

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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How To Improve The Bioreceptivity of Concrete Infrastructure Used in Marine Ecosystems? Literature Review for Mechanisms, Key Factors and Colonization Effects Mahmoud Hayek† , Marie Salgues† , Jean-Claude Souche† , Klaartje De Weerdt‡ , Sylvain Pioch§ † LMGC, IMT Mines Alès Univ Montpellier, CNRS Alès, France ‡ Department of Structural Engineering Norwegian University of Science and Technology NTNU Trondheim, Norway 12 SLAGAM University Paul Valéry Montpellier 3 Montpellier, France sylvain.pioch@univ-montp3.fr **LRH: Hayek, Salgues, Souche, De Weerdt, Pioch RRH: Bioreceptivity of marine concrete**

ABSTRACT

 With 39,400 km² of coastal and marine areas artificialized and an increasing demand due to the growing global population – 9 billion by 2050 – we need to find ways to mitigate futures construc- tions impacts on biodiversity. In this study, we explore how civil engineering can take further tech- nical measures to enhance marine biodiversity, in a real and valuable "win-win" strategy. The global aim is to integrate eco-engineering practices within coastal projects and include ecological targets (*e.g*. the diversity and speed of biological colonization) early, at the project design stage, with the same level efforts for technical, social and economic studies. Concrete is the most useful material for coastal infrastructures constructions, therefore enhance its positive impact on coloni- zation is by far one of the key-point for developers and coastal managers. To this end, we've first reviewed the latest research regarding the bioreceptivity of concrete, focusing on the characteristics of the marine environment that affect the colonization of concrete and the organisms involved. From this base of publications, we've updated the intrinsic and environmental parameters that can influence the intrinsic and the extrinsic bioreceptivity of concrete and operate the link with the mechanisms leading to the colonization of concrete and biofilm formation, which wasn't done be- fore. Based on the persistence of their significant effect (after 78 days of immersion in seawater), the intrinsic parameters that support greater biocolonization are classified from more to less effec- tive in the following order; surface roughness (190 %) > chemical composition (slag cement instead 36 Portland cement) (136%) > chemical composition (presence of formwork oil) (106%) . We've

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

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

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

 Therefore, reducing artificialization and land use is critical to reduce biodiversity loss and preserve the future of humanity. Yet predictions indicate a clear acceleration of urbanization around the world, on land and at sea. Between 2019 and 2040, more than US\$60,000 billion is forecast to be invested in infrastructure in the 56 countries representing 88% of global GDP (Global Infrastructure Hub 2017–2018). More than 1.2 million km² will be urbanized by 2030 – just 8 years from now – representing an increase of more than 185% compared to the period from 1970 to 2000 (Seto *et al.*, 2012). By 2050, 3 to 4.7 million km² of roads will be constructed, which represents an increase of more than 25% compared to the current annual rate (Meijer *et al.*, 2018). This process will be particularly significant along coasts, where eight of the ten largest megalopolises in the world are located (such as Lagos, Tokyo, Jakarta and New York). Around the world, the population living within 100 km of a coast is estimated to approach 7.5 billion people by 2050 (de Fraiture *et al.*, 2007), leading to a need for increased coastal development and potentially disturbing essential coastal habitats (Weilgart, 2007). Global marine infrastructure extended over approximately 32,000 km² in 2018 (Bugnot *et al.*, 2020) and is expected to reach 39,400 km² by 2028: a surface area equivalent to the country of Bhutan. The area of seascapes impacted by these structures was estimated to be between 1 and 3.4 million km² in 2018, which is expected to increase 50–70% by 2028 (*Ibid*). The phenomenon of 'coastal squeeze', a term introduced by Doody (2004) to refer to the threat to the existence of coastal habitats caused by the compound impacts of sea-level rise and human activities, will not abate under current development conditions. Most of these structures are made from concrete, the most durable materials for the aggressive marine environment. Table 1 summarize the types of concrete structures found in coastal areas.

 From the 1970s through the 1990s, coastal ecology literature dealing with biodiversity focused mainly on artificial substrates use to promote restoration, studied from colonization or settlement phenomena problematics (Relini *et al.*, 1998; Zobel, 1974). However, since the 2000s, hard artificial infrastructure made with concrete, has been implicated as a major risk factor for local and native biodiversity, by introducing invasive species with ubiquitous and rapid growing characteristics, in comparison with surrounding natural rocky area (Bulleri and Airoldi, 2005; Bulleri and Chapman, 2010, 2004; Jackson *et al.*, 2001). In contrast, several studies about ecological design of coastal infrastructure published since 2010 has clearly demonstrated that it is 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 Coastal defense structures (protection of coastlines and management of wave action effects)** *Offshore breakwater* 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 *Groin* Coastal defense structure to protect the coast from erosion and sediment transport Rock fill *Harbor breakwater, jetty* Structure to protect a harbor area from wave action and currents Rock fill and blocks of concrete (blocks, tetrapodes, accropodes) **Harbor structures** *Finger pier* Mooring structure for loading and unloading boats Reinforced or prestressed concrete apron on caisson or gabion-type piles **Boom wharf** Structure on piles making up a finger pier (wharf perpendicular to the coast) Reinforced concrete apron on metal or reinforced concrete piles *Quay* 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 *Floating dock* Floating structure enabling docking and mooring of boats in a harbor Metal or reinforced concrete, held in place by piles or reinforced concrete moorings *Floating breakwater* Floating structure to protect a harbor from wave action Reinforced concrete, held in place by piles or reinforced concrete moorings *Lock / gate* Structure enabling boats to change level and/or to keep a dock filled with water Infrastructure in reinforced concrete, equipment in steel

Harbor structures for maintenance and services

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 Pragmatically, most current coastal concrete infrastructure cannot be readily removed. But there is now increasing research efforts into innovative ways that future new infrastructures can be designed and built to meet engineering requirements, while also increasing its value as an ecological habitat for marine life (Dafforn *et al.*, 2015; Firth *et al.*, 2014; Lacroix and Pioch, 2011; Naylor and Coombes, 2015; Patranella *et al.*, 2017; Perkol-Finkel and Sella, 2014; Phan, van Thiel de Vries and Stive, 2015; Pioch and Souche, 2021). This "win-win" approach, should ensure benefits both for human society and nature (Chapman and Underwood, 2011; Ferrario *et al.*, 2014; Perkol-Finkel *et al.*, 2018; Pioch *et al.*, 2018, 2011; Temmerman *et al.*, 2013). This paradigm support the idea that we have to construct structures with a minimal ecological impact on the existing environment, maximizing their potential for colonization, and restore original habitats or create new habitats adapted for local species ecological requirements (Browne and Chapman, 2011; Souche *et al.*, 2018). In this way, combining engineering techniques with ecological understanding can provide, with a cost-effective ways, the maintenance or development of native biodiversity (Airoldi *et al.*, 2020; Martins *et al.*, 2010; Pioch *et al.*, 2018; Scheres and Schüttrumpf, 2019; Souche et al., 2019).

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

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

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

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

to take into account in order to promote the bioreceptivity of concrete structures.

Scope and research significance

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

- Afterward, to achieve the purpose of this paper, i) some of the most significant quantitative results
- about the bioreceptivity of concrete in the marine environment are presented ii) the ecological
- impact and the effect of biological colonization on the durability of concrete are discussed and iii)
- the perspectives to design a more bioreceptive concrete (green concrete) are mentioned.
- Web of science and google scholar are the two databases used in this study for collecting the relevant materials (211 studies) from the literature. The keywords used were: Biofilm formation,

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

METHODS: BIOLOGICAL FACTORS AFFECTING THE

COLONIZATION OF CONCRETE IN THE MARINE

ENVIRONMENT

Colonization and the development of biofilm on surface concrete

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

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

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

the surface is considered a key step in biofilm formation (Herrmann *et al.*, 1988; Tang *et al.*, 2006).

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

 Therefore, the main stages of biofilm formation are (i) reversible and irreversible adhesion of bacterial cells to the surface of the cement-based material; (ii) growth and maturing of the biofilm

with the secretion of EPS; and (iii) partial detachment of the biofilm and the dispersion of bacterial

cells (Hinsa and O'Toole, 2006; O'Toole, Kaplan and Kolter, 2000).

 The adhesion of bacteria to a surface first requires that they move toward the surface to be colonized. This movement can be due to environmental phenomena (gravitational and hydrodynamic forces in the surrounding fluid) and/or by appendages on the bacteria that assist their 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 $\lt 1$ nm), low-intensity interactions take place between the bacterium and the surface, e.g. electrostatic forces, Van der Waals forces and hydrophobic interactions (Carpentier and Cerf, 1993; Dunne, 2002). Depending on these various interactions, the bacterium may either adhere or be repelled by the effect of hydrodynamic actions. If the interactions are favorable, the bacterium adheres to the surface and this step is then considered the reversible adhesion phase (An and Friedman, 1998; Berne *et al.*, 2018; Busscher and Weerkamp, 1987; van Loosdrecht *et al.*, 1989). The factors that influence this phase concern interactions between the bacterial cells and the surface of the cement-based material: (i) the specific characteristics of the bacteria, such as its cell surface charge, cell surface hydrophobicity and the chemical composition of the cell wall; (ii) the intrinsic parameters of the host surface, such as its physicochemical nature, polarity, porosity and roughness; and (iii) the environmental conditions, such as pH level, temperature and flow conditions (Bunt *et al.*, 1993; Scheuerman *et al.*, 1998; Speranza *et al.*, 2004). According to Carpentier and Cerf (1993), host surface porosity and roughness are believed to increase bacterial adhesion, while a negatively charged surface may have a repulsive effect as most bacteria are negatively charged.

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

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

A mature biofilm has a three-dimensional structure surrounded by a matrix made up of mineral

elements and EPS: proteins, polysaccharides, nucleic acids, lipids and other cellular components.

This matrix forms a barrier that protects biofilm microorganisms against external threats (Ha and

O'Toole, 2015; Soleimani *et al.*, 2013).

The last step in the development of the biofilm is the dispersion of cells in the environment after

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

Colonizing organisms of cement-based materials

In the marine environment, there are many ubiquitous colonizing microorganisms likely to form a

- biofilm on the surface of objects under suitable conditions. The main colonizing microorganisms of cement-based materials are bacteria, fungi, cyanobacteria and algae (Gaylarde and Gaylarde,
- 2005).

Bacteria

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

 The deterioration of cement-based materials by microbial induced corrosion has been known since 1945 following the work of Parker, who was the first to isolate from concrete samples a sulfuric acid-producing bacterium species initially called *Thiobacillus concretivorus*, then subsequently called *Acidithiobacillus thiooxidans* (Parker, 1945). This phenomenon has mainly been studied in sanitation networks, where the sulfate- and nutrient-rich environment provides optimal conditions for the development of microbial fauna belonging to the genus *Thiobacillus* (Yuan *et al.*, 2015). *Thiobacilli* are bacteria that oxidize sulfur compounds into sulfuric acid, a substance that is particularly aggressive to concrete (De Belie, 2010). However, the biochemical processes leading 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 (H_2SO_4) and nitric acid (HNO_3) (Cwalina, 2008). These acids dissolve calcareous components, resulting in a loss of cohesion in the material and the appearance of cracks or corrosion of the reinforcement bars in the concrete (Chatterjee and Goyns, 2013; Poonguzhali *et al.*, 2008). These effects are common in the sulfate- and ammoniac-

rich environment of sanitation networks.

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

Algae and cyanobacteria

 Microscopic algae (microalgae) and cyanobacteria are unicellular or multicellular species, generally between 0.5 μm and 1 mm in size. These microorganisms are characterized by their 267 capacity to perform oxygenic photosynthesis (Leclerc,).¹ As algae and cyanobacteria are capable of generating their own organic matter from mineral elements, they are autotrophic organisms (Harper and Pendleton, 1993; Krumbein, 1979; Subashchandrabose et al., 2011).

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

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

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

Fungi

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

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

¹ Photosynthesis is the reaction enabling certain living organisms to synthesize organic matter from $CO₂$ and an electron donor under the effect of sunlight.

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

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

METHODS: PHYSICOCHEMICAL FACTORS INFLUENCING THE COLONIZATION OF CEMENT-BASED MATERIALS IN MARINE ENVIRONMENTS

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

Intrinsic bioreceptivity (Material factors)

Chemical composition

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

Surface roughness or topography

 The roughness of the surface of a material is one of the main physical factors influencing its bioreceptivity. Roughness consists of bumps and irregularities on the surface of the material. These irregularities can act as anchoring sites and micro-refuges for microorganisms, thus favoring colonization (Ammar *et al.*, 2015). These micro-refuges protect microorganisms from the hydrodynamic forces that tend to detach them during the highly precarious reversible adhesion phase (Bellon-Fontaine *et al.*, 2016). Kerr *et al.* (1999) had observed an increase in bacterial adhesion on glass and acrylic samples immersed in natural seawater when roughness was raised from 0.25 to 30 µm. Teughels *et al.* (2006) had showed that the formation of dental plaque is favored by a rough surface. Concerning cement-based materials, several studies have demonstrated the effect of surface roughness on biofilm formation (Govin *et al.*, 2013; Hayek *et al.*, 2021; Manso *et al.*, 2014; Manso and Aguado, 2016; Miller *et al.*, 2009; Souche *et al.*, 2019; Tomaselli *et al.*, 2000; Tran *et al.*, 2014, 2012). These studies show that rough cement-based materials have higher bioreceptivity than smooth surfaces under both laboratory and natural conditions. To enhance colonization, the target is to obtain multiscale rugosity; it is possible to reach the required quality using special elastomeric formworks (Souche *et al.*, 2019). ²

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

Total porosity to water

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

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

Surface pH

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 The pH of the surface of the material can influence the type of microorganism and its capacity to adhere to the material (Shi and Zhu, 2009). Generally, each species has an optimum pH for growth 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 ($pH >$ 8).

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

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

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

Surface hydrophobicity

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

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

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

Extrinsic bioreceptivity (Environmental factors)

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

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

modification of the surface pH arises from the adsorption of mineral and organic compounds and

the interaction of the concrete with seawater, which contains high concentrations of several

dissolved species (Vavrenyuk, Efimenko and Vavrenyuk, 2020). De Weerdt, Justnes and Geiker

- (2014) have shown that, within about 20 days, cement-based binders immersed in seawater undergo
- phase changes, whatever the type of binder tested (De Weerdt, Justnes and Geiker, 2014; Jakobsen,
- De Weerdt and Geiker, 2016).

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

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

Nutrients availability

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

 Seawater contains micro- and macronutrients essential for the growth of the cells of living organisms. Macronutrients, which contain carbon (C), phosphorus (P), nitrogen (N) and silicon (Si), provide the nutritive requirements indispensable to the metabolic functions of living organisms. Micronutrients, containing iron (Fe), Zinc (Zn), Copper (Cu) or Manganese (Mn), while consumed in much lower quantities, act as enzyme cofactors for metabolic reactions (de Fommervault, 2016). The existence of these nutrients in seawater make it rich not only in macro- organisms, but also microorganisms such as bacteria, cyanobacteria and microalgae *(Poli et al*., 2017). The concentration and diversity of marine organisms also depend on other environmental conditions, such as pressure, temperature and salinity. Biodiversity is lower where these conditions

- are more drastic and hence more selective (Das, Lyla and Khan *et al.*, 2006; Sheehan, 2001;
- Zeppilli *et al.*, 2018).

In laboratory tests, the use of artificial seawater as a culture medium has been shown to be sufficient

- for marine bacteria to form a mature biofilm on a glass surface (Guillonneau *et al.*, 2018; Hayek *et al.*, 2019; Mireille Ayé *et al.*, 2015). This medium lacks organic substances, indicating that marine
- bacteria can form a biofilm without the need organic matter.

Temperature

 Temperature is one of the most important factors affecting the growth of microorganisms (Mesquita *et al.*, 2020). The optimum temperature for most microorganisms is between 15°C and 35°C (Lors, Feugeas and Tribollet, 2019; Tran, 2011). A temperature that is too low reduces the kinetics of the enzymatic reactions that condition the metabolism of microorganisms and thereby their growth (González-Figueredo et al., 2018; Huston, Krieger‐Brockett and Deming, 2000; Robinson, 2015). A temperature that is too high damages microbial cells by denaturing the enzymes 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 70°C (Brock, 2012). Moreover, each microorganism has an optimum growth temperature dependent on its original environment (Lors, Feugeas and Tribollet, 2019). For example, marine 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^oC and 30°C, but they can grow at 20°C and withstand a temperature of 4°C (Brian-Jaisson *et al.*, 2014; Guillonneau *et al.*, 2018; Hau and Gralnick, 2007; Hayek *et al.*, 2019; Makemson *et al.*, 1997; Mireille Ayé *et al.*, 2015).

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

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RESULTS: BIOLOGICAL COLONIZATION AND THE DURABILITY OF CONCRETE

 The effect of the materials factors (cited below) on the intrinsic bioreceptivity of cement-based materials in the marine environment was studied in situ by Hayek *et al.*, (2021). In this paper, the effect of several parameters in the two main stages (bacterial biofilm and colonization by algae and other marine organisms) of cementitious material biocolonization in the marine environment was tested. The main parameters studied were i) the surface roughness, ii) the surface hydrophobicity and iii) the chemical composition. Figure 3 present the results obtained after 6 (Figure 3a) and 78 (Figure 3b) days of immersion in seawater. The results obtained in this study are in keeping with the literature cited above, in which a similar effect of materials factors on the intrinsic bioreceptivity has been reported. Based on the persistence of their significant effect (after 78 days of immersion in seawater), the intrinsic parameters that support greater biocolonization are classified from more to less effective in the following order; surface roughness (190 %) > chemical composition (slag cement instead Portland cement) (136 %) > chemical composition (presence of formwork oil) (106 %). Therefore, at the material scale, surface roughness is the most effective factor in designing bioreceptive concrete that enhances marine biodiversity (Browne and Chapman 2011; Dafforn *et al*., 2015; Hayek *et al*., 2021).

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

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

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

estimated from ferrous lactate coating.

 Moreover, the colonization of cementitious material differs depending on the season (temperature effect) (Choi *et al*., 2006; Manso *et al*., 2015). In a study carried out by Rica et al. (2016) focusing

on the effect of temperature on the colonizing biomass of a concrete surface in a marine

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

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

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

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

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

Goyal and Reddy, 2018; Risinger, 2012).

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

 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).

 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).

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

- 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

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

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

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

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