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# How to Improve the Bioreceptivity of Concrete Infrastructure Used in Marine Ecosystems? Literature Review for Mechanisms, Key Factors, and Colonization Effects

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37 finally analyzed both the ecological effect and the positive and negative effects of biofilm for-  
38 mation on the durability of concrete, in the scope to provide clear and operational results for future  
39 concrete coastal construction implementation for decision maker.

40

### **ADDITIONAL INDEX WORDS**

41 *Eco-engineering practices, marine biodiversity, concrete, colonization, eco-design, bioreceptivity,*  
42 *biofilm, durability.*

43

44

## INTRODUCTION

46 The global ecological crisis is the concomitant with the Anthropocene, a new geological era in  
47 which humans have become the central actors on the planet (Crutzen, 2006; Shukla *et al.*, 2021;  
48 Valipour, Bateni, and Jun, 2021). The recent report (2019) of the Intergovernmental Science-Policy  
49 Platform on Biodiversity and Ecosystem Services (IPBES) presents a devastating assessment of  
50 the impact on biodiversity since the start of the industrial era two centuries ago: in terms of coastal  
51 and marine ecosystems, more than 85% of wetlands have been destroyed, and human activities  
52 threaten 66% of the oceans. The causes leading to this situation are today well known and include  
53 (in order of impact): (1) artificialization and land use, (2) overexploitation of resources (fishing,  
54 forestry, etc.), (3) climate change, (4) pollution (plastics, chemical residues, etc.) and (5) invasive  
55 species (*Ibid*).

56 Therefore, reducing artificialization and land use is critical to reduce biodiversity loss and preserve  
57 the future of humanity. Yet predictions indicate a clear acceleration of urbanization around the  
58 world, on land and at sea. Between 2019 and 2040, more than US\$60,000 billion is forecast to be  
59 invested in infrastructure in the 56 countries representing 88% of global GDP (Global  
60 Infrastructure Hub 2017–2018). More than 1.2 million km<sup>2</sup> will be urbanized by 2030 – just 8 years  
61 from now – representing an increase of more than 185% compared to the period from 1970 to 2000  
62 (Seto *et al.*, 2012). By 2050, 3 to 4.7 million km<sup>2</sup> of roads will be constructed, which represents an  
63 increase of more than 25% compared to the current annual rate (Meijer *et al.*, 2018). This process  
64 will be particularly significant along coasts, where eight of the ten largest megalopolises in the  
65 world are located (such as Lagos, Tokyo, Jakarta and New York). Around the world, the population  
66 living within 100 km of a coast is estimated to approach 7.5 billion people by 2050 (de Fraiture *et*  
67 *al.*, 2007), leading to a need for increased coastal development and potentially disturbing essential  
68 coastal habitats (Weilgart, 2007). Global marine infrastructure extended over approximately  
69 32,000 km<sup>2</sup> in 2018 (Bugnot *et al.*, 2020) and is expected to reach 39,400 km<sup>2</sup> by 2028: a surface  
70 area equivalent to the country of Bhutan. The area of seascapes impacted by these structures was  
71 estimated to be between 1 and 3.4 million km<sup>2</sup> in 2018, which is expected to increase 50–70% by  
72 2028 (*Ibid*). The phenomenon of ‘coastal squeeze’, a term introduced by Doody (2004) to refer to  
73 the threat to the existence of coastal habitats caused by the compound impacts of sea-level rise and  
74 human activities, will not abate under current development conditions. Most of these structures are  
75 made from concrete, the most durable materials for the aggressive marine environment. Table 1  
76 summarize the types of concrete structures found in coastal areas.

77 From the 1970s through the 1990s, coastal ecology literature dealing with biodiversity focused  
78 mainly on artificial substrates use to promote restoration, studied from colonization or settlement  
79 phenomena problematics (Relini *et al.*, 1998; Zobel, 1974). However, since the 2000s, hard  
80 artificial infrastructure made with concrete, has been implicated as a major risk factor for local and  
81 native biodiversity, by introducing invasive species with ubiquitous and rapid growing  
82 characteristics, in comparison with surrounding natural rocky area (Bulleri and Airoidi, 2005;  
83 Bulleri and Chapman, 2010, 2004; Jackson *et al.*, 2001). In contrast, several studies about  
84 ecological design of coastal infrastructure published since 2010 has clearly demonstrated that it is  
85 feasible to enhance biodiversity through changes in concrete chemical composition, roughness,

86 surface treatment, or inclusion of variously sized pits or holes (Chapman and Underwood, 2011;  
 87 Evans, Bax and Bernal, 2016; Evans, Bax and Smith, 2018; Martins *et al.*, 2010; Phan, van Thiel  
 88 de Vries and Stive, 2015; Souche *et al.*, 2016). In addition, other studies also has indicated that  
 89 physical alteration and damage to artificial structures due to natural processes (*e.g.* weathering,  
 90 wave action, chemical erosion) could be reduced by the positive protective role of marine  
 91 organisms (Coombes *et al.*, 2017, 2013; Gowell, Coombes and Viles, 2015; La Marca *et al.*, 2014;  
 92 Naylor and Coombes, 2015; Perkol-Finkel and Sella, 2014).

93 Table 1. Types of structures in coastal areas, their functions and the materials used

Types of structure	Socio-technical objectives	Materials
<b>Coastal defense structures (protection of coastlines and management of wave action effects)</b>		
<i>Offshore breakwater</i>	Coastal defense structure built near the coast to protect it from the actions of the sea by reducing wave-action intensity	Rock fill Concrete blocks
<i>Groin</i>	Coastal defense structure to protect the coast from erosion and sediment transport	Rock fill
<i>Harbor breakwater, jetty</i>	Structure to protect a harbor area from wave action and currents	Rock fill and blocks of concrete (blocks, tetrapodes, accropodes)
<b>Harbor structures</b>		
<i>Finger pier</i>	Mooring structure for loading and unloading boats	Reinforced or prestressed concrete apron on caisson or gabion-type piles
<i>Boom wharf</i>	Structure on piles making up a finger pier (wharf perpendicular to the coast)	Reinforced concrete apron on metal or reinforced concrete piles
<i>Quay</i>	Continuous open-platform structure for mooring, loading and unloading boats	Reinforced concrete caissons, metal sheet-pile gabions, curtain walls and screens in metal or reinforced concrete
<i>Floating dock</i>	Floating structure enabling docking and mooring of boats in a harbor	Metal or reinforced concrete, held in place by piles or reinforced concrete moorings
<i>Floating breakwater</i>	Floating structure to protect a harbor from wave action	Reinforced concrete, held in place by piles or reinforced concrete moorings
<i>Lock / gate</i>	Structure enabling boats to change level and/or to keep a dock filled with water	Infrastructure in reinforced concrete, equipment in steel

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**Harbor structures for maintenance and services**

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<b><i>Dry dock</i></b>	Waterproof basin that can be drained or filled with water to enable maintenance and repair of boats/caissons	Unreinforced concrete, reinforced concrete / reinforced concrete, prestressed concrete
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<b><i>Slipway</i></b>	Slope enabling a boat to be launched or landed for maintenance	Reinforced concrete
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<b><i>Self-elevating platform, jack-up barge</i></b>	Floating deck using a wet dock to raise boats out of the water for maintenance or refitting	Infrastructure in reinforced concrete, equipment in steel
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**Open-sea structures**

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<b><i>Offshore structure</i></b>	Structure out as sea such as an oil rig or wind turbine	Reinforced concrete and steel
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<b><i>Anchorage, ballast</i></b>	Underwater structure for anchoring boats or floating infrastructure	Reinforced concrete
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<b><i>Artificial reef</i></b>	Underwater structure for protecting or hosting fauna and flora or for leisure activities	Reinforced concrete, metal, diverse materials
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95 Pragmatically, most current coastal concrete infrastructure cannot be readily removed. But there is  
96 now increasing research efforts into innovative ways that future new infrastructures can be  
97 designed and built to meet engineering requirements, while also increasing its value as an  
98 ecological habitat for marine life (Dafforn *et al.*, 2015; Firth *et al.*, 2014; Lacroix and Pioch, 2011;  
99 Naylor and Coombes, 2015; Patranella *et al.*, 2017; Perkol-Finkel and Sella, 2014; Phan, van Thiel  
100 de Vries and Stive, 2015; Pioch and Souche, 2021). This “win-win” approach, should ensure  
101 benefits both for human society and nature (Chapman and Underwood, 2011; Ferrario *et al.*, 2014;  
102 Perkol-Finkel *et al.*, 2018; Pioch *et al.*, 2018, 2011; Temmerman *et al.*, 2013). This paradigm  
103 support the idea that we have to construct structures with a minimal ecological impact on the  
104 existing environment, maximizing their potential for colonization, and restore original habitats or  
105 create new habitats adapted for local species ecological requirements (Browne and Chapman,  
106 2011; Souche *et al.*, 2018). In this way, combining engineering techniques with ecological  
107 understanding can provide, with a cost-effective ways, the maintenance or development of native  
108 biodiversity (Airoldi *et al.*, 2020; Martins *et al.*, 2010; Pioch *et al.*, 2018; Scheres and Schüttrumpf,  
109 2019; Souche *et al.*, 2019).

110 Artificial marine structures modify, positively or negatively, the natural environment (Azevedo *et*  
111 *al.*, 2006) to become part of a new ecosystem (Giraudel, Garcia and Ledoux, 2014). Then to avoid  
112 high negatives impacts, as we have seen, the ecological mechanism of settlement on concrete needs  
113 to be assessed before their implementation (Baine, 2001; Bulleri and Chapman, 2010). Impacts  
114 parameters are divided in five main fields: localization for the immersion zone, implantation  
115 (Giraudel, Garcia and Ledoux, 2014), seasons and immersion time (Firth *et al.*, 2014; Vaz-Pinto *et*  
116 *al.*, 2014), design of the structure (Baine, 2001; Pioch *et al.*, 2011), and the type of materials used

117 (Anderson and Underwood, 1994; Fitzhardinge and Bailey-Brock, 1989; Lee *et al.*, 2008). There  
118 are also interdependent relationships that change over time between the type of structure, exposure  
119 conditions, substrate, seasonality and colonization by living organisms (Anderson and Underwood,  
120 1994; C. Choi *et al.*, 2006; Somsueb, Ohno and Kimura, 2001).

121 The way to associate civil engineering and ecological consideration is now a common subject of  
122 research with eco-engineering, and specifically within the construction field the eco-design  
123 strategies. Recent research applications, have demonstrated that it's possible to integrate  
124 environmental objectives in the design of functional concrete marine infrastructures (Bulleri and  
125 Chapman, 2010; Lacroix and Pioch, 2011; Pioch *et al.*, 2011; Pioch and Souche, 2021). Thus,  
126 understanding the interactions between microorganisms and cementitious materials is a crucial step  
127 toward constructing more durable, safer and more ecological structures (Lors, Feugeas and  
128 Tribollet, 2019; Ottel  *et al.*, 2011; Perini and Ottel , 2014; Perkol-Finkel and Sella, 2014). As the  
129 colonization of cementitious material by microorganisms is an essential step for the adhesion of  
130 other organisms (Mieszkin, Callow and Callow, 2013; Rajitha *et al.*, 2020; Salta *et al.*, 2013), the  
131 aim is to favor this colonization in order to reduce the ecological impact of the structure and  
132 consequently increase biodiversity, without impacting the structure's strength and durability. Then,  
133 additional studies are necessary to determine the different factors that can influence the  
134 colonization of cementitious materials in the marine environment, but the main parameters seem  
135 to be the water pH, the materials chemical composition and surface roughness or topography  
136 (Grosseau *et al.*, 2015; Hayek *et al.*, 2020a, 2021; McManus *et al.*, 2018; Miller *et al.*, 2009, 2012).

137 The objective of this study was to identify key parameters to take into account to promote the  
138 bioreceptivity of concrete structures in coastal and marine environments to identify key parameters  
139 to take into account in order to promote the bioreceptivity of concrete structures.

## 140 **Scope and research significance**

141 The interaction of cement-based materials with microorganisms has been widely studied in  
142 building and sewer network. To the best of our knowledge, in the marine environment, the present  
143 literature review is the first one in the field of concrete bioreceptivity and concrete biocolonization  
144 by marine bacteria. In this review, a significant amount of research has been performed on the first  
145 stage of colonization of concrete in seawater with significant research on this topic including i) the  
146 early colonization process and the detailed mechanisms involved in the biofilm formation on the  
147 surface of marine concrete ii) the colonizing organisms of cement-based materials and iii) the  
148 physicochemical factors influencing the colonization of cement-based materials in marine  
149 environments.

150 Afterward, to achieve the purpose of this paper, i) some of the most significant quantitative results  
151 about the bioreceptivity of concrete in the marine environment are presented ii) the ecological  
152 impact and the effect of biological colonization on the durability of concrete are discussed and iii)  
153 the perspectives to design a more bioreceptive concrete (green concrete) are mentioned.

154 Web of science and google scholar are the two databases used in this study for collecting the  
155 relevant materials (211 studies) from the literature. The keywords used were: Biofilm formation,

156 Concrete biocolonization, Ecological impact, Eco-engineering *practices* Artificial reef, Marine  
157 environment, Concrete Durability, Bioreceptivity and marine biodiversity.

158

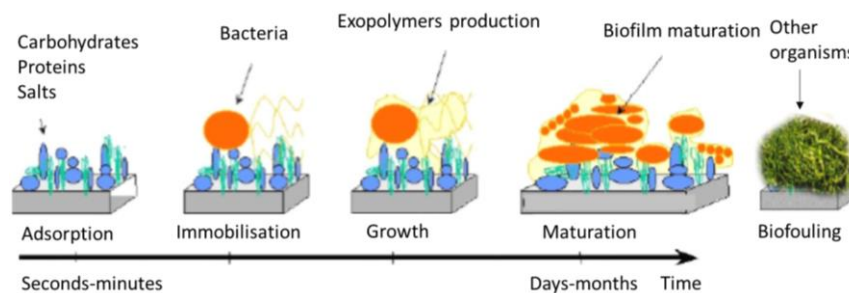
## 159 METHODS: BIOLOGICAL FACTORS AFFECTING THE 160 COLONIZATION OF CONCRETE IN THE MARINE 161 ENVIRONMENT

### 162 **Colonization and the development of biofilm on surface concrete**

163 From the first hours of the immersion of an object in seawater, a cement-based material is covered  
164 with a biofilm consisting of one or more microbial communities (Dang and Lovell, 2015;  
165 Dobretsov, Teplitski and Paul, 2009). By definition, the biofilm is considered to be a sessile  
166 microbial community irreversibly attached to a substrate or an interface and included in a coating  
167 of extracellular polymeric substances (EPS). Biofilm cells have modified phenotypes compared to  
168 plankton cells that float freely in the environment (Donlan and Costerton, 2002).

169 The biofilm is the first stage in colonization, which enables the establishment of other marine  
170 organisms via the EPS synthesized on the surface (Hinsa and O'Toole, 2006; Pasmore and  
171 Costerton, 2003; Wood *et al.*, 2016). Bacteria (bacterial biofilm) are the first colonizers, which  
172 facilitate the adhesion of other micro- and macro-organisms such as fungi, microalgae, macroalgae  
173 and invertebrates (Briand *et al.*, 2018; Brian-Jaisson *et al.*, 2014; Dobretsov and Rittschof, 2020;  
174 Salta *et al.*, 2013) (Figure 1).

175 After immersion, an induction phase allows the surface to be conditioned (through the formation  
176 of the conditioning film), resulting in exponential accumulation, characterized by the adhesion and  
177 growth of bacteria and other microorganisms on the surface. The length of the induction phase  
178 depends on both the environmental parameters and the intrinsic parameters of the host material  
179 (Hayek *et al.*, 2019; Karunasagar *et al.*, 1996; Katsikogianni and Missirlis, 2004). A submerged  
180 solid object adsorbs organic and inorganic molecules and ions, leading to a modification of the  
181 physicochemical properties of the surface, thus forming a nutrient-rich micro-niche (on a small  
182 scale in comparison to the material) that favors the adhesion of bacterial cells. In this way, surface  
183 conditioning impacts the adhesion of bacterial cells (Loeb and Neihof, 1975), which can be  
184 stimulated or inhibited by the nature of the adsorbed molecules. This is why the conditioning of  
185 the surface is considered a key step in biofilm formation (Herrmann *et al.*, 1988; Tang *et al.*, 2006).



186

187

Figure 1. Biofouling in the marine environment (Ogunola and Onada, 2016).



188 Therefore, the main stages of biofilm formation are (i) reversible and irreversible adhesion of  
189 bacterial cells to the surface of the cement-based material; (ii) growth and maturing of the biofilm  
190 with the secretion of EPS; and (iii) partial detachment of the biofilm and the dispersion of bacterial  
191 cells (Hinsa and O'Toole, 2006; O'Toole, Kaplan and Kolter, 2000).

192 The adhesion of bacteria to a surface first requires that they move toward the surface to be  
193 colonized. This movement can be due to environmental phenomena (gravitational and  
194 hydrodynamic forces in the surrounding fluid) and/or by appendages on the bacteria that assist their  
195 movement, such as flagella or pili (Ha and O'Toole, 2015; Katsikogianni and Missirlis, 2004).

196 Once a bacterium is within a critical distance of the object (generally  $< 1$  nm), low-intensity  
197 interactions take place between the bacterium and the surface, e.g. electrostatic forces, Van der  
198 Waals forces and hydrophobic interactions (Carpentier and Cerf, 1993; Dunne, 2002). Depending  
199 on these various interactions, the bacterium may either adhere or be repelled by the effect of  
200 hydrodynamic actions. If the interactions are favorable, the bacterium adheres to the surface and  
201 this step is then considered the reversible adhesion phase (An and Friedman, 1998; Berne *et al.*,  
202 2018; Busscher and Weerkamp, 1987; van Loosdrecht *et al.*, 1989). The factors that influence this  
203 phase concern interactions between the bacterial cells and the surface of the cement-based material:  
204 (i) the specific characteristics of the bacteria, such as its cell surface charge, cell surface  
205 hydrophobicity and the chemical composition of the cell wall; (ii) the intrinsic parameters of the  
206 host surface, such as its physicochemical nature, polarity, porosity and roughness; and (iii) the  
207 environmental conditions, such as pH level, temperature and flow conditions (Bunt *et al.*, 1993;  
208 Scheuerman *et al.*, 1998; Speranza *et al.*, 2004). According to Carpentier and Cerf (1993), host  
209 surface porosity and roughness are believed to increase bacterial adhesion, while a negatively  
210 charged surface may have a repulsive effect as most bacteria are negatively charged.

211 Adhesion subsequently becomes irreversible when strong covalent interactions are established  
212 between the bacteria and the surface of the cement-based material. The covalent interactions are  
213 formed by macromolecules on the bacteria surface and include polysaccharides, proteins or  
214 lipopolysaccharides (Berne *et al.*, 2018).

215 Once established on the object, the bacteria multiply and the EPS they secrete develop a three-  
216 dimensional structure to form colonies that cover all or part of the surface. This varies depending  
217 on the bacteria present and (i) their capacity to communicate with each other, i.e. their cell-to-cell  
218 communication, or quorum sensing, system (Branda *et al.*, 2005; Dobretsov *et al.*, 2011); (ii) the  
219 intracellular concentration of cyclic di-GMP (a high intracellular concentration of c-di-GMP  
220 promotes the expression of genes involved in biofilm formation) (Barraud *et al.*, 2009; Chao *et al.*,  
221 2013; Dang and Lovell, 2015); (iii) environmental conditions, and (iv) the properties of the host  
222 material, especially hydrophobic properties ( O'Toole, Kaplan and Kolter, 2000).

223 A mature biofilm has a three-dimensional structure surrounded by a matrix made up of mineral  
224 elements and EPS: proteins, polysaccharides, nucleic acids, lipids and other cellular components.  
225 This matrix forms a barrier that protects biofilm microorganisms against external threats (Ha and  
226 O'Toole, 2015; Soleimani *et al.*, 2013).

227 The last step in the development of the biofilm is the dispersion of cells in the environment after  
228 detaching from the surface. This dispersion is an essential part of the biofilm cycle as it enables the  
229 bacteria to colonize new surfaces and develop new biofilms (Kaplan, 2010). In general, biofilm  
230 dispersion mechanisms can be divided into two broad categories: (i) active dispersion based on  
231 mechanisms initiated by the bacteria; and (ii) passive dispersion due to external forces, such as  
232 shearing forces caused by hydrodynamic conditions (Lawrence *et al.*, 2002). Several factors are  
233 involved in the complex process of biofilm dispersion, such as environmental signals, quorum  
234 sensing systems, and the bacteria's production of extracellular enzymes that can degrade the matrix  
235 of biofilm components and facilitate dissociation (Boyd and Chakrabarty, 1994; Kaplan, 2010).

## 236 **Colonizing organisms of cement-based materials**

237 In the marine environment, there are many ubiquitous colonizing microorganisms likely to form a  
238 biofilm on the surface of objects under suitable conditions. The main colonizing microorganisms  
239 of cement-based materials are bacteria, fungi, cyanobacteria and algae (Gaylarde and Gaylarde,  
240 2005).

### 241 **Bacteria**

242 Bacteria are medium-sized unicellular microorganisms that vary in size from 0.5 to 1.5  $\mu\text{m}$  (Yañez  
243 *et al.*, 2010) and develop on all types of surface materials; they are generally pioneers in the  
244 colonization process (Meng *et al.*, 2020; Romani *et al.*, 2019; Scatigno *et al.*, 2016).

245 The deterioration of cement-based materials by microbial induced corrosion has been known since  
246 1945 following the work of Parker, who was the first to isolate from concrete samples a sulfuric  
247 acid-producing bacterium species initially called *Thiobacillus concretivorus*, then subsequently  
248 called *Acidithiobacillus thiooxidans* (Parker, 1945). This phenomenon has mainly been studied in  
249 sanitation networks, where the sulfate- and nutrient-rich environment provides optimal conditions  
250 for the development of microbial fauna belonging to the genus *Thiobacillus* (Yuan *et al.*, 2015).  
251 *Thiobacilli* are bacteria that oxidize sulfur compounds into sulfuric acid, a substance that is  
252 particularly aggressive to concrete (De Belie, 2010). However, the biochemical processes leading  
253 to the degradation of concrete under the influence of bacteria are essentially based on the synthesis  
254 (bacterial metabolism) of acids such as sulfuric acid ( $\text{H}_2\text{SO}_4$ ) and nitric acid ( $\text{HNO}_3$ ) (Cwalina,  
255 2008). These acids dissolve calcareous components, resulting in a loss of cohesion in the material  
256 and the appearance of cracks or corrosion of the reinforcement bars in the concrete (Chatterjee and  
257 Goyns, 2013; Poonguzhali *et al.*, 2008). These effects are common in the sulfate- and ammoniac-  
258 rich environment of sanitation networks.

259 Despite the large number of concrete structures present in the waters of seas, rivers and lakes,  
260 studies concerning the colonization of cementitious materials by bacteria in these environments  
261 remain relatively rare (Chlayon, Iwanami et Chijiwa, 2020; Cooke *et al.*, 2020; Costa and  
262 Appleton, 2001; Rica *et al.*, 2016; Scheres and Schüttrumpf, 2019; Sosa *et al.*, 2011; Souche *et al.*,  
263 2016).

### 264 **Algae and cyanobacteria**

265 Microscopic algae (microalgae) and cyanobacteria are unicellular or multicellular species,  
266 generally between 0.5  $\mu\text{m}$  and 1 mm in size. These microorganisms are characterized by their  
267 capacity to perform oxygenic photosynthesis (Leclerc, 1975).<sup>1</sup> As algae and cyanobacteria are  
268 capable of generating their own organic matter from mineral elements, they are autotrophic  
269 organisms (Harper and Pendleton, 1993; Krumbein, 1979; Subashchandrabose et al., 2011).

270 According to the literature, green algae (Chlorophyta) and cyanobacteria (Cyanophyta) produce  
271 greenish, reddish or blackish stains on concrete, depending on the composition of the biofilm. In  
272 addition, the secretion of organic acids, resulting from the metabolism of these organisms, can lead  
273 to the dissolution of the calcium-rich phases of calcareous stones, concrete and mortar. In this way,  
274 algae and cyanobacteria can cause the alteration or degradation of cementitious materials  
275 (Escadeillas *et al.*, 2008, 2007).

276 Cyanobacteria are prokaryotic microorganisms, whereas microalgae are eukaryotic. Microalgae  
277 are divided into several phylogenetic groups, such as chlorophytes, rhodophytes and diatoms (Al-  
278 Handal and Wulff, 2008). Chlorophytes mainly develop in freshwater, such as rivers, and are the  
279 largest group of green algae (Lueangthuwapranit, Sampantarak and Wongsai, 2011). Rhodophytes  
280 are mainly macroscopic multicellular marine organisms and are known as red algae (Bengtson *et*  
281 *al.*, 2017). Diatoms are microscopic unicellular organisms characterized by a silica cell wall, called  
282 a ‘frustule’, that surrounds them. They are abundant in the marine environment (Robert *et al.*,  
283 2004).

284 The colonization of cement-based materials in situ by cyanobacteria and algae has been confirmed  
285 in various studies by measuring the quantity of chlorophyll and/or by the appearance of different-  
286 colored stains on the samples tested (Silva and Naik, 2013; Souche *et al.*, 2016; Tran, 2011;  
287 Vishwakarma *et al.*, 2014). However, in most of these studies, the colonizing species were not  
288 identified and described, calling for further research to obtain a deeper understanding of the  
289 interactions between cement materials and microorganisms in the marine environment.

## 290 **Fungi**

291 Micro-fungi, whose size varies from 1 to 100  $\mu\text{m}$ , are chemotrophic eukaryotes. They are not  
292 capable of photosynthesis to produce their own organic matter. Fungi require wet or moist  
293 environments to develop, and their energy is provided by redox reactions. They are not very  
294 sensitive to pH variation but develop more easily in acidic conditions.

295 Fungi are heterotrophs. They can only synthesize their cellular components from a source of  
296 organic carbon. Thus, fungal colonization of a cement-based material requires an accumulation of  
297 organic matter on the surface. In the case of successful colonization, fungi can then lead to the  
298 alteration or deterioration of the colonized material. Certain fungi produce pigments (for example,  
299 melanin) that cause dark stains on the cement-based material (Giannantonio et al., 2009). In  
300 addition, fungi produce a large quantity of organic acids (acetic, formic, oxalic and tartaric acid),

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<sup>1</sup> Photosynthesis is the reaction enabling certain living organisms to synthesize organic matter from CO<sub>2</sub> and an electron donor under the effect of sunlight.

301 which are responsible for the dissolution of lime in mineral matrices and the chelation of calcium  
302 ions, leading to the deterioration of the material.

303 The marine environment is considered to contain a large number of fungal organisms (Escadeillas  
304 *et al.*, 2007). However, only a few studies have focused on the colonization by fungi of cement-  
305 based materials in the marine environment. Submerged surfaces are often covered by one or more  
306 layers of algae and other protozoan organisms (Dürr and Thomason, 2009), which makes it difficult  
307 to identify and isolate sessile fungi colonizers in the marine environment without the use of  
308 advanced molecular biology techniques such as the extraction and sequencing of 18S rRNA.

## 309 **METHODS: PHYSICOCHEMICAL FACTORS INFLUENCING** 310 **THE COLONIZATION OF CEMENT-BASED MATERIALS IN** 311 **MARINE ENVIRONMENTS**

312 The biological colonization of a surface is governed by the interaction of three main factors: the  
313 type of material, the microorganisms and the environment (Lors, Feugeas and Tribollet, 2019).  
314 Then, two types of bioreceptivity are defined: (i) intrinsic bioreceptivity, linked only to the  
315 physicochemical properties of the concrete surface, such as porosity, roughness and chemical  
316 composition; and (ii) extrinsic bioreceptivity, related to the environment (Lors, Feugeas and  
317 Tribollet, 2019).

