The role of environmental biotechnology in exploring, exploiting, monitoring, preserving, protecting and decontaminating the marine environment

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In light of the Marine Strategy Framework Directive (MSFD) and the EU Thematic Strategy on the Sustainable Use of Natural Resources, environmental biotechnology could make significant contributions in the exploitation of marine resources and addressing key marine environmental problems. In this paper 14 propositions are presented focusing on (i) the contamination of the marine environment, and more particularly how to optimize the use of biotechnology-related tools and strategies for predicting and monitoring contamination and developing mitigation measures; (ii) the exploitation of the marine biological and genetic resources to progress with the sustainable, eco-compatible use of the maritime space (issues are very diversified and include, for example, waste treatment and recycling, anti-biofouling agents; bio-plastics); (iii) environmental/marine biotechnology as a driver for a sustainable economic growth.

Introduction

The most recent international strategies to re-launch global (bio)economy consider the marine environment as the last frontier. Baseline scenarios identify successful trends for high technology marine sectors that operate in a truly global market place. Such a fast developing and diversifying maritime industry can seriously threaten the marine environment.

Environmental biotechnology may provide important knowledge and tools that will help to protect the resource base upon which marine-related economic and social activities depend. Environmental biotechnology can play a significant role in addressing marine environmental problems. These are summarized in the following paragraphs grouped in five focus areas.

Early warning systems to foresee marine threats (natural and anthropogenic)

The EU has adopted several environmental directives, strategies, recommendations, and agreements aimed at protecting the marine environment and its resources.

The EU Marine Strategy Framework Directive (MSFD; 2008/56/EC [1]) includes 11 qualitative descriptors for determining good environmental status (GES). Descriptors 5 (includes harmful algae

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blooms), 8 (contaminants and their effects), 9 (contaminants in seafood) and 10 (marine litter) are intimately related; however, their assessments often follow different approaches and are based on unrelated technologies. Biotechnology can provide a bridge to harmonize procedures and optimize resources for MSFD monitoring programs as well as to implement nearly real time early warning systems for natural and anthropogenic threats to the marine environment.

MSFD focuses on biological endpoints with ecosystem health at the center of regulation and management decision-making. Thus, the MSFD requires criteria and methodological standards to allow consistency in approach in evaluating the extent to which GES is being achieved [2]. Establishing criteria and methods to determine GES is therefore a priority challenge for basic research, aimed at establishing solid foundations to achieve harmonized assessment and monitoring procedures.

More recently, chief research efforts have been addressed toward an omics approach for the diagnosis of environmental syndromes due to, for example, pollution and climate change in marine biota and ecosystems [3–5]. In this case, biomedical advances are followed more closely by environmental and marine scientists but much remains still to be done. For instance, the use of biomarkers; a biomarker, or biological marker, refers to any characteristic, which can be measured and serves as an indicator of some biological state or condition. Molecular biomarkers measuring gene expression alterations (e.g. microarrays) after chemical exposure are to-date the front line of research in marine ecotoxicology [6–9]. We need sequence information for relevant pollution sentinel species that could be employed for the design, fabrication and commercialization of oligonucleotide high-density microarrays. One weak point in transcriptomic studies is the lack of information on the organismal/environmental relevance of alterations in gene expression profiles. High-throughput transcriptomic studies need to be assessed together to functional endpoints in order to link molecular mechanisms with phenotypic alterations. These functional endpoints should be also high throughput such as proteomics and metabolomics, and although the application of proteomics and metabolomics in the marine environment is still in its beginning, – omic studies are already in progress for marine flora, fauna and microorganisms [6–13]. Interestingly, – omics biomarkers represent a continuum of cellular responses to chemical exposure and to multiple sources of environmental stress, and provide linkages to mechanisms of cell injury/cell death or carcinogenic transformation [14].

Overall, improving mechanistic understanding, determining natural variability and baseline values, standardizing sampling and analytical procedures, integrating biomarkers among them and with chemical endpoints and relating biomarkers to ecological effects are issues of major concern for implementing biotools (biomarkers and omic diagnosis data sets) for the MSFD [15]. Criteria and methodological standards are urgently needed to allow consistency in the biomarker + omic approach for evaluating the extent to which GES is achieved [16], as well long-time series that relate pollutant exposure to effects on organisms and ecosystems at long-term scale [17].

Learning from biomedical sciences to speed up the development and use of advanced biomarkers and high-throughput technologies suitable to foresee marine threats will provide scientists, environmentalists and decision-makers with up-to-date early warning systems for the monitoring of marine chemical pollution and its effects.

MSFD descriptors 8 and 9 deal with the environmental risk assessment of chemical contaminants. Likewise, the Water Framework Directive (WFD; 2000/60/EC [18]) and the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH; 2006/1907/EC [19]) deal with the compliance of environmental quality standards established for chemical substances at European level. Chemical monitoring can be done with a combination of chemical technology and biosensors. Two major FP7 calls were recently launched on the development of biosensors for the marine environment for continuous monitoring of priority pollutants, emerging contaminants and biotoxins (funded projects: BRAAVOO and SMS). Chemical biomonitoring, that is, methylmercury contamination can also be done by measuring the behavior of fish in response to an external stimulus; in this case, the response of the fish is the biomarker. This is a very promising area where research is still very scarce.

Whole cell biosensors are detection tools based on a live bacteria that can sense a signal (of interest is the presence of a petroleum hydrocarbon), and deliver an output response that can be detected and quantified using a suitable detector device. Biosensors can be very useful for a fast and cost-effective first-line screening of the presence of particular pollutants [20–22]. Although biosensors are not aimed at substituting analytical techniques such as gas chromatography or high-pressure liquid chromatography, they can be attractive complementary tools to detect pollutants *in situ* in a cheap and flexible way, and do not need heavy and expensive equipment. Importantly, biosensors respond to the amount of the pollutant that is bioavailable (available to the cells), while chemical methods detect the total amount of the compound present (bioavailable and not bioavailable), which may overestimate the real risks in terms of toxicity [23]. Therefore, biosensors can be very useful for measuring the ecotoxicity of contaminants, as well as for monitoring bioremediation processes. For example, a whole-cell biosensor based on an engineered *Escherichia coli* bacterial strain has been successfully shown to be useful in field tests for the detection of arsenic salts in groundwater [24].

Remediation of marine oil spills

In the recent BP's Deepwater Horizon incident in the Gulf of Mexico, it was the first time that large-scale release of dispersants (COREXIT 9500) was attempted in the deep subsurface [25] directly at the wellhead at a water depth of about 1500 m. The experience collected through monitoring and dispersant use in this incident [26] is very valuable in designing new products for subsea use that address the problem of dissolved hydrocarbon gases released as the oil is moving upwards. It was the first time that a cloud of micro-droplets was monitored several kilometers from the well-head at a depth of about 1000–1300 m. Fortunately, such accidents are rare and highly unlikely to occur, however, given the recent interest in oil and gas present in the Eastern Mediterranean Sea, the potential of a well blowout accident during exploration at depths 2000–3000 m is high enough to force policy makers and end users to have suitable contingency plans in place. Based on the experience of the Deepwater Horizon accident, it is important to develop multifunctional dispersants comprised of biosurfactants with sufficient amounts of oleophilic biostimulants to boost 'cloud' biodegradation at an enhanced rate. Although the dispersion related processes are not fully understood at these depths, multifunctional agents (i.e. dispersant and biostimulant) are expected to be a much better choice. It is noted that there is even the potential of gas hydrate formation from the gas evolving from the crude oil at the prevailing high pressures (>100 bar) which is further enhanced in the presence of surface active agents [27].

Despite their higher production cost, biosurfactants are an important alternative bioremediation agent. Large numbers of microbial strains producing biosurfactants have already been isolated from diverse environments, including marine environments. Potential applications for the biosurfactants are being actively investigated by a range of major international companies with interests in a wide range of market niches and potential applications [28]. The biosurfactants produced by microorganisms, bacteria, yeasts and fungi fall into a variety of different categories depending on their size and structure [29]. There are several recent reviews which have summarized the range and potential applications for microbial biosurfactants and have all stressed the high level of commercial interest and future potentials for these molecules [30].

The biosurfactants eliciting most current interest are the glycolipids types which are molecules comprising alkyl chains linked to sugar molecules giving them hydrophilic and hydrophobic regions and producing stable but readily biodegradable structures. The variety of possible structures within this category of biosurfactants offers the potential for a wide range of different functionalities [31]. Biosurfactants can play a significant role in enhancing hydrocarbon biodegradation in the marine environment [32,33]. Further advances in enhancing bioremediation of marine oil spills can be accomplished by using multi-functional bioremediation agents, which combine biosurfactants with biostimulants.

Microbial biodiversity of pollutant degraders in the marine environment

In the recent years significant progress has been accomplished in research on petroleum hydrocarbon microbial degraders in marine environments. Several studies have identified novel obligate hydrocarbon degraders typical of marine habitats like *Alkanivorax* and *Cycloclasticus* that are now well studied. These powerful and specialized microorganisms have been shown to play important roles in HC degradation yet in conditions of standard oil spills. However, there are still several unexplored frontiers for the research including oil degrading microbes adapted to extreme conditions like the deep sea and novel microbes from neglected highly polluted sites. These extreme environments can be studied and exploited by coupling advanced microbial cultivation through the high throughput cultivation systems and the advanced 'omics'-based research technologies.

On the other hand, sediments are the main repositories for many of the more toxic chlorinated chemicals that enter the marine environment, especially polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs), that cause habitat impairments and pose an unacceptable risk to aquatic organisms, aquatic-dependent wildlife and humans. Anaerobic microbial reductive dechlorination processes can transform these highly chlorinated pollutants into less chlorinated, less harmful compounds and might thus be potentially responsible for a significant and cost effective decontamination/detoxification of polluted sediments, when actively taking place *in situ* [34–36]. However, relatively little is known yet about the actual relevance of such processes *in situ* and the possible strategies/approaches suitable to efficiently and safely stimulate them, in particular in the large number of marine contaminated habitats. This is dramatically limiting the opportunities and perspectives of biological approaches in the management of the huge amounts of contaminated sediments.

Main research efforts on chloroaromatics reductive dechlorination have focused so far on freshwater and, to less extent, estuarine sediments, leading to the characterization of several dechlorination processes, the enrichment of chloroaromatics-dechlorinating cultures in defined media and the identification of several, not yet isolated, dechlorinating microorganisms with the use of molecular techniques. Much less is known on microbial reductive dechlorination and dechlorinating microorganisms in marine environments, which typically display biogeochemical (e.g. salinity and availability of sulfate) and microbiological features quite different from those of freshwater and estuarine habitats and where the occurrence of a wide variety of halogenated organic compounds naturally produced by indigenous organisms might favor the enrichment of dehalogenating microorganisms with different dechlorination activity. As an example, although the only few PCB dechlorinating bacteria identified so far in marine sediment cultures display high phylogenetic similarity to dehalorespiring *Dehalococcoides*-like bacteria previously identified in estuarine environments [37–39], they can exhibit a wider PCB dechlorination activity and specificity [38]. The chloroaromatics biodegradation potential or marine microorganisms is still mainly unexplored and should thus be further investigated.

Aquaculture – environmental and sustainability issues

Aquaculture has been the fastest growing food production sector globally the last decade (6.3% per annum [40]), and further growth is needed to meet the dietary requirements of the growing human population [41]. A considerable amount of the feed used in aquaculture is released into the surrounding waters as respiratory products, faeces and uneaten feed, and there is an increasing concern regarding the potentially negative impacts that this nutrient load may have [42]. Concerns are particularly directed at the monoculture of species using feed containing fish meal and fish oil [43], as this form of aquaculture may lead to depletion of natural fish resources used as feed ingredients [44,45]. One of the major challenges for the sustainable development of freshwater and marine aquaculture in Europe is therefore to maximize the biomass production per unit of feed used and at the same time minimize waste discharges that may lead to degradation of the environment. Cultivation of species from different trophic levels (e.g. vegetables, macroalgae, bivalves and detritus bottom feeders) in the effluent from fish farms in closed or semi-closed freshwater and open marine systems can consume and convert excess nutrients into valuable biomass with many applications in, for example, food, feed, energy, pharmaceuticals and cosmetics, thus adding value to the feed investments at the same time contributing to a more sustainable aquaculture [46–50]. By such means, integrated waste recycling using the principles of polyculture complies with the strategy to maintain the nutrient state of inshore and coastal waters with respect to the EU Water Framework Directive [18].

The growth of the aquaculture industry depends on a corresponding growth in the feed production [51]. The large demand for marine fishmeal and oils for this feed is a challenge and new, less exploited feed sources like macro- and microalgae are thus needed to contribute to the further increase and to enhance the sustainability in fish farming. As primary producers algae extract nutrients from the water and convert CO_2 into biomass. The cultivation of macroalgae in seawater can produce large biomass without input of extra energy, proteins or nutrients. Furthermore, as new technology develops the importance of microalgae for biomass production in land based cultivation systems increases rapidly. The high number of different algal species represents a raw material for extraction of a long range of special compounds with beneficial nutritional properties [52,53]. Some compounds are suitable for use in feeds as is, whereas existing or new, advanced extraction and conversion technology can be used for modification of components into high value products with special properties. The cultivation conditions influence on the composition of the algal biomass and are a tool for inducing enhanced synthesis of the demanded components. Increased utilization of proteins, lipids and high-valuable components from the so far under-exploited marine resource represented by cultivated algae would support a sustainable increase in the production of aquaculture feed and thus of healthy seafood.

Industrial development – challenges and opportunities

Traditionally, only few countries, namely United States and Western Europe, have demonstrated inventive strengths in marine biotechnology. In the last years, biotechnology breakthroughs coming from developing countries (i.e. China, South Korea and India) are making a significant impact on the global marketplace. In order to boost its leadership in the marine biotechnology sector, EU needs innovative strategies leaning on its strong assets and new models of Research and Industry cooperation. It is proposed to promote test case studies to optimize strategies promoting entrepreneurships and to develop new programs for the new generation.

In this position paper, fourteen (14) propositions are presented and briefly analyzed in the following sections.

Main propositions

Early warning systems to foresee marine threats (natural and anthropogenic)

Proposition-1.
Biosensors for hydrocarbon contamination: the next step.

The nature and advantages of biosensors have been described in detail in the Introduction. Several biosensors have been reported to date for diverse applications. Although many perform well in the laboratory, only a few have finally reached the market. The *sensitivity, selectivity, speed of the response, and signal to noise ratio* of most currently available biosensors still needs further optimization. This requires a fundamental understanding of the mechanisms involved in the uptake of the chemical to be sensed by the cell, of its interaction with the sensing molecule (typically a protein acting as transcriptional regulator), and of the way the sensing protein regulates transcription of the gene delivering the output response. Also, the way in which the output response (i.e. light or fluorescence) is converted into a quantitative signal is of prime importance. All these issues can be tackled. The success will probably depend on the fruitful convergence of molecular microbiology, synthetic biology, ecotoxicology and engineering [20]. The outcome will be the rational design of a bacterial cell able to respond to a given chemical in a sensitive and selective way, delivering an output signal that can be efficiently and unequivocally monitored with an electronic recording system that should be small and cost-effective.

In the case of marine environments, several whole-cell biosensors able to detect contaminations by hydrocarbons are already available [54–56]; however, they still suffer from several problems. These problems mainly derive from the very low water-solubility of hydrocarbons, particularly those of high molecular weight. Since reporter bacteria are intended to detect the hydrocarbon molecules present the aqueous phase, a very low water solubility of the hydrocarbon leads to a serious mass-transfer problem, resulting in bioreporter cells that have very long response times and relatively low signal to noise ratios. Creative solutions to these problems are needed. Probably the main issue here is to find ways to facilitate the uptake of the hydrocarbon by the reporter cell, which in turn needs a deep understanding of the uptake processes involved. A non-excluding alternative is to encapsulate cells into polymer matrixes tailor-made to facilitate these mass transfer processes, a solution that also requires specific research. Obtaining whole-cell biosensors useful in real-life field applications requires solving two additional issues. One is the specificity of the response. Environmental samples may contain not only the contaminant to be detected, but also additional molecules that can interfere with the response. It is therefore important to increase the specificity of the sensing protein. Fortunately, specificity may be improved or modified in many cases using molecular approaches [57]. A final key issue is how to store the reporter cells in a way that is suitable both for an optimum response to the hydrocarbons and for an easy transport, distribution and manipulation for real life applications. Cells encapsulated into suitable matrixes are a promising solution to this problem although for hydrocarbons more hydrophobic matrixes will probably be needed to facilitate the mass-transfer problems derived from their low water solubility. Recent advances in the development of organic hydrogels and of inorganic matrixes using the sol–gel technology will allow the development of biocompatible matrixes of controlled hydrophobicity that preserve cell viability [58,59]. It is anticipated that this will strongly facilitate the practical use of whole-cell biosensors in the near future.

Proposition-2.
Early warning bio-tools to assess ecosystem health status (*Biomarkers*).

Marine ecosystem health assessment is aimed at providing information necessary to protect biodiversity and integrity of marine communities and habitats, at limiting human influences on bioresources, and at safeguarding human health [16,60]. Though ecological and chemical endpoints have been conventionally used for this assessment, these responses are manifestations of damage rather than prognostic indices. In contrast, responses at molecular, cellular, tissue-level underlie effects at complex biological levels, for which causality can be established, and may thus provide early warning of ecosystem health deterioration [16]. Biomarkers are these early responses that indicate the presence of pollutants (exposure biomarkers), the magnitude of the biological response to pollutant exposure (effect biomarkers) or the susceptibility of organisms to environmental insult (susceptibility biomarkers) [14,61]. As a result, molecular, cell and tissue-level biomarkers have been developed over the last 30 years to provide early warning systems for the monitoring of chemical pollution and its effects as well as for environmental risk and ecosystem health assessment [60]. In parallel, impressive advances have been accomplished in the research and application of biomarkers in the areas of biomedicine (pharmacology, toxicology, pathology); however, the advances in the area of marine and environmental sciences have progressed more slowly [14].

It is proposed to optimize the use of biotechnological tools (biotools), including specimen banking and data storage and meta-analysis, for predicting and monitoring marine chemical pollution and its effects as well as for marine environmental risk and ecosystem health assessment. For these purposes the following research needs have been identified:

(1) Understanding variability and responsiveness of biomarkers including those produced by omic studies to identify background levels of biological response. Seasonal variability, natural factors and global stressors affecting biotools are poorly known at present, and the existence of distinct response-times and adaptive capacities of the biological responses is commonly neglected.
(2) Optimizing existