, biocides, or both. Water repellents slow the adsorption of water by reducing the surface energy and capillary forces of the concrete, thus decreasing the bioreceptivity of the material. Biocides focus on decreasing biological activity. While the use of hydrophobic compounds alone was shown to be insufficient in inhibiting microbial growth, the application of a combination of both

treatments has been reported as effective (Urzì and De Leo, 2007; De Muynck et al., 2009). The order of application of the different products influences the efficiency of the final treatment; this must be considered to avoid negative effects (Malagodi et al., 2000; Nugari and Salvadori, 2003; Moreau et al., 2008).

Biocide treatments have one of two mechanisms: the elimination of the microorganisms already present on the material, and the prevention and control of microbial re-colonisation on clean surfaces. Many compounds have been analysed and tested over the years, including zeolites supporting heavy metal ions (such as copper and silver), zinc oxide, silver nanoparticles, pyridine biocides, silver nitrate, and quaternary ammonium compounds, or Quats (Urzì and De Leo, 2007; Alum et al., 2008; De Muynck et al., 2009; Eyssautier-Chuine et al., 2015). Quats are most frequently used because they have good efficacy as algaecides (Nugari et al., 2009). Nevertheless, this issue is far from being solved from a scientifically and technically point of view.

The main aim of this paper is to present the results obtained in a research devoted to the development of cement-based materials with biocide activity to be used as a biocide mortar layout in existing concrete structures (Vaquero, 2013; Aguado et al., 2014). Therefore, different commercially available antimicrobial products were evaluated for effectiveness against microbial colonisation in concrete. These products were chosen for cost-effectiveness, non-toxicity, and capacity as disinfectants in other applications, such as floor and wall coverings. Different combinations of the commercial biocides were considered to increase their antimicrobial effect obtaining a set of biocide formulations. The experimental campaign was designed to evaluate the influence of the incorporation of the biocide formulations in concrete properties and their antimicrobial activity. Furthermore, the durability of the biocide

activity was also evaluated through accelerated aging processes. The performances of the different combinations studied are shown and analysed, with special attention to the reasons responsible for the failure of the formulations.

2. Materials and methods

2.1 Concrete specimens

Concrete specimens were fabricated with the dosage shown in Table 1. The cement selected to produce the different mixtures was a CEM II A/V 42.5R. All aggregates were siliceous with a low fraction of particles under 0.125 mm sieve. The largest aggregate size was selected according to the size of the Petri dishes utilized for the microbiological tests. Furthermore, a large water-to-cement (w/c) ratio of the mixes (0.7) was used to obtain samples with high porosities that favoured microbiological colonisation. Lastly, a plasticiser additive (Pozzolith 475N, BASF Construction Chemicals) was added to each mixture based on percentage over cement weight (% ocw). All concrete samples were made according to EN 12390-2 (AENOR, 2009a). The samples were cured in a curing chamber (20° ± 2°C; 95 ± 5% relative humidity) for 28 days.

Table 1. Concrete dosages for reference and biocide mixtures

Compound	**Dosage (kg/m^3)**
Cement	350
Fine aggregate (0-5 mm)	1074
Coarse aggregate (5-12 mm)	724
Plasticiser	3.5 (1%)
Water	244

2.2 Design of biocide formulations

Ten commercially available antimicrobial products (Table 2) were tested during the experimental campaign. These products were chosen for cost-effectiveness, non-toxicity, and capacity as disinfectants in other applications, such as floor and wall coverings. The antimicrobial products shown in Table 2 were used to develop different formulations for cement-based biocides. These biocide formulations were composed of one (monocomponent) or several antimicrobial products (multicomponent) as well as incorporating different additives in some cases (see Table 3). Multicomponent formulations were designed to achieve as wide a spectrum of antimicrobial action as possible. The content of each component of the formulations is given between brackets, expressed as percentage of the formulation.

Table 2. General information on the different antimicrobial products evaluated

Number	Active	Commercial name	Effect	Physical state
1	bis(2-pyridylthio)zinc 1,1'-dioxide (Zinc pyrithione 10%)	ACTICIDE SR 1288®	Fungicide and bactericide	Liquid
2	1,2,3,6-Tetrahydro-N-(trichloromethylthio)phthalimide	BIOPOL D 212®	Fungicide	Powder
3	Dithio-2,2'-bis(benzmethylamide)	DENSIL P®	Fungicide and bactericide	Liquid
4	2-pyridinethiol-1-NaO + 1, 2-benzisothiazole-3 (2H) -one	P 24-81	Fungicide and bactericide	Powder
5	bis(2-pyridylthio)zinc 1,1'-dioxide (Zinc pyrithione 10%)	BC 98-56	Fungicide and bactericide	Powder
6	n-Butyl-1,2-benzisothiazolin-3-one	VANQUISH 100®	Fungicide, bactericide and algaecide	Liquid
7	Poly(hexamethylene biguanide) hydrochloride	VANTOCIL IB®	Bactericide	Liquid
8	2-octyl-2H-isothiazol-3-one + Terbutryn	ACTICIDE SR 1453®	Algaecide and fungicide	Powder
9	n-Tert-butyl-N-cyclopropyl-6-(methylthio)-1,3,5-triazine-2,4-diamine + 2 Octyl-2H-isothiazol-3-one	ACTICIDE MBP®	Bactericide and fungicide	Powder
10	2,4,4'-trichloro-2'-hydroxy-diphenyl ether	IRGASAN DP 300®	Bactericide and fungicide	Powder

Table 3. General information on the biocide formulations

Formulation name		Active number	Additives
Monocomponent	BXT	1	Calcium filler (20%)
	BIOPOL D212	2	-
	DENSIL P	3	-
	P24-81	4	-
	BC 98-56	5	-
	VANQUISH 100	6	-
	VANTOCIL IB	7	-
	ACTICIDE MBP	9	-
	BXT/11	10	PG (59.7%), TIBP (0.3%), water (15%)
	BXT/AB	10	PG (74.44%), TIBP (0.45%), BUBLEX® (0.11)
Multicomponent	BXT/1	1 (16%), 8 (59%)	MOUSSEX® (4%), water (21%)
	BXT/5	1 (8%), 10 (3.45%)	PG (10%), PEG (10%), TIBP (0.15%), OPTIGEL CR® (1.5%)
	BXT/10P	9 (15%), 10 (4%)	MOUSSEX® (0.3%), Calcium filler (80.7)
	BXT/12C	1 (5.12%), 10 (5%)	PG (12.23%), PEG (77.34%), TIBP (0.21%), BUBLEX® (0.1%)
	PL-UV-H-2B	8 (30%), 10 (15%)	MOUSSEX® (4%), Calcium filler (51%)

As Table 3 shows, some additives were incorporated to improve the physical properties of the mixture. MOUSSEX®, BUBLEX®, and tri-isobutyl phosphate (TIBP) are used as defoamer compounds to avoid air entrapment in the concrete mix; propylene and polyethylene glycol (PG and PEG, respectively) are used as solvents instead of water to allow the solubilisation of the antimicrobial products and the collection of liquid admixtures; OPTIGEL CR® is an activated bentonite product used for anti-settling and stabilizing in water-based systems. Finally, calcium filler is used as a dispersive matrix for the biostatic agents. The biocide formulations were incorporated in the concrete mix in different amounts, as listed in Table 4; the dosage is expressed as percentage over dry weight of the mix (% odw). Concrete specimens were fabricated for each formulation dosage. Furthermore, reference samples were made for each biocide formulation without since cement samples may vary over time. These quantities were chosen based on the recommendations of the manufacturers of the products. Table 4 also provides information of the content of active principle in the formulations; in

case of multicomponent formulations, individual and total contents are given for each antimicrobial product.

Table 4. Dosage of the biocide formulations and active contents

Formulation name		Dosage (% odw)	Active content (kg/m3)		
			A	B	Total
Monocomponent	BXT	0.05	0.859	-	0.859
		0.15	2.578	-	2.578
	BIOPOL D212	0.1	2.148	-	2.148
		0.2	4.296	-	4.296
		0.3	6.444	-	6.444
	DENSIL P	0.2	4.296	-	4.296
		0.5	10.740	-	10.740
		1	21.480	-	21.480
		2	42.960	-	42.960
	P24-81	0.3	6.444	-	6.444
	BC 98-56	0.3	6.444	-	6.444
	VANQUISH 100	0.002	0.043	-	0.043
		0.013	0.279	-	0.279
		0.025	0.537	-	0.537
		0.037	0.795	-	0.795
		0.05	1.074	-	1.074
	VANTOCIL IB	0.3	6.444	-	6.444
		0.6	12.888	-	12.888
		1	21.480	-	21.480
	ACTICIDE MBP	0.1	2.148	-	2.148
		0.4	8.592	-	8.592
		0.8	17.184	-	17.184
	BXT/11	0.15	0.806	-	0.806
		0.18	0.967	-	0.967
		0.2	1.074	-	1.074
		0.22	1.181	-	1.181
	BXT/AB	0.01	0.054	-	0.054
		0.05	0.269	-	0.269
		0.1	0.537	-	0.537
		0.15	0.806	-	0.806
Multicomponent	BXT/1	2.5	8.592	31.683	40.275
		3	10.310	38.020	48.330
		3.5	12.029	44.356	56.385
	BXT/5	0.075	0.056	0.129	0.184
		0.15	0.111	0.258	0.369
		0.2	0.148	0.344	0.492
		0.4	0.296	0.687	0.984
	BXT/10P	0.8	0.687	2.578	3.265
		1	0.859	3.222	4.081
	BXT/12C	0.1	0.110	0.107	0.217
		0.6	0.660	0.644	1.304
	PL-UV-H-2B	0.15	0.483	0.967	1.450
		0.2	0.644	1.289	1.933
		0.3	0.967	1.933	2.900

2.3 Experimental campaign

The experimental campaign was designed as a flowchart, as described in Figure 1 with three consecutive main phases. Firstly, the influence of the different biocide formulations on physical properties of concrete was determined. Secondly, those biocide concretes that passed the first phase were subjected to a release test to determine the amount of active released from the sample. Finally, microbiological tests were conducted in those samples that passed the previous phases. The evaluation of antimicrobial properties was conducted twice, before and after an accelerated aging test, to simulate adverse conditions and thus estimate the durability of the tested treatment.

Figure 1. Flowchart of the experimental campaign

2.3.1 Physical characterisation of concrete specimens

Concrete specimens were characterised to determine the influence of the biostatic formulations on the physical properties of the samples. Several parameters were considered in both the fresh and hardened state. Workability and density were characterised in the fresh concrete. The workability of the mixes was determined according to the ISO 1920-2 standard (ISO, 2005). The characterisation of the hardened concrete samples included density and mechanical properties determinations (compressive and flexural strength). The compressive and flexural strength measurements were made following EN 12390-4 and 5, respectively (AENOR, 2009b; c).

2.3.2 Release of active

Following the physical characterization of the concrete samples, release tests were performed to determine the amount of the active released from the samples. The method described in

prEN 16105:2010 (AENOR, 2010) is usually applied to evaluate leaching of substances from coatings and paints. Thus, it was selected to evaluate the release of active from the concrete samples, as the cement-based biocide developed is intended to be used as an overlay mortar for existing structures. This method subjects the samples to wetting-drying cycles to simulate the effects of rain and environmental conditions. After the wetting-drying cycles, the water samples are analysed to determine the amount of active released from the samples.

Samples for the release tests were obtained from the same concrete dosages described previously. All samples presented at least 100 cm^2 of surface area and with weights characterised both before and after the tests. For each analysis, duplicate samples of each biocide mixture were studied. Furthermore, one reference sample was also analysed. All samples were maintained for 7 days at 23° ± 2° C at a relative humidity of 50 ± 5%. The subsequent immersion process lasted 24 days. During that time, nine wetting-drying cycles were performed on days 1, 3, 7, 9, 14, 16, 18, 22, and 24. Each cycle contained the following steps: 1 h immersion, 4 h drying, 1 h immersion, and 48 h drying. At the end of the cycle, the water samples were collected and analysed by HPLC analyses and pH measurements.

The amount of active present in the solution after the release tests can be expressed according to Eq. (1). Here, M is the amount of active expressed in mg/m^2·day, c is the measured concentration of active in the eluate in mg/L, V_{real} is the real volume of eluate after each immersion day in L, A is the effective surface area exposed to the releasing test in m^2, and t is the day of immersion.

$$M = \frac{c \cdot V_{real}}{A \cdot t} \quad (1)$$

2.3.3 Evaluation of antimicrobial properties

The different concrete samples were tested to determine their antimicrobial properties both before and after accelerated aging processes to estimate the durability of the treatments. Several samples from the different concrete mixes were exposed to suspensions containing different microorganisms. The characteristics of the microorganisms used are described in Table 5. The microorganisms were collected from different culture media from the American Type Culture Collection (ATCC) and the Sammlung von Algenkulturen Göttingen (SAG).

Table 5. Microorganisms used for to evaluate antimicrobial properties and characteristics of incubation periods thereof

Type of microorganism	Microorganism	Reference code	Incubation period		
			Agar type	Temperature (°C)	Time (days)
Fungus	*Aspergillus niger*	ATCC 6275	Malt extract agar	25 ± 2	5-7
Bacteria	*Staphylococcus aureus*	ATCC 6538P	Nutrient agar	30 ± 2	2
Bacteria	*Escherichia coli*	ATCC 8739		30 ± 2	2
Algae	*Scenedesmus vaculatus*	SAG 211-8b	Kuhl solution	20 ± 2	21
Algae	*Stichococcus bacillaris*	SAG 379-1a		20 ± 2	21

The concrete samples were located in Petri dishes containing agar media specialized for each microorganism. The concrete samples were of 10 mm thickness and variable sizes, obtained from the samples described in section 2.3. The different microorganism suspensions were spread over the agar surface. Each suspension contained at least 5×10^6 organisms per mL. The Petri dishes with the concrete samples and the suspensions were incubated as detailed in Table 5. At the end of the incubation period, the antimicrobial properties of the samples were evaluated. The samples were visually analysed to determine the growth of the microorganisms and classified by the following scale: 0 – no visible growth of

microorganisms; 1 – slight observable growth of microorganisms; 2 – evident growth of microorganisms and presence of spores.

As mentioned above, after the first evaluation of the antimicrobial properties an accelerated aging process was conducted to simulate the adverse conditions concrete can experience, thus evaluating antimicrobial efficiency over time. The exposure test Xenotest 150S was performed. This test was designed to evaluate the durability of paints and varnishes, and consists of an uninterrupted irradiation of the samples for 2000 h with 340 nm radiation at a radiant flux of 0.5 W/m^2, in UNE-EN ISO 4892-2 (AENOR, 2014) standard conditions. This treatment is equivalent to 6-8 months of continuous strong sunlight. By using ultraviolet fluorescent lamps and irrigation sprinklers, the process simulates environmental wear accrued over a much longer period than actually experienced. The samples exposed to the accelerated aging process and others with the same characteristics without aging were evaluated a second time with respect to antimicrobial properties, and the differences in the capacity of the samples to inhibit microbial growth were compared and examined.

3. Results and discussion

3.1 General evaluation

During the experimental campaign, the biocide formulations shown in Table 3 were submitted to the flowchart process described in section 2.2. Those that did not present good results in a given phase were immediately discarded from the experiment to save time and cost. At first, only monocomponent formulations were evaluated. None of them was able to pass the whole evaluation process, as shown in Table 6. Some of the formulations failed in the physical properties evaluation phase (BXT/AB, BXT/11, VANQUISH 100, DENSIL P and BC 98-56);

formulations BIOPOL D212 and VANTOCIL IB did not passed releasing tests; finally, ACTICIDE MBP, P24-82 and BXT were discarded after the antimicrobial test.

Considering these results, multicomponent formulations were tested to increase the antimicrobial activity and/or to overcome the problems observed during the first evaluation phase. Five multicomponent formulations were designed and analysed. Only one of the multicomponent formulations (PL-UV-H-2B) presented good results during the complete evaluation process. Table 6 presents a summary of the phase at which the formulation was discarded.

Table 6. Failure phases and qualification of each formulation during the experimental campaign

Formulation		**Failure phase**
Monocomponent	BXT/AB	Physical properties
	BXT/11	Physical properties
	ACTICIDE MBP	Antimicrobial properties (before aging process)
	BIOPOL D212	Releasing test
	VANTOCIL IB	Releasing test
	VANQUISH 100	Physical properties
	DENSIL P	Physical properties
	P24-81	Antimicrobial properties (before aging process)
	BC 98-56	Physical properties
	BXT	Antimicrobial properties (after aging process)
Multicomponent	BXT/12C	Physical properties
	BXT/10P	Physical properties
	BXT/1	Physical properties
	BXT/5	Antimicrobial properties (before aging process)
	PL-UV-H-2B	None

3.2 Physical characterisation

Firstly, the results obtained by the monocomponent formulations will be analysed. Five out of ten of the monocomponent formulations affected the physical properties of the concrete samples, mainly with respect to strength. Only the results of these formulations will be analysed here. The results of mechanical performance of these samples are shown in Table 7, along with the measurements of workability and density in fresh state.

Table 7. Mechanical properties of the monocomponent formulations failing first phase tests

Formulation name	Dosage (% odw)	Flow (mm)	Fresh density (kg/m^3)	Mechanical properties (MPa)			
				Compressive strength		Flexural strength	
				1 d	28d	1d	28d
DENSIL P	0	154	2450	19.1	47.8	4.8	8.2
	0.2	154	2435	19.3	48.3	4.6	9.4
	0.5	155	2435	8.5	41.1	2.2	7.2
	1.0	155	2435	4.4	42.1	1.2	8.3
	2.0	159	2422	n.d.	34.2	n.d.	7.3
BC 98-56	0	150	2432	12.8	33.5	3.5	6.8
	0.3	135	2465	n.d.	36.6	n.d.	7.6
VANQUISH 100	0	155	2450	15.4	33.6	3.3	6.5
	0.002	152	2392	14.2	33.1	2.9	6.1
	0.013	152	2401	13.1	29.2	2.8	5.6
	0.025	153	2417	13.4	27.4	3.0	5.5
	0.037	156	2439	12.8	30.9	3.0	5.4
	0.050	150	2427	12.8	27.4	2.5	5.8
BXT/11	0	117	2389	15.5	35.1	3.2	5.6
	0.15	118	2005	5.3	16.0	2.1	3.3
	0.18	122	2004	7.2	21.4	2.5	5.2
	0.20	121	2122	6.9	20.9	2.7	5.3
	0.22	127	2174	9.6	24.3	2.4	5.8
BXT/AB	0	117	2389	15.5	35.1	3.2	5.6
	0.01	120	2420	15.1	29.9	2.6	5.4
	0.05	125	2395	14.8	34.3	2.8	5.4
	0.10	128	2467	13.0	31.8	2.3	5.0
	0.15	124	2397	11.9	29.8	2.1	4.9

The results shown in Table 7 direct evidence the biocide formulations affected the concrete samples in two different ways. The first phenomenon observed was the influence of the active

principles in the cement hydration process. Most of the formulations exhibited this phenomenon. The formulations BXT/AB, VANQUISH 100 and DENSIL P mainly influenced early-age properties of the concrete samples. Concrete samples made with these formulations presented results similar to those from the reference concrete regarding workability and density, with variations lower than 5% in these properties. However, the mechanical resistances decreased significantly, especially at lower ages. The reduction of compressive strength increases with increased biocide formulation dosages, e.g.: concrete samples made with BXT/AB formulation reached a 23% reduction of compressive strength with a dose of 0.15% at 1 day, and a 35% reduction of flexural strength at the same dose and age. In this case, the active principles are retarding the cement hydration process thus reducing compressive and flexural strengths in relation to the reference samples. The strengths obtained at 28 days showed slight recovery, although they remained below the reference value.

BC 98-56 formulation also influenced the cement hydration process but in a different way. This formulation modified the workability of the concrete samples by increasing the viscosity of the fresh mix. A strong diminution of the resistances at 24 h was observed and the samples could not be demoulded. Mechanical properties at 28 days were higher than those of the reference concrete were. This behaviour could be explained by the influence of the active principle on the cement hydration process, generating smaller but more proliferate crystals. Consequently, the crystallization process would be slower and the resistances would increase with age.

The second phenomenon observed was air-entrapment, evidenced in concrete samples made with BXT/11 formulation. This formulation was designed with the same active principle as

BXT/AB, but with a reduced amount of propylene glycol and water substituted to improve the stability of the solution. With this reduction, the amount of defoamer compound was also decreased; only TIBP was used. In these samples, significant reductions were observed in their densities. The addition of IRGASAN DP 300® produced air entrapment in the concrete mix and diminished the density, thus requiring increased amounts of defoamer to release entrapped air. Another important aspect was observed regarding to the densities of the antimicrobial samples. The increased amount of biostatic agent in the mix did not produce a proportional reduction of the densities, but quite the opposite; as the content of biocide increased, the density also increased. This phenomenon may result from the increased amount of biostatic agent being accompanied by an increased amount of defoamer compounds in the concrete, thus reducing air entrapment. The influence of the formulation on the densities of the mixtures produced significant reductions in the strength values, with a maximum diminution of 66% in compressive strength with a dose of 0.15% at 1 day.

Regarding the multicomponent formulations evaluated, most of them did not present good results during the evaluation of their mechanical performance. This was the case for BXT/1, BXT/10P, and BXT/12C; the compressive and flexural strengths of these compounds are shown in Table 8, as well as the workability and density measurements in fresh state. A trend in both compressive and flexural strengths of the concrete samples is observed; the decreases in strength are directly proportional to the increases of biocide contents in the mixtures. The controlling factor in this decrease of strength is again the influence of the active principles in the cement hydration process. Minor influences are observed in workability and density in fresh state of the concrete samples as compared with the reference samples. Nevertheless, large reductions in compressive strength are observed for the samples containing BXT/1,

BXT/10P, and BXT/12C formulations. Furthermore, samples with BXT/1 formulation could not be demoulded at the age of 24 hours.

Table 8. Mechanical properties of the multicomponent formulations

Formulation name	Dosage (% odw)	Flow (mm)	Fresh density (kg/m^3)	Mechanical properties (MPa)			
				Compressive strength		Flexural strength	
				1 d	28d	1d	28d
BXT/1	0	157	2426	12.5	36.7	3.7	7.5
	0.4	150	2465	n.d.	34.0	n.d.	6.7
	0.45	153	2439	n.d.	31.2	n.d.	6.5
	0.5	149	2468	n.d.	34.3	n.d.	6.3
BXT/5	0	165	2343	15.8	42.3	4.8	8.7
	0.08	170	2337	14.9	40.5	4.3	8.1
	0.15	167	2381	15.1	40.0	3.8	8.4
	0.2	165	2330	14.5	39.0	3.5	7.9
	0.4	168	2327	14.0	39.0	3.3	7.7
BXT/10P	0	125	2473	19.1	35.0	4.8	7.2
	0.8	117	2266	10.3	30.6	2.8	6.1
	1	120	2200	8.2	29.0	2.5	6.0
BXT/12C	0	117	2389	12.8	33.5	3.5	6.8
	0.6	120	2420	8.1	28.4	2.8	5.6
	1	125	2395	6.2	22.7	2.3	4.4
PL-UV-H-2B	0	148	2523	14.3	37.1	4.0	9.4
	0.15	152	2501	12.7	36.9	3.5	8.6
	0.2	145	2479	11.3	35.7	2.8	8.2
	0.3	150	2484	14.0	34.9	3.2	8.5

Only BXT/12C and PL-UV-H-2B formulations did not significantly change the physical properties of the concrete mixtures. Both fresh and apparent densities are in the same range of values, with differences under 2%. Minor reductions in the fresh density of samples elaborated with the highest dosage of PL-UC-H-2B formulation are observed. This reduction is related with an air-entrapping process, since the formulation was designed as a solid powder because of the difficulties of obtaining a stable suspension of the antimicrobial products. Although minor variations of compressive and flexural strength are observed, these

are negligible and do not evidence shortcomings in the mechanical performances of the concretes.

3.3 Release of active

Releasing tests were made in those monocomponent formulations that succeeded the first phase of physical characterization (ACTICIDE MBP, BIOPOL D212, VANTOCIL IB, P24-81, and BXT). The releasing curves of the actives from concrete samples containing each formulation are shown in Figure 2; in the case of P24-81 formulation, separated curves are shown for each active (NaP: 2-pyridinethiol-1-NaO; BIT: 1,2-benzisothiazole-3(2H)-one). For each formulation, daily and accumulated concentrations are given on the right and left side of the figure, respectively). According to the same procedure used in the physical characterization tests, the formulations that did not present good results in this phase were discarded, as occurred with the monocomponent formulations Biopol D212 and Vantocil IB (see Figure 2b and 2c). Although the releasing levels of Biopol D212 are far from toxicity, as the value considered detrimental for humans of this product is LD_{50} > 9000 ppm, this high solubility in water could quickly decrease the antimicrobial efficiency of the treated concrete. The formulation seemed excessively soluble for the intended purpose and thus it was discarded. Similar behaviour was observed with the formulation Vantocil IB in the releasing test. The amount of active released increases as the dosage of the antimicrobial mixture is increased, for both formulations.

Figure 2. Releasing curves of the active from concrete fabricated with monocomponent formulations: a) ACTICIDE MBP, b) BIOPOL D212, c) VANTOCIL IB, d) P24-81, and e) BXT

Regarding the multicomponent formulations, releasing tests were made only in PL-UV-H-2B formulations since BXT/5 formulation is comparable to monocomponent formulations ACTICIDE MBP and BXT. The releasing curves of the active from concrete samples containing the formulation PL-UV-H-2B are shown in Figure 3; separated curves are provided for each active present in the formulation (OIT: 2-octyl-2H-isothiazol-3-one; TER: terbutryn). As expected, an increase in the actives released is observed with increasing dosages of the formulation. For the sample with 0.15% dosage of the formulation, the actives are mainly released during the first 9 days of the test. Afterwards, only minor releasing is observed. The sample with 0.2% dosage presents higher released quantities of actives throughout the releasing test. An anomalous measurement at 14 days is much higher for OIT than the rest (0.45 mg/m^2). If this value is not considered, the total amount of active released from the sample is proportional to the initial content of the active in the concrete sample.

Figure 3. Releasing curves of the active from concrete fabricated with PL-UV-H-2B formulation

For the highest tested dosage of PL-UV-H-2B formulation (0.3%), the quantities of active released are much larger than the ones for the other dosages. The release of active from the sample during the first 7 days is approximately six times greater compared to the results of the 0.15% sample, thus increasing until the end of the process. The total active released from the 0.3% sample is more than nine times larger than that from the 0.15% sample. This behaviour suggests possible interactions between the active and the cementitious matrix. However, as the content of formulation is increased above 0.2%, there is more free active in the pore solution; hence, the active released increases during the duration of the test. The total amount

of active released throughout the test is far below toxic values. The sample with the largest content of formulation releases a total amount of 1.52 mg/m^2 of actives, which is equivalent to 0.3 ppm. This antimicrobial product is considered toxic for humans at LD_{50} > 2360 ppm, which is almost four orders of magnitude larger than the total amount of active released from the sample during the releasing test.

3.4 Evaluation of the antimicrobial properties

The final phase of the experimental campaign was only performed on those formulations that had presented good results in both previous phases, as described in sections 3.2 and 3.3. Three monocomponent formulations (ACTICIDE MBP, P 24-81, and BXT) were evaluated, but none presented good results after the process. Two multicomponent formulations (BXT/5 and PL-UV-H-2B) were also subjected to the microbiological test.

Firstly, the results obtained by concrete samples fabricated with ACTICIDE MBP formulation will be presented. This monocomponent formulation was expected to have bactericide and fungicide activity. Therefore, it was tested against three microorganisms (*Escherichia coli*, *Staphylococcus aureus*, and *Aspergillus niger*). Figure 6 depicts images of the evaluation of the antimicrobial tests performed with this formulation. The concrete samples show good efficiency against *Staphylococcus aureus*, preventing biological growth on the surface with a growth index of 0 in all treated samples; the reference sample presents a growth index of 2. However, the concrete samples do not inhibit surface growth of *Escherichia coli* or *Aspergillus niger*, presenting a growth index of 2 at all dosages. Since the antimicrobial activity against two of the three microorganisms is negligible, the product is considered not valid.

Figure 4. Effectiveness of concrete made with Acticide MBP formulation against different microorganisms

Secondly, concrete samples made with P 24-81 formulation were analysed; the results of the antimicrobial tests are shown in Figure 5. This formulation was also expected to present bactericide and fungicide activity; it was tested against the same microorganisms as samples made with ACTICIDE MBP formulation. The treated samples contained 0.3% odw. After the microbiological test, the compound presented no antimicrobial properties against any microorganism, with no inhibition of biological growth on the surface of the sample. The visual analysis (see Figure 5) showed a growth index of 2 in all cases; hence, it was also discarded before the accelerated aging process.

Figure 5. Effectiveness of concrete made with P 24-81 formulation against different microorganisms

Finally, BXT formulation was analysed. This product was expected to have biocide and fungicide activity. In initial antimicrobial tests, shown in Figure 6a, the product exhibits good antimicrobial properties against both types of bacteria, with a growth index of 0 in every case. Nevertheless, the effect against the *Aspergillus niger* fungus is only acceptable with the dosage of 0.15% with a growth index of 1; this index has a value of 2 for the sample with the lowest dose of 0.05%. The results are considered sufficient for validity, and the samples were submitted to the accelerated aging test. A second antimicrobial test was conducted following the same procedure. After aging, the inhibition of bacterial growth is retained, as seen in Figure 6b, with a growth index of 0 in all cases. However, the antimicrobial activity against

Aspergillus niger is not preserved, as the growth index reaches 2 with both dosages; thus, the product is considered not valid.

Figure 6. Effectiveness of concrete made with BXT formulation against different microorganisms: a) before and b) after the accelerated aging process

Regarding the multicomponent formulations, BXT/5 formulation is composed of the active principles IRGASAN DP 300® and ACTICIDE SR 1288®, and it was tested against all microorganisms listed in Table 5 to evaluate its antimicrobial efficiency. After the biological tests, the treatment showed great efficiency against the bacteria *Staphylococcus aureus* and the two types of algae, *Scenedesmus vaculatus* and *Stichococcus bacillaris*, with growth indexes of 0 for all dosages, as seen in Figure 7. In these cases, no biological growth occurred on the surfaces, and inhibition halos surrounding the samples were observed. However, the formulation did not present antimicrobial activity against *Escherichia coli* at any dose. This may be due to the low content of ACTICIDE SR 1288® in the mixtures; BXT, which had the same active principle but in greater amounts, presented highly antimicrobial behaviour. The growth indexes against this microorganism with BXT/5 were 2 in all cases (see Figure 7). The treatment was also inefficient against the fungus *Aspergillus niger*, reaching only an acceptable level of activity, with a growth index of 1, at the highest dose (0.4%). At all other doses, the growth index was 2, with no inhibition of biological growth. The unsatisfactory behaviour of the treatment against *Escherichia coli* and *Aspergillus niger* justified the discarding of the treatment without accelerated aging.

Figure 7. Effectiveness of concrete made with BXT/5 formulation against different microorganisms: a) before and b) after the accelerated aging process

Finally, concrete samples made with PL-UV-H-2B formulation were analysed. Figure 8a shows the results of microorganism colonisation after the incubation period of the formulation. As noted in section 3.1, because the formulation is expected to present algaecide, bactericide, and fungicide activity, the compound was tested against all microorganisms listed in Table 5 to evaluate its antimicrobial efficiency. The concrete samples present efficiency against all microorganisms evaluated at all dosages; it prevents biological growth on the surface and, in most cases, forms an inhibition halo surrounding the sample. With both bacteria and algae, the concrete shows high antimicrobial activity with growth indexes of 0 in all cases. With *Aspergillus niger*, higher formulation dosages of 0.2 and 0.3% are required to obtain the same effect, as the lowest dose of 0.1% presents a growth index of 1 with no inhibition halo surrounding the sample surface. The growth indexes for the reference samples against the different microorganisms vary from 1 to 2.

Figure 8. Effectiveness of concrete made with PL-UV-H-2B formulation against different microorganisms: a) before and b) after the accelerated aging process

According to the results of the initial antimicrobial test, the formulation PL-UV-H-2B was considered valid and the samples were then subjected to the accelerated aging test. A second antimicrobial test was conducted; images of the microbiological cultures are shown in Figure 8b. The antimicrobial activities against both bacteria and algae prevail after the aging process, although larger inhibition halos surrounding the samples are observed at higher dosages of the formulation. The growth index in all cases is zero. For the efficiency against fungal growth, a slight loss of the antimicrobial activity is perceived because the inhibition halo is not formed

at any dosage. However, no biological growth occurs on the concrete samples; the growth index is 1 for the samples with different biocide doses.

4. Conclusions

Fifteen biocide formulations were studied during this campaign to assess their biocide effects when incorporated into concrete. These formulations were elaborated from different commercially available antimicrobial products that were chosen for cost-effectiveness, non-toxicity, and capacity as disinfectants in other applications, such as floor and wall coverings. The biocide formulations were composed of one (monocomponent) or more antimicrobial products (multicomponent) as well as incorporating different additives in some cases.

A three-phase process was performed to evaluate the physical properties, release of active and antimicrobial properties of the concrete samples incorporating the different formulations. The study showed that monocomponent formulations were insufficient to inhibit biological growth in concrete without either significant detriment to the mechanical properties of the material or losing efficient biostatic activity after a short period. To obtain a long-lasting effect against a wide spectrum of microorganisms, the use of multicomponent formulations was necessary.

The use of existing commercial products such as Vanquish 100®, Densil P®, and BC 98-56 presented significant reductions in the mechanical resistances of concrete, typically with influences during the hardening process. The formulations BXT/AB and BXT/11, which incorporated IRGASAN DP 300®, also showed decreases in the strengths of the concrete. This active principle, in certain compositions, caused a decrease in density through air entrapment in the concrete mix. Some multicomponent formulations, such as BXT/12C, BXT/10P, and BXT/1, also presented poor performance regarding mechanical properties. The

effect of the biostatic agents in the cement hydration process was the main reason for the mechanical degradation of the concrete, especially at low ages.

Many of the formulations affected significantly the physical and mechanical properties of the concrete samples, thus presenting lower mechanical performances. Two different behaviour were observed. According to the results obtained, some formulations presented an interaction of the actives with the cement hydration process. The low mechanical performance of the samples at early ages may be related with the retardation of the hydration reactions. Furthermore, air-entrapping was also observed. The formulations Vanquish 100®, Densil P®, and BC 98-56, as well as BXT/AB and BXT/11, which incorporated IRGASAN DP 300®, were discarded from the evaluation process.

The addition of the chemicals Biopol D212® and Vantocil IB® did not affect the strengths of the concrete mixtures. However, when submitted to release tests, the modified samples presented high solubility in water. This behaviour implied a rapid loss of antimicrobial activity, which would compromise the duration of protection.

In the microbiological tests, the chemicals ACTICIDE MBP® and P 24-81 presented poor results with almost no inhibition of biological growth. BXT offered good results against both types of bacteria and the tested fungus before the accelerated aging process. However, after aging, the activity against the fungus was not maintained. The multicomponent formulation BXT/5 showed a high antimicrobial efficiency against both algae species and the bacteria *Staphylococcus aureus*. Despite these results, the treatment did not present any biocide effect against *Escherichia coli* and *Aspergillus niger*.

Among all the formulations evaluated, the formulation PL-UV-H-2B was the only one to succeed in all the evaluation process. This multicomponent formulation, when mixed with concrete, did not affect the physical or mechanical properties of the material. Only slight reductions in density, compressive strength, and flexural strength were observed in comparison with those of the reference samples, although the reductions were not significant.

Concerning the release tests, the quantity of active released by this formulation was very low. The quantities released are approximately four orders of magnitude below the amount that may be considered toxic for humans. This result also indicates a greater duration of the biostatic effect of the treatment.

Moreover, concrete samples fabricated with PL-UV-H-2B formulation demonstrated high effectiveness in antimicrobial tests against algae, fungi, and bacteria, both before and after accelerated aging processes. The compound prevented biological growth in concrete, and in most cases formed inhibition halos surrounding the samples.

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References

AENOR, 2009a. UNE-EN 12390-2:2009 Testing hardened concrete - Part 2: Making and curing specimens for strength tests

AENOR, 2009b. UNE-EN 12390-3:2009 Testing hardened concrete - Part 3: Compressive strength of test specimens

AENOR, 2009c. UNE-EN 12390-5:2009 Testing hardened concrete - Part 5: Flexural strength of test specimens

AENOR, 2010. prEN 16105:2010 Paints and varnishes - Leaching of substances from coatings - Laboratory immersion method

AENOR, 2014. UNE-EN ISO 4892-2:2014 Plastics - Methods of exposure to laboratory light sources - Part 2: Xenon-arc lamps

Aguado, A., Segura, I., Vaquero, J.M., Servos, F., 2014. Additive to inhibit biological growth in concrete structures and use, Spanish Patent reference number P201431036 (under review).

Alum, A., Rashid, A., Mobasher, B., Abbaszadegan, M., 2008. Cement-based biocide coatings for controlling algal growth in water distribution canals. Cement & Concrete Composites, 30: 839-847.

Andrewartha, J.M., Sargison, J.E., and Perkins, K.J., 2007. The effect of Gomphonema and filamentous algae streamers on hydroelectric canal capacity and turbulent boundary layer structure. 16th Australasian Fluid Mechanics Conference.

Chambers, L.D., Stokes, K.R., Walsh, F.C., and Wood, R.J.K., 2006. Modern approaches to marine antifouling coatings. Surface and Coatings Technology 201: 3642–3652.

De Muynck, W., Maury Ramirez, A., De Belie, N., 2009. Evaluation of strategies to prevent algal fouling on white architectural and cellular concrete. International Biodeterioration & Biodegradation, 63: 679-689.

Eyssautier-Chuine, S., Vaillant-Gaveau, N., Gommeaux, M., Thomachot-Schneider, C., Pleck, J., Fronteau, G., 2015. Efficacy of different chemical mixtures against green algal growth on limestone: A case study with *Chlorella vulgaris*. International Biodeterioration & Biodegradation, 103: 59-68.

Giannantonio, D.J., Kurth, J.C., Kurtis, K.E., and Sobecky, P. A., 2009. Effects of concrete properties and nutrients on fungal colonization and fouling. International Biodeterioration and Biodegradation, 63: 252–259.

ISO, 2005 ISO 1920-2:2005. Testing of concrete - Part 2: Properties of fresh concrete.

Lancar, L., and Krake, K., 2002. Aquatic Weeds and their Management. International Commission on Irrigation and Drainage.

Malagodi, M., Nugari, M.P., Altieri, A., Lonati, G., 2000. Effects of combined application of biocides and protectives. In: Fassina, V. (Ed.), 9th International Congress on Deterioration and Conservation of Stone. Elsevier, Amsterdam, the Netherlands, 225-233.

Moreau, C., Vergès-Belmin, V., Leroux, L., Orial, G., Fronteau, G., Barbin, V., 2008. Water-repellent and biocide treatments: assessment of the potential combinations. Journal of Cultural Heritage, 9: 394–400.

Nugari, M.P., Salvadori, O., 2003. Biocides and treatment of stone: limitations and future prospects. In: Koestler, R.J., Koestler, V.H., Charola, E.A.E., Nieto-Fernandez, F.E. (Eds.), Art, Biology and Conservation: Biodeterioration of Works of Art. The Metropolitan Museum of Art, New York, USA, 519–535.

Nugari, M.P., Pietrini, A.M., Caneva, G., Imperi, F., Visca, P., 2009. Biodeterioration of mural paintings in a rocky habitat: the Crypt of the Original Sin (Matera, Italy). International Biodeterioration & Biodegradation, 63: 705-711.

Perkins, K. J., Sargison, J., and Hallegraeff, G. M., 2009. Diatom fouling problems in a Tasmanian hydro canal, including the description of Gomphonema Tarraleahae sp. nov. Diatom Research, 24 (2): 377-391.

Urzì, C., De Leo, F., 2007. Evaluation of the efficiency of water-repellent and biocide compounds against microbial colonisation of mortars. International Biodeterioration & Biodegradation, 60: 25-34.

Vaquero, J. M., 2013. Microorganism-concrete interactions: evaluation methods and possible solutions. PhD Thesis. Department of Construction Engineering, Universitat Politècnica de Catalunya - Barcelona Tech. http://www.tdx.cat/handle/10803/116930

Wei, S., Jiang, Z., Liu, H., Zhou, D., and Sanchez-Silva, M., 2013. Microbiologically induced deterioration of concrete - A Review. Brazilian Journal of Microbiology, 44(4): 1001–1007.

Table

Table 1. Concrete dosages for reference and biocide mixtures

Compound	**Dosage (kg/m^3)**
Cement	350
Fine aggregate (0-5 mm)	1074
Coarse aggregate (5-12 mm)	724
Plasticiser	3.5 (1%)
Water	244

Table 2. General information on the different antimicrobial products evaluated

Number	Active	Commercial name	Effect	Physical state
1	bis(2-pyridylthio)zinc 1,1'-dioxide (Zinc pyrithione 10%)	ACTICIDE SR 1288®	Fungicide and bactericide	Liquid
2	1,2,3,6-Tetrahydro-N-(trichloromethylthio)phthalimide	BIOPOL D 212®	Fungicide	Powder
3	Dithio-2,2'-bis(benzmethylamide)	DENSIL P®	Fungicide and bactericide	Liquid
4	2-pyridinethiol-1-NaO + 1, 2-benzisothiazole-3 (2H) -one	P 24-81	Fungicide and bactericide	Powder
5	bis(2-pyridylthio)zinc 1,1'-dioxide (Zinc pyrithione 10%)	BC 98-56	Fungicide and bactericide	Powder
6	n-Butyl-1,2-benzisothiazolin-3-one	VANQUISH 100®	Fungicide, bactericide and algaecide	Liquid
7	Poly(hexamethylene biguanide) hydrochloride	VANTOCIL IB®	Bactericide	Liquid
8	2-octyl-2H-isothiazol-3-one + Terbutryn	ACTICIDE SR 1453®	Algaecide and fungicide	Powder
9	n-Tert-butyl-N-cyclopropyl-6-(methylthio)-1,3,5-triazine-2,4-diamine + 2 Octyl-2H-isothiazol-3-one	ACTICIDE MBP®	Bactericide and fungicide	Powder
10	2,4,4'-trichloro-2'-hydroxy-diphenyl ether	IRGASAN DP 300®	Bactericide and fungicide	Powder

Table 3. General information on the biocide formulations

Formulation name		Active number	Additives
Monocomponent	BXT	1	Calcium filler (20%)
	BIOPOL D212	2	-
	DENSIL P	3	-
	P24-81	4	-
	BC 98-56	5	-
	VANQUISH 100	6	-
	VANTOCIL IB	7	-
	ACTICIDE MBP	9	-
	BXT/11	10	PG (59.7%), TIBP (0.3%), water (15%)
	BXT/AB	10	PG (74.44%), TIBP (0.45%), BUBLEX® (0.11)
Multicomponent	BXT/1	1 (16%), 8 (59%)	MOUSSEX® (4%), water (21%)
	BXT/5	1 (8%), 10 (3.45%)	PG (10%), PEG (10%), TIBP (0.15%), OPTIGEL CR® (1.5%)
	BXT/10P	9 (15%), 10 (4%)	MOUSSEX® (0.3%), Calcium filler (80.7)
	BXT/12C	1 (5.12%), 10 (5%)	PG (12.23%), PEG (77.34%), TIBP (0.21%), BUBLEX® (0.1%)
	PL-UV-H-2B	8 (30%), 10 (15%)	MOUSSEX® (4%), Calcium filler (51%)

Table 4. Dosage of the biocide formulations and active contents

Formulation name		Dosage (% odw)	Active content (kg/m3)		
			A	B	Total
Monocomponent	BXT	0.05	0.859	-	0.859
		0.15	2.578	-	2.578
	BIOPOL D212	0.1	2.148	-	2.148
		0.2	4.296	-	4.296
		0.3	6.444	-	6.444
	DENSIL P	0.2	4.296	-	4.296
		0.5	10.740	-	10.740
		1	21.480	-	21.480
		2	42.960	-	42.960
	P24-81	0.3	6.444	-	6.444
	BC 98-56	0.3	6.444	-	6.444
	VANQUISH 100	0.002	0.043	-	0.043
		0.013	0.279	-	0.279
		0.025	0.537	-	0.537
		0.037	0.795	-	0.795
		0.05	1.074	-	1.074
	VANTOCIL IB	0.3	6.444	-	6.444
		0.6	12.888	-	12.888
		1	21.480	-	21.480
	ACTICIDE MBP	0.1	2.148	-	2.148
		0.4	8.592	-	8.592
		0.8	17.184	-	17.184
	BXT/11	0.15	0.806	-	0.806
		0.18	0.967	-	0.967
		0.2	1.074	-	1.074
		0.22	1.181	-	1.181
	BXT/AB	0.01	0.054	-	0.054
		0.05	0.269	-	0.269
		0.1	0.537	-	0.537
		0.15	0.806	-	0.806
Multicomponent	BXT/1	2.5	8.592	31.683	40.275
		3	10.310	38.020	48.330
		3.5	12.029	44.356	56.385
	BXT/5	0.075	0.056	0.129	0.184
		0.15	0.111	0.258	0.369
		0.2	0.148	0.344	0.492
		0.4	0.296	0.687	0.984
	BXT/10P	0.8	0.687	2.578	3.265
		1	0.859	3.222	4.081
	BXT/12C	0.1	0.110	0.107	0.217
		0.6	0.660	0.644	1.304
	PL-UV-H-2B	0.15	0.483	0.967	1.450
		0.2	0.644	1.289	1.933
		0.3	0.967	1.933	2.900

Table 5. Microorganisms used for to evaluate antimicrobial properties and characteristics of incubation periods thereof

Type of microorganism	Microorganism	Reference code	Incubation period		
			Agar type	Temperature (°C)	Time (days)
Fungus	*Aspergillus niger*	ATCC 6275	Malt extract agar	25 ± 2	5-7
Bacteria	*Staphylococcus aureus*	ATCC 6538P	Nutrient agar	30 ± 2	2
Bacteria	*Escherichia coli*	ATCC 8739		30 ± 2	2
Algae	*Scenedesmus vaculatus*	SAG 211-8b	Kuhl solution	20 ± 2	21
Algae	*Stichococcus bacillaris*	SAG 379-1a		20 ± 2	21

Table 6. Failure phases and qualification of each formulation during the experimental campaign

Formulation		Failure phase
Monocomponent	BXT/AB	Physical properties
	BXT/11	Physical properties
	ACTICIDE MBP	Antimicrobial properties (before aging process)
	BIOPOL D212	Releasing test
	VANTOCIL IB	Releasing test
	VANQUISH 100	Physical properties
	DENSIL P	Physical properties
	P24-81	Antimicrobial properties (before aging process)
	BC 98-56	Physical properties
	BXT	Antimicrobial properties (after aging process)
Multicomponent	BXT/12C	Physical properties
	BXT/10P	Physical properties
	BXT/1	Physical properties
	BXT/5	Antimicrobial properties (before aging process)
	PL-UV-H-2B	None

Table 7. Mechanical properties of the monocomponent formulations failing first phase tests

Formulation name	Dosage (% odw)	Flow (mm)	Fresh density (kg/m^3)	Mechanical properties (MPa)			
				Compressive strength		Flexural strength	
				1 d	28d	1d	28d
DENSIL P	0	154	2450	19.1	47.8	4.8	8.2
	0.2	154	2435	19.3	48.3	4.6	9.4
	0.5	155	2435	8.5	41.1	2.2	7.2
	1.0	155	2435	4.4	42.1	1.2	8.3
	2.0	159	2422	n.d.	34.2	n.d.	7.3
BC 98-56	0	150	2432	12.8	33.5	3.5	6.8
	0.3	135	2465	n.d.	36.6	n.d.	7.6
VANQUISH 100	0	155	2450	15.4	33.6	3.3	6.5
	0.002	152	2392	14.2	33.1	2.9	6.1
	0.013	152	2401	13.1	29.2	2.8	5.6
	0.025	153	2417	13.4	27.4	3.0	5.5
	0.037	156	2439	12.8	30.9	3.0	5.4
	0.050	150	2427	12.8	27.4	2.5	5.8
BXT/11	0	117	2389	15.5	35.1	3.2	5.6
	0.15	118	2005	5.3	16.0	2.1	3.3
	0.18	122	2004	7.2	21.4	2.5	5.2
	0.20	121	2122	6.9	20.9	2.7	5.3
	0.22	127	2174	9.6	24.3	2.4	5.8
BXT/AB	0	117	2389	15.5	35.1	3.2	5.6
	0.01	120	2420	15.1	29.9	2.6	5.4
	0.05	125	2395	14.8	34.3	2.8	5.4
	0.10	128	2467	13.0	31.8	2.3	5.0
	0.15	124	2397	11.9	29.8	2.1	4.9

Table 8. Mechanical properties of the multicomponent formulations

Formulation name	Dosage (% odw)	Flow (mm)	Fresh density (kg/m^3)	Mechanical properties (MPa)			
				Compressive strength		Flexural strength	
				1 d	28d	1d	28d
BXT/1	0	157	2426	12.5	36.7	3.7	7.5
	0.4	150	2465	n.d.	34.0	n.d.	6.7
	0.45	153	2439	n.d.	31.2	n.d.	6.5
	0.5	149	2468	n.d.	34.3	n.d.	6.3
BXT/5	0	165	2343	15.8	42.3	4.8	8.7
	0.08	170	2337	14.9	40.5	4.3	8.1
	0.15	167	2381	15.1	40.0	3.8	8.4
	0.2	165	2330	14.5	39.0	3.5	7.9
	0.4	168	2327	14.0	39.0	3.3	7.7
BXT/10P	0	125	2473	19.1	35.0	4.8	7.2
	0.8	117	2266	10.3	30.6	2.8	6.1
	1	120	2200	8.2	29.0	2.5	6.0
BXT/12C	0	117	2389	12.8	33.5	3.5	6.8
	0.6	120	2420	8.1	28.4	2.8	5.6
	1	125	2395	6.2	22.7	2.3	4.4
PL-UV-H-2B	0	148	2523	14.3	37.1	4.0	9.4
	0.15	152	2501	12.7	36.9	3.5	8.6
	0.2	145	2479	11.3	35.7	2.8	8.2
	0.3	150	2484	14.0	34.9	3.2	8.5

Figure

Figure 1

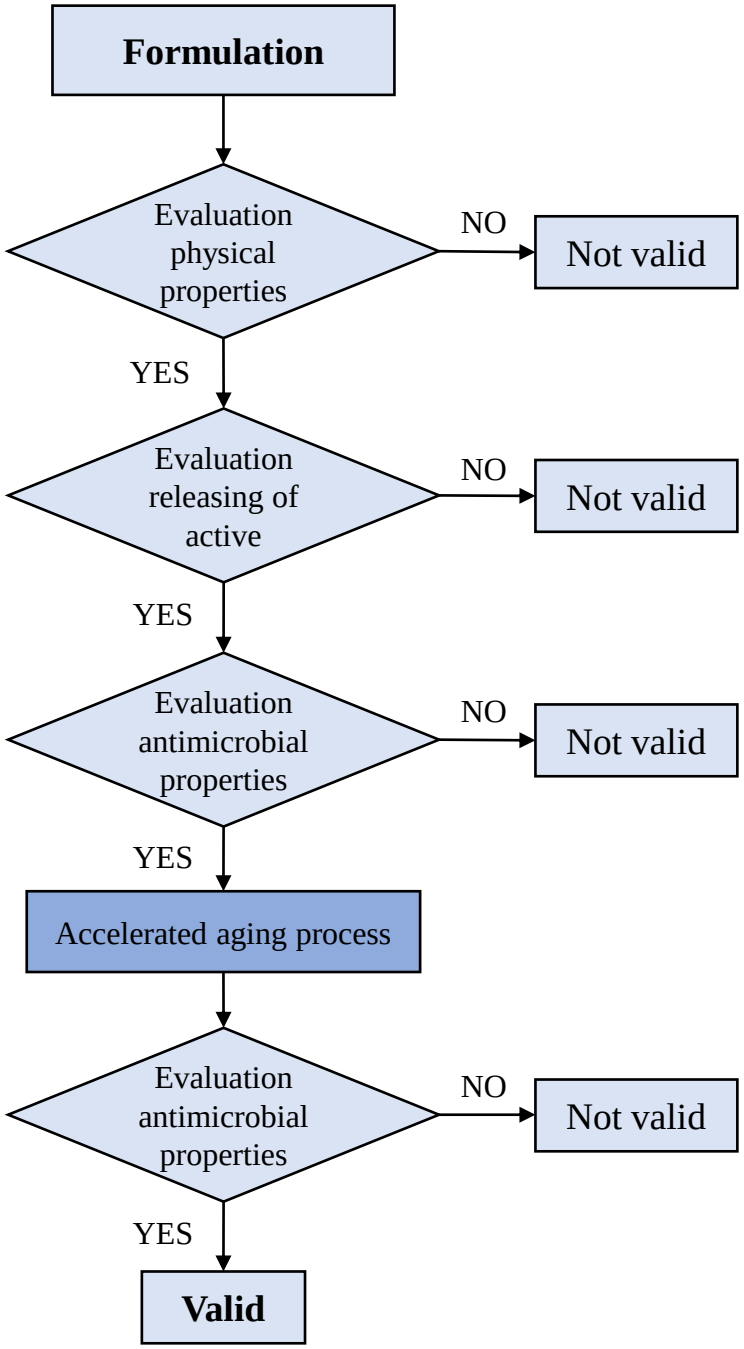
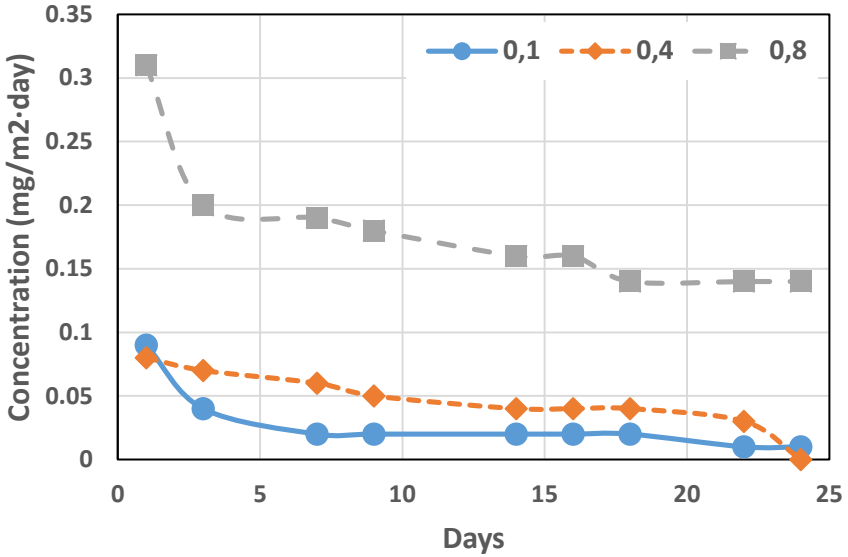

Formulation
Evaluation physical properties
NO
Not valid
YES
Evaluation releasing of active
NO
Not valid
YES
Evaluation antimicrobial properties
NO
Not valid
YES
Accelerated aging process
Evaluation antimicrobial properties
NO
Not valid
YES
Valid

Figure 2a


0,1
0,4
0,8
Concentration (mg/m2·day)
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
Days

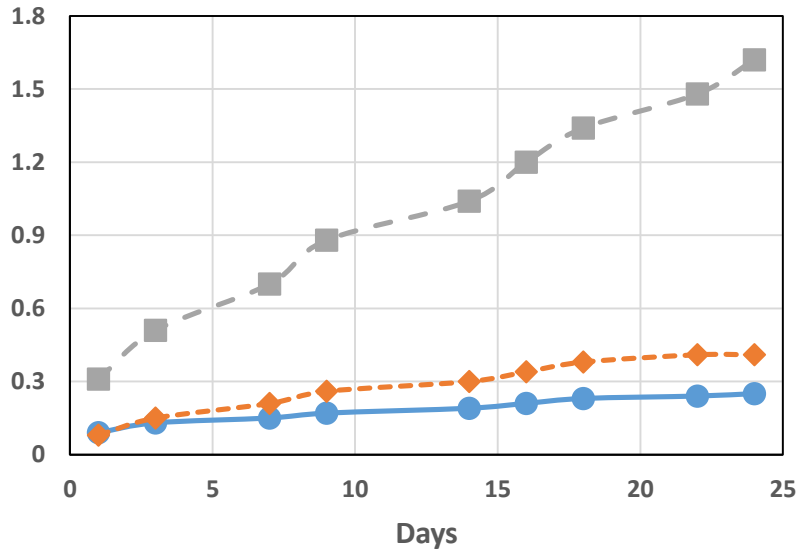

1.8
1.5
1.2
0.9
0.6
0.3
0
0
5
10
15
20
25
Days

Figure 2b

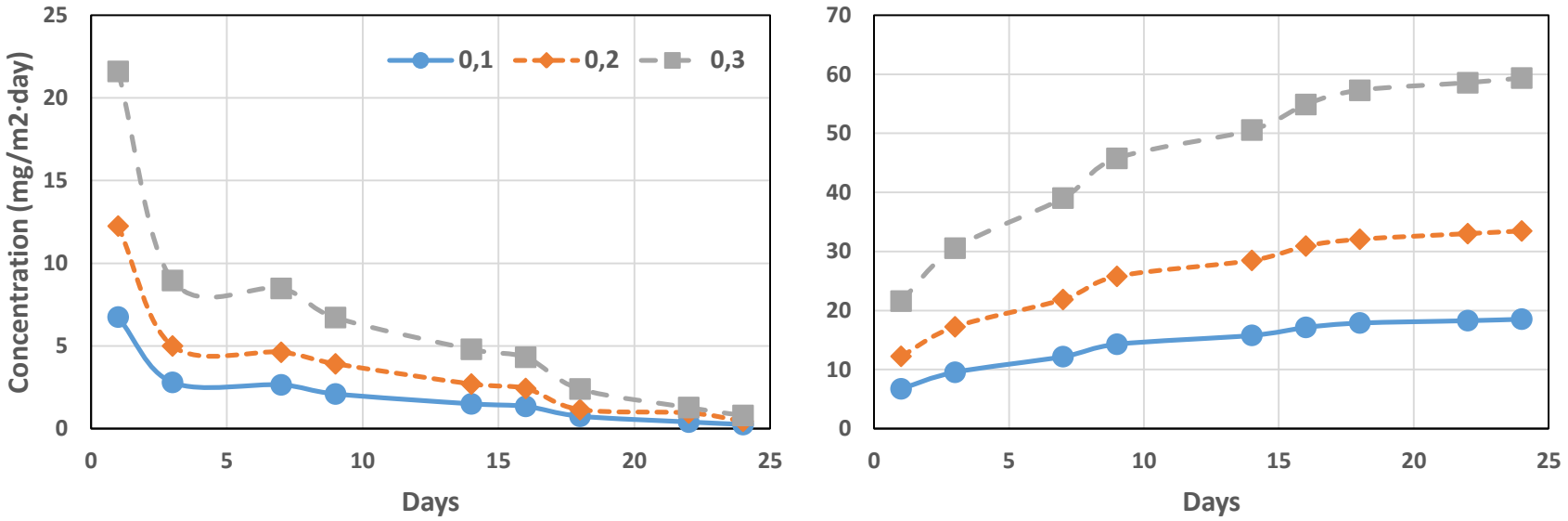


Figure 2c

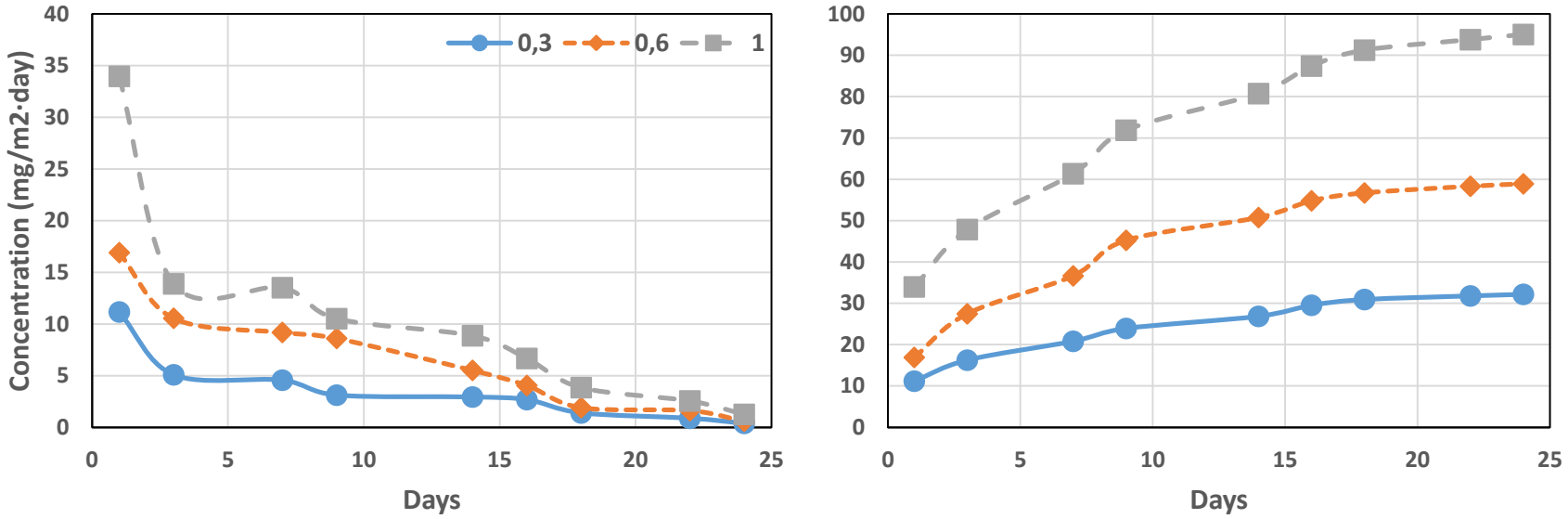


Figure 2d

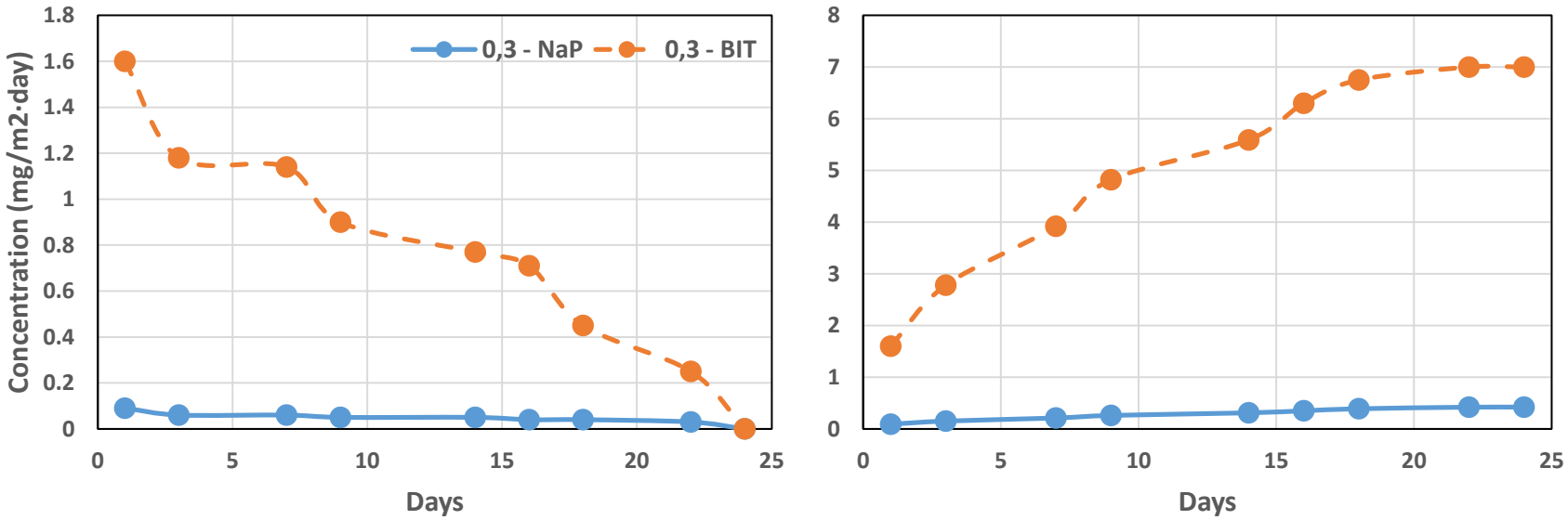


Figure 2e

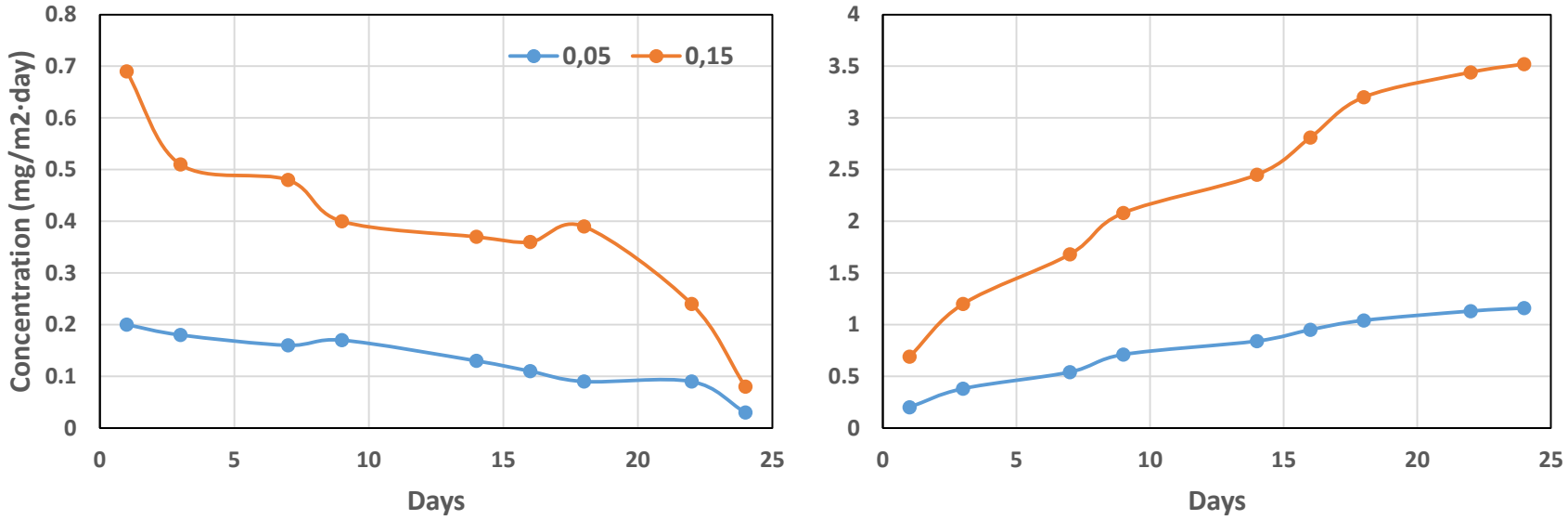


Figure 3

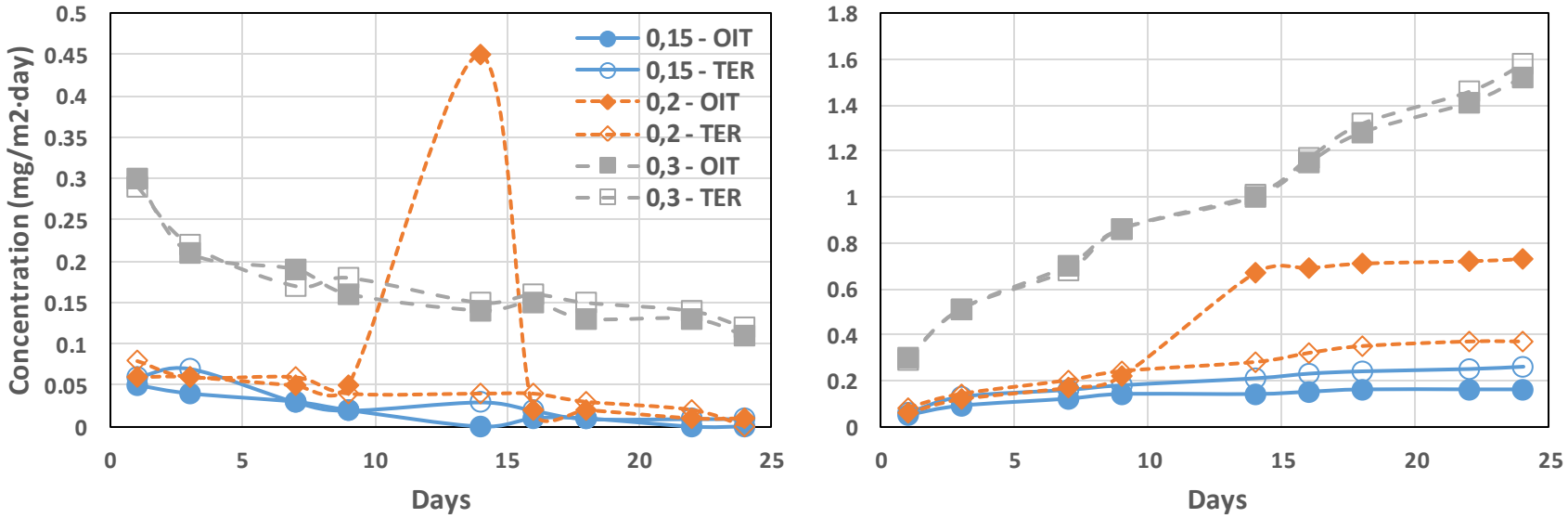

Concentration (mg/m2·day)
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
0
5
10
15
20
25
Days
0,15 - OIT
0,15 - TER
0,2 - OIT
0,2 - TER
0,3 - OIT
0,3 - TER
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
5
10
15
20
25
Days

Figure 4

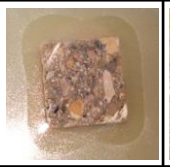
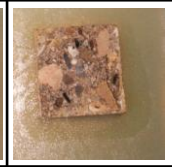
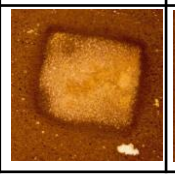
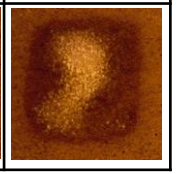
Microorganism	Formulation content (% odw)			
	Reference	0.1	0.4	0.8
Escherichia coli				
Staphylococcus aureus				
Aspergillus niger				

Figure 5

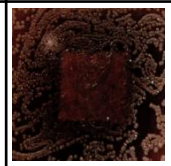
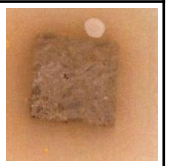
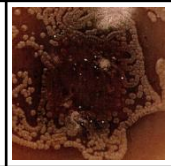
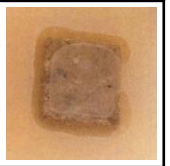
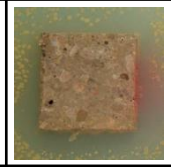
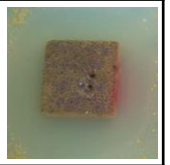
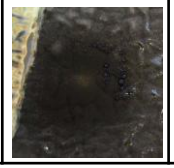
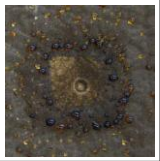
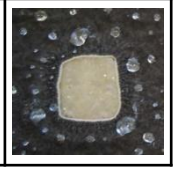
Microorganism	Formulation content (% odw)	
	Reference	0.3
Escherichia coli		
Staphylococcus aureus		
Aspergillus niger		

Figure 6

a)

Microorganism	Formulation content (% odw)		
	Reference	0.05	0.15
Escherichia coli			
Staphylococcus aureus			
Aspergillus niger			

b)

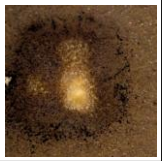
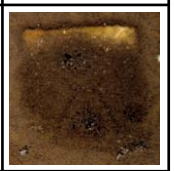
Microorganism	Formulation content (% odw)		
	Reference	0.05	0.15
Escherichia coli			
Staphylococcus aureus			
Aspergillus niger			

Figure 7

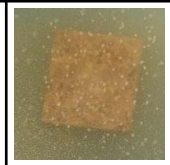
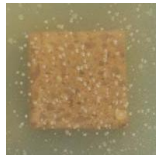
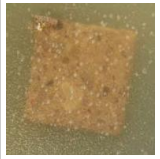
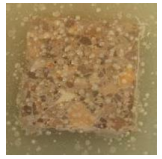
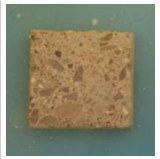
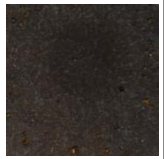
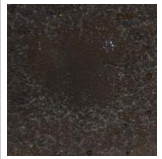
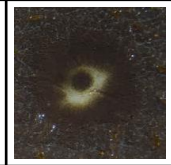
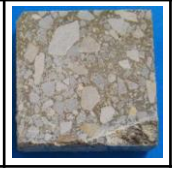
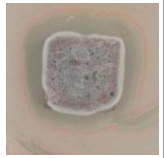
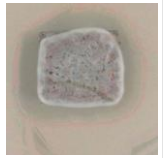
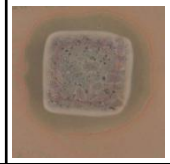
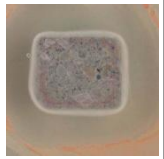
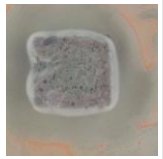
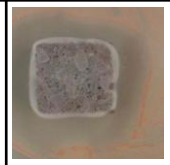
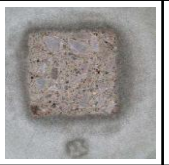
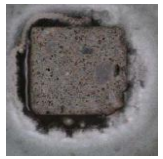
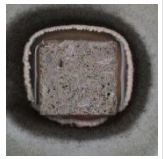
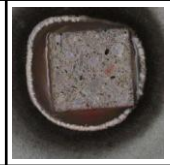
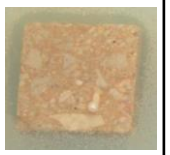
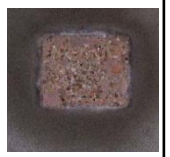
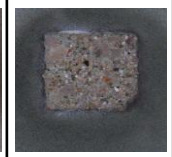
Microorganism	Formulation content (% odw)				
	Reference	0.075	0.15	0.2	0.4
Escherichia coli					
Staphylococcus aureus					
Aspergillus niger					
Stichococcus bacillaris Scenedesmus vaculatus					

Figure 8

a)

Microorganism	Formulation content (% odw)			
	Reference	0.15	0.2	0.3
Escherichia coli				
Staphylococcus aureus				
Aspergillus niger				
Stichococcus bacillaris *Scenedesmus vaculatus*				

b)

Microorganism	Formulation content (% odw)			
	Reference	0.15	0.2	0.3
Escherichia coli				
Staphylococcus aureus				
Aspergillus niger				
Stichococcus bacillaris *Scenedesmus vaculatus*