### 318 **Intrinsic bioreceptivity (Material factors)**

#### 319 **Chemical composition**

320 Depending on its chemical composition, a material can contain substances that favor or inhibit the  
321 development of biofilm (Ly *et al.*, 2021). For example, the presence of certain heavy metals, in  
322 sufficient quantities, can inhibit the growth and development of microorganisms (Dalod *et al.*,  
323 2014; Dubosc, Escadeillas and Blanc 2001). In a comparison of the bacterial colonization of  
324 Portland cement (CEM I) and slag cement (CEM III) in seawater, CEM III mortar was shown to  
325 be more bioreceptive than CEM I mortar (Hayek *et al.*, 2020a, 2020b). Thus, the type of cement  
326 influences the kinetics and the level of development of bacterial biofilm on a concrete surface. The  
327 chemical composition of a concrete can also influence other important parameters, such as  
328 roughness, porosity and pH (Veeger *et al.*, 2021).

#### 329 **Surface roughness or topography**

330 The roughness of the surface of a material is one of the main physical factors influencing its  
331 bioreceptivity. Roughness consists of bumps and irregularities on the surface of the material. These  
332 irregularities can act as anchoring sites and micro-refuges for microorganisms, thus favoring  
333 colonization (Ammar *et al.*, 2015). These micro-refuges protect microorganisms from the  
334 hydrodynamic forces that tend to detach them during the highly precarious reversible adhesion  
335 phase (Bellon-Fontaine *et al.*, 2016). Kerr *et al.* (1999) had observed an increase in bacterial  
336 adhesion on glass and acrylic samples immersed in natural seawater when roughness was raised  
337 from 0.25 to 30  $\mu\text{m}$ . Teughels *et al.* (2006) had showed that the formation of dental plaque is  
338 favored by a rough surface. Concerning cement-based materials, several studies have demonstrated

339 the effect of surface roughness on biofilm formation (Govin *et al.*, 2013; Hayek *et al.*, 2021; Manso  
340 *et al.*, 2014; Manso and Aguado, 2016; Miller *et al.*, 2009; Souche *et al.*, 2019; Tomaselli *et al.*,  
341 2000; Tran *et al.*, 2014, 2012). These studies show that rough cement-based materials have higher  
342 bioreceptivity than smooth surfaces under both laboratory and natural conditions. To enhance  
343 colonization, the target is to obtain multiscale rugosity; it is possible to reach the required quality  
344 using special elastomeric formworks (Souche *et al.*, 2019).<sup>2</sup>

345 In addition to its effect on the adhesion of microorganisms, surface roughness also influences water  
346 flow rates and the adsorption of organic matter (Dubosc, Escadeillas and Blanc 2001; El-Naggar  
347 *et al.*, 2010). This directly impacts the formation of the conditioning film during the induction  
348 phase. Some studies nuance the effect of roughness on the bioreceptivity of a material, specifying  
349 that this depends on the characteristics of the microorganisms involved, e.g. the type of  
350 microorganism, geometry, size and shape (Whitman *et al.*, 1998). For example, filamentous algae  
351 such as *Klebsormidium flaccidum* attach to rough surfaces, whereas algae with thick cell walls  
352 adhere to smooth surfaces (Lors, Feugeas and Tribollet, 2019; Rindi, Guiry and López-Bautista,  
353 2008; Tran, 2011).

#### 354 **Total porosity to water**

355 In the case of surfaces not immersed in water, bacteria preferentially colonize porous surfaces. This  
356 can mainly be explained by the influence of porosity on the absorption and retention of water by  
357 the material, a determining factor for the development of microorganisms (Nuhoglu *et al.*, 2006).  
358 Crevasses that are the same size as the microorganism concerned appear to be ideal. Smaller pits  
359 assist anchoring but do not protect from flows, while pits that are too large do not enable the  
360 optimum transfer of nutrients.

361 In the case of submerged concrete, it is difficult to determine the effect of porosity on bioreceptivity  
362 (Govin *et al.*, 2013; Tran *et al.*, 2014, 2012). This difficulty has been explained by the permanent  
363 saturation of samples tested under water and therefore the high humidity throughout the  
364 experiment.

#### 365 **Surface pH**

366 The pH of the surface of the material can influence the type of microorganism and its capacity to  
367 adhere to the material (Shi and Zhu, 2009). Generally, each species has an optimum pH for growth  
368 and can develop within a more or less limited range around this value. Based on these pH ranges,  
369 microorganisms are classed as acidophilic (pH < 6), neutrophilic (6 < pH < 8) or basophilic (pH >  
370 8).

371 The metabolic activities, growth and adhesion of microorganisms and the synthesis of EPS are  
372 influenced by the pH of the surface of the material. The reduction of the surface pH of concrete  
373 after immersion in seawater (from 13 to about 9) accelerates colonization of the surface and  
374 improves the bioreceptivity of the material (Dalod *et al.*, 2014; Hayek *et al.*, 2020b; Roberts *et al.*,  
375 2002; Tran *et al.*, 2014; Vincke *et al.*, 2001). However, even concrete with a highly basic surface

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<sup>2</sup> <http://www.grouplib.com/les-moules-et-produits-en-elastomeres.html>

376 could be colonized by extremophilic bacteria that can resist environmental conditions such as  
377 extreme temperature, pH or pressure.

### 378 **Surface hydrophobicity**

379 Surface hydrophobicity is one of the factors that affect the patterns and nature of bacterial  
380 colonization of a surface, especially during the early stages of biofilm formation (Dang and Lovell,  
381 2015; Scheuerman *et al.*, 1998). Unsurprisingly, hydrophilic microorganisms attach more easily to  
382 hydrophilic surfaces, whereas hydrophobic microorganisms prefer hydrophobic surfaces (Shi and  
383 Zhu, 2009). As hydrophobicity and surface charge depend on the material's chemical composition  
384 (Katsikogianni and Missirlis, 2004), this is one of the ways that composition influences the types  
385 and diversity of colonizing microorganisms (Giannantonio *et al.*, 2009; Guillitte, 1995; Miller *et*  
386 *al.*, 2012).

387 However, a hydrophobic surface exhibits low wettability and low surface energy, whereas a  
388 hydrophilic surface exhibits high wettability and high surface energy. Water droplets on a  
389 hydrophobic surface will 'bead up' while droplets on a hydrophilic surface will spread out evenly.  
390 The degree of hydrophobicity is determined by contact angle measurements between water droplet  
391 and surface; the surface is super-hydrophilic, hydrophilic, hydrophobic and superhydrophobic  
392 when the contact angle is respectively less than 10°, between 10° and 90°, between 90° and 150°  
393 and between 150° and 180° (Bixler and Bhushan, 2012).

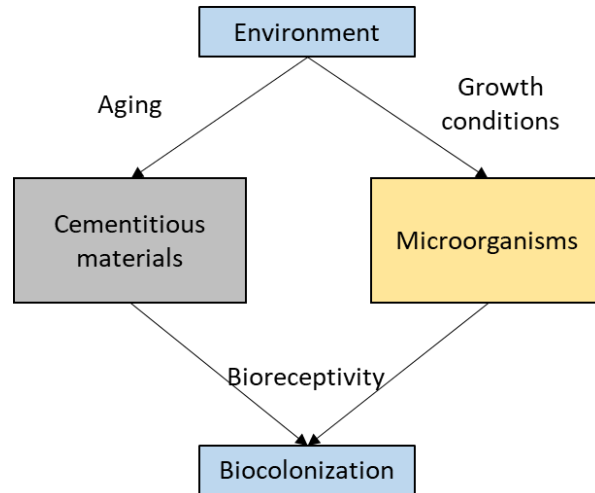
394 Despite the importance of this parameter on marine biodiversity as well as on the colonization of  
395 submerged surfaces, studies that relate to the surface hydrophobicity of cementitious materials and  
396 its effects on the biocolonization in the marine environment seem to be very rare. Hayek *et al.*,  
397 (2021) showed that the use of curing compound inhibits the biocolonization of concrete surface in  
398 seawater because of its effect on surface hydrophobicity (the cure compound forms a  
399 superhydrophobic film on the concrete surface). This study is in agreement with the literature,  
400 which has been proven that the use of a hydrophobic surface coating (low adhesive surface) is a  
401 successful strategy to inhibit biofouling in the marine environment (Blainey and Marshall, 1991;  
402 Huggett *et al.*, 2009; Razavi *et al.*, 2019).

### 403 **Extrinsic bioreceptivity (Environmental factors)**

404 Several studies have reported that the environment has a strong impact on the colonization of  
405 cementitious materials in the marine context (Giraudel, Garcia and Ledoux, 2014; Lors, Feugeas  
406 and Tribollet, 2019; Souche *et al.*, 2019). The medium influences the development and growth of  
407 microorganisms as well as the aging of the material. Indeed, interactions between the environment  
408 and microorganisms define the conditions in which microbial growth can occur (Figure 2).

409 Generally, the development of biofilm on the surface of a cement-based material is relatively slow  
410 as concrete has a high pH of about 13 (Codina, 2007; Manso and Aguado, 2017). This high  
411 alkalinity is unfavorable for biological colonization because few microorganisms can develop  
412 under such basic pH conditions. For that reason, an initial induction step that reduces the pH from  
413 13 to 9.5 will favor the colonization of cement-based materials (Roberts *et al.*, 2002). This  
414 modification of the surface pH arises from the adsorption of mineral and organic compounds and

415 the interaction of the concrete with seawater, which contains high concentrations of several  
416 dissolved species (Vavrenyuk, Efimenko and Vavrenyuk, 2020). De Weerd, Justnes and Geiker  
417 (2014) have shown that, within about 20 days, cement-based binders immersed in seawater undergo  
418 phase changes, whatever the type of binder tested (De Weerd, Justnes and Geiker, 2014; Jakobsen,  
419 De Weerd and Geiker, 2016).



420  
421 Figure 2. Interactions between the environment, microorganisms and cementitious materials  
422 (Lors, Feugeas and Tribollet, 2019)

423 There are a number of environmental factors that act on the colonization of cementitious materials  
424 in the marine environment, including luminosity, pH, temperature, nutrients and hydrodynamic  
425 conditions (Azevedo, Carloni and Carvalheira, 2006; Choi *et al.*, 2006; Färber *et al.*, 2015; Firth *et*  
426 *al.*, 2014; Qvarfordt *et al.*, 2006; Sherman, Giliam and Spieler, 2001; Witt *et al.*, 2012). Of these,  
427 it seems that the availability of nutrients and the temperature are the most likely factors to differ  
428 between marine environments. Thus, these are considered the major factors influencing the  
429 extrinsic bioreceptivity of cementitious materials in these contexts (Färber *et al.*, 2015; Gong, Song  
430 and Warren 2005; Salta *et al.*, 2013).

### 431 **Nutrients availability**

432 The medium is the means of propagation of nutrients allowing microorganisms to develop. The  
433 environment can be the source of nutrients, as is the case in sanitation networks, where the  
434 environment consists of wastewater rich in nutrients such as organic matter and sulfur compounds.  
435 Or the medium can simply be a means of transporting nutrients through the dissolution of organic  
436 matter. The concentration of nutrients present in the environment may favor the development of  
437 certain bacterial species. In the case of cementitious materials, these do not provide nutrients to the  
438 microorganisms, so the medium must be the source of nutrients to allow bacterial growth.

439 Seawater contains micro- and macronutrients essential for the growth of the cells of living  
440 organisms. Macronutrients, which contain carbon (C), phosphorus (P), nitrogen (N) and silicon  
441 (Si), provide the nutritive requirements indispensable to the metabolic functions of living  
442 organisms. Micronutrients, containing iron (Fe), Zinc (Zn), Copper (Cu) or Manganese (Mn), while  
443 consumed in much lower quantities, act as enzyme cofactors for metabolic reactions (de



444 Fommervault, 2016). The existence of these nutrients in seawater make it rich not only in macro-  
445 organisms, but also microorganisms such as bacteria, cyanobacteria and microalgae (*Poli et al.*,  
446 2017). The concentration and diversity of marine organisms also depend on other environmental  
447 conditions, such as pressure, temperature and salinity. Biodiversity is lower where these conditions  
448 are more drastic and hence more selective (Das, Lyla and Khan *et al.*, 2006; Sheehan, 2001;  
449 Zeppilli *et al.*, 2018).

450 In laboratory tests, the use of artificial seawater as a culture medium has been shown to be sufficient  
451 for marine bacteria to form a mature biofilm on a glass surface (Guillonneau *et al.*, 2018; Hayek *et*  
452 *al.*, 2019; Mireille Ayé *et al.*, 2015). This medium lacks organic substances, indicating that marine  
453 bacteria can form a biofilm without the need organic matter.

#### 454 **Temperature**

455 Temperature is one of the most important factors affecting the growth of microorganisms  
456 (Mesquita *et al.*, 2020). The optimum temperature for most microorganisms is between 15°C and  
457 35°C (Lors, Feugeas and Tribollet, 2019; Tran, 2011). A temperature that is too low reduces the  
458 kinetics of the enzymatic reactions that condition the metabolism of microorganisms and thereby  
459 their growth (González-Figueredo *et al.*, 2018; Huston, Krieger-Brockett and Deming, 2000;  
460 Robinson, 2015). A temperature that is too high damages microbial cells by denaturing the enzymes  
461 and proteins of transport systems (Shehadul Islam, Aryasomayajula and Selvaganapathy, 2017).  
462 However, certain microorganisms can resist temperatures close to 0°C and greater than or equal to  
463 70°C (Brock, 2012). Moreover, each microorganism has an optimum growth temperature  
464 dependent on its original environment (Lors, Feugeas and Tribollet, 2019). For example, marine  
465 bacteria isolated from sediment, water or biofilm samples (adhered to a surface) are mesophilic,  
466 growing best in moderate temperatures. Their optimum growth temperatures are between 25°C and  
467 30°C, but they can grow at 20°C and withstand a temperature of 4°C (Brian-Jaisson *et al.*, 2014;  
468 Guillonneau *et al.*, 2018; Hau and Gralnick, 2007; Hayek *et al.*, 2019; Makemson *et al.*, 1997;  
469 Mireille Ayé *et al.*, 2015).

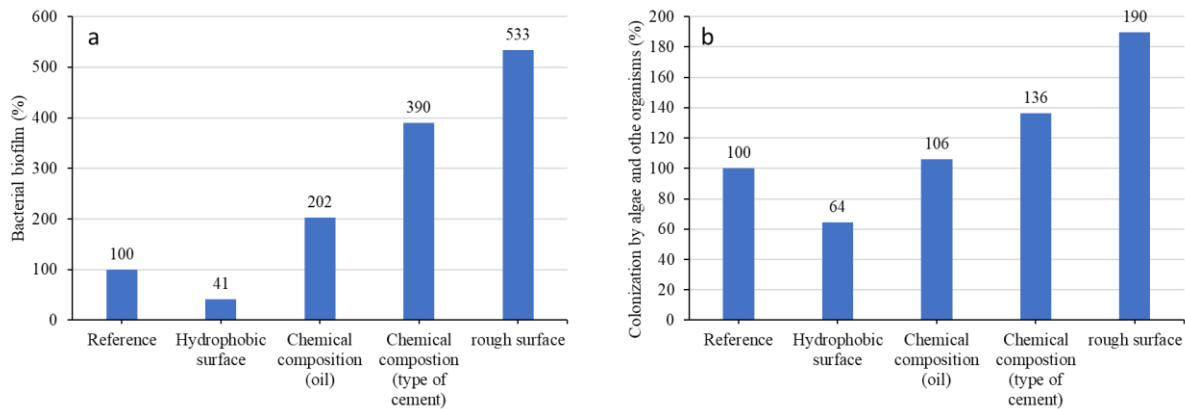
470 Likewise, the temperature of the medium affects the bioreceptivity of cementitious materials  
471 through the mechanisms of adhesion between microorganisms and material. The surface tension of  
472 a liquid medium varies with temperature, which influences the three-way equilibrium between  
473 material, medium and microorganism and can thus affect microorganism adhesion. In the same  
474 effect category, Van der Waals forces also vary with temperature (Nedwell, 1999).

## 475 **RESULTS: BIOLOGICAL COLONIZATION AND THE** 476 **DURABILITY OF CONCRETE**

477 The effect of the materials factors (cited below) on the intrinsic bioreceptivity of cement-based  
478 materials in the marine environment was studied in situ by Hayek *et al.*, (2021). In this paper, the  
479 effect of several parameters in the two main stages (bacterial biofilm and colonization by algae and  
480 other marine organisms) of cementitious material biocolonization in the marine environment was  
481 tested. The main parameters studied were i) the surface roughness, ii) the surface hydrophobicity  
482 and iii) the chemical composition. Figure 3 present the results obtained after 6 (Figure 3a) and 78



483 (Figure 3b) days of immersion in seawater. The results obtained in this study are in keeping with  
 484 the literature cited above, in which a similar effect of materials factors on the intrinsic  
 485 bioreceptivity has been reported. Based on the persistence of their significant effect (after 78 days  
 486 of immersion in seawater), the intrinsic parameters that support greater biocolonization are  
 487 classified from more to less effective in the following order; surface roughness (190 %) > chemical  
 488 composition (slag cement instead Portland cement) (136 %) > chemical composition (presence of  
 489 formwork oil) (106 %). Therefore, at the material scale, surface roughness is the most effective  
 490 factor in designing bioreceptive concrete that enhances marine biodiversity (Browne and Chapman  
 491 2011; Dafforn *et al.*, 2015; Hayek *et al.*, 2021).



492  
 493 Figure 3. The effect of material factors on the intrinsic bioreceptivity of cement-based materials  
 494 in the marine environment. Data collected from the study of Hayek *et al.* (2021). (a) colonization  
 495 by bacteria after 6 days of immersion. (b) colonization by algae and other marine organisms after  
 496 78 days of immersion in seawater.

497 Hayek *et al.* (2020a) also showed that the carbonation of cementitious materials before immersion  
 498 in seawater plays a primary role in surface colonization by marine bacteria. Carbonated concrete  
 499 and mortar were shown to be more bioreceptive (200 % after 6 days of immersion in seawater)  
 500 than non-carbonated cement-based materials.

501 However, regarding the environmental factors, several authors have mentioned the importance of  
 502 nutrients availability. For example, in the case of artificial reef structures whose objective is the  
 503 colonization of the material, several ‘pro-biofouling’ coatings have been tried. The constituents of  
 504 these coatings are today known as chemo-attractors that allow microorganisms to adhere and grow  
 505 on the material (Lee *et al.*, 2008; Souche *et al.*, 2016). Lee *et al.* (2008) have found that the use of  
 506 chemoattractant coatings such as ferrous sulfate, zinc oxide, ammonium nitrate, sodium phosphates  
 507 and ferrous lactate increase the colonization of cementitious materials in the marine environment.  
 508 A maximum biocolonization with green algae 25%, red algae 11.3% and brown algae 63.7% was  
 509 estimated from ferrous lactate coating.

510 Moreover, the colonization of cementitious material differs depending on the season (temperature  
 511 effect) (Choi *et al.*, 2006; Manso *et al.*, 2015). In a study carried out by Rica *et al.* (2016) focusing  
 512 on the effect of temperature on the colonizing biomass of a concrete surface in a marine

513 environment, a decrease in temperature from 18°C to 13°C led to a regression (from 100% to 33%)  
514 in the biomass of the biofilm formed on concrete immersed in seawater.

515 Biological colonization can have positive or negative effects on a structure, either protecting or  
516 degrading the material (Coombes *et al.*, 2011; Georges *et al.*, 2021; Harshada, Desai and Gupta,  
517 2017). Relatively little attention has been paid to the biodegradation of concrete in seawater, despite  
518 the fact that the colonization of marine concrete structures is spontaneous. This can be explained  
519 by the difficulty of separating biodeterioration from other processes that damage cementitious  
520 materials in seawater (e.g. aging, chemical erosion by chlorides, sulfate, *etc.*). In 2018, a laboratory  
521 experiment demonstrated that the colonization of cementitious materials by microorganisms such  
522 as the diatom *Cylindrotheca closterium* allowed greater penetration of chloride and sulfate ions,  
523 which accelerated the degradation of the material. The author posited that the creation of cracks in  
524 the material by diatoms could explain this phenomenon (Ferrero, 2018).

525 Yet as marine environments are extremely diverse, the results of experiments carried out in the  
526 laboratory do not always reflect reality. A colonization effect determined in the laboratory is highly  
527 dependent on the choice of microorganisms, the studied material (concrete, mortar, cement paste,  
528 Portland cement, *etc.*) and the experimental conditions. According to Lv *et al.* (2015), the  
529 colonization of concrete may actually improve its lifespan depending on the substrate and  
530 microorganisms involved. These authors showed that the biofilm formed by bacteria of the genus  
531 *Pseudoalteromonas* on the surface of the cement-based material improved its durability by  
532 reducing the quantity of aggressive ions such as Cl<sup>-</sup> able to penetrate the material and cause  
533 biodeterioration. This protective effect of biofilm has been identified not only in the laboratory  
534 with bacteria, but also in situ with biofilm made up of other marine microorganisms and macro-  
535 organisms such as cyanobacteria, algae, lichens and barnacles (Bertron, 2014; Coombes *et al.*,  
536 2011; de la Rosa, Warke and Smith, 2013). In a number of studies, these living organisms have  
537 been found to reduce the permeability of the cement-based material, resulting in greater durability  
538 (Chlayon, Iwanami and Chijiwa, 2018; Coombes *et al.*, 2017; Patil *et al.*, 1994; Perkol-Finkel and  
539 Sella, 2014; Soleimani *et al.*, 2013).

540 In this way, the biological colonization of concrete could provide structures with additional  
541 protection against chemical or even mechanical degradation, through the effects of the physical  
542 barrier offered by living organisms colonizing the surface of cementitious material (Ariño *et al.*,  
543 1995; Bartoli *et al.*, 2014; Carter and Viles, 2003; Chlayon, Iwanami and Chijiwa, 2020;  
544 Mottershead and Lucas, 2000; Viles and Cutler, 2012). According to the literature, a beneficial  
545 biofilm (bioprotection) can protect the material by reducing the quantity of water and nutrients  
546 available, synthesizing anti-microbial agents (thus inhibiting the growth of other microorganisms  
547 having a harmful effect on cementitious materials) or forming a protective layer of synthesized  
548 exopolysaccharides (Chlayon *et al.*, 2018; Soleimani *et al.*, 2013).

549 A microbial biofilm can also facilitate the attachment of other living organisms to the surface of  
550 the structure. In the case of cementitious materials, the attachment of living organisms such as  
551 oysters, tubeworms, barnacles and corals can lead to the formation of calcite precipitated in the  
552 gaps of the cement matrix, making the concrete denser and stronger (Chlayon *et al.*, 2018; Joshi,  
553 Goyal and Reddy, 2018; Risinger, 2012).

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## **DISCUSSION: ECOLOGICAL CONSIDERATIONS AND PERSPECTIVES TO DESIGN MORE BIORECEPTIVE CONCRETE (GREEN CONCRETE)**

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It has been shown that the marine diversity decreases with the loss of coastal wetlands and nearshore communities when marine infrastructure such as seawalls are constructed. The loss of horizontal extent of coastal communities occurs with seawalls, contributing to poor ecological performance, exacerbated by increased land-based sources of pollution. One of the major reasons for the poor ecological performance of the marine infrastructure is the structural design which is less complex than the natural communities replaced (Hosseinzadeh *et al.*, 2022). However, if the marine infrastructure is designed considering ecological features, natural marine habitats could be replicated which supports some species of marine life (Dyson and Yocom, 2015).

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While ideally coastal development should be minimized or avoided, as artificialization is the primary cause of biodiversity loss in the world (IPBES, 2019), in the short term the global demand for this is set to continue. To reduce the environmental impact and the disturbance of coastal ecosystems, we recommend using a new design method for coastal and marine structures. One way to do this is to consider the use of bioreceptive concrete at the site of impact, conceived with the optimal surface characteristics for the local ecosystem. This new design approach would aim to enhance biological colonization of coastal engineering structures and should be considered at the project outset (Pioch *et al.*, 2018). The geometry or the physical shape of marine infrastructure is one of the parameters that show a direct ecological effect; fishes and benthic invertebrates populate and interact in greater numbers on materials with rugose and irregular surfaces rather than a smooth, flat wall (Hosseinzadeh *et al.*, 2022; Koraim, 2013; Perkol-Finkel and Sella, 2014; Yip, Zhang and Chwang, 2002).

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Moreover, based in the present paper and the literature cited above, the following recommendations aim to meet the objective of the new design approach:

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1. Map the ecosystem at the site where the structure will be built and identify sensitive species.
2. Test the compatibility of the proposed concrete composition with the ecosystem where the project is planned, using the test method proposed by Hayek *et al.* (2020a).
3. Design surface roughness to ensure adhesion.
4. Pretreat the surface (e.g. carbonation), if possible, to accelerate growth.
5. Adjust the timing of the project (e.g. season) so that the biophysical conditions (dependent on the area) are favorable for the ecosystem to start colonization quickly.
6. Develop a monitoring plan to ensure the reestablishment of the ecosystem after construction, as well as a solution to fight against invasive species if needed.
7. Do not apply a hydrophobic coating such as the curing product on the concrete surface.
8. Use slag cement (CEM III) instead of Portland cement (CEM I) to prepare the concrete.

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## **CONCLUSIONS**

594 This review of literature presents some of the latest research results that contributes to enhancing  
595 the colonization of marine infrastructure built with concrete. Biological colonization can reduce  
596 negative impacts on natural and local ecosystems and lower ubiquitous exotic species invasion. To  
597 encourage surface colonization by marine microorganisms, the carbonation of cementitious  
598 materials before their immersion in seawater play the primary role. The type of cement influences  
599 the kinetics and the level of surface colonization. Moreover, latest findings clearly indicate that  
600 slag cement (CEM III) enhances marine biodiversity as compared to Portland cement (CEM I). At  
601 the material scale, surface roughness is the most effective factor in designing bioreceptive concrete  
602 that enhances marine biodiversity. Based on the persistence of their significant effect (after 78 days  
603 of immersion in seawater), the intrinsic parameters that support greater biocolonization are  
604 classified from more to less effective in the following order; surface roughness, CEM III as binder,  
605 chemical composition (presence of formwork oil).

606 But, adopting new methods of construction often requires overcoming a certain inertia: insurance  
607 and construction companies, as well as resource managers, tend to try to avoid technical issue or  
608 legal repercussions by repeating historically proven methods, ignoring the ecological aspect or  
609 hiding negatives impacts. Nonetheless, there is no question that coastal areas need to be considered  
610 as tremendous reservoirs of biodiversity (IPBES, 2019). Ecological engineering inspiration to eco-  
611 design concrete infrastructures, is one way to mitigate negative impact from human construction,  
612 and should help to restore marine biodiversity which could be saved by 2050, if appropriate policies  
613 and projects in the field start quickly (Duarte *et al.*, 2020). This review highlights some of the most  
614 significant results demonstrating that concrete can be an appropriate support for biological  
615 colonization, making it a candidate for green design strategies. Of course, such an approach should  
616 not be used as a ‘greenwashing’ excuse to allow unregulated construction anywhere, thus becoming  
617 a Trojan horse to ongoing massive artificialization (Firth *et al.*, 2020). For real and fair progress to  
618 be made, it will be essential to develop robust assessment methods to measure the ecological gains  
619 of bioreceptive designed infrastructure and prove their potential benefits.

620 However, to increase the understanding of biofilm formation on concrete surfaces in the marine  
621 environment and to analyses the ecological benefits of this biocolonization, further investigation is  
622 needed using the molecular biology tools such as the amplicon sequencing of the 16S rRNA gene  
623 in order to investigate succession of the biofilm communities and their interaction with the concrete  
624 material and the flowing water.

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629

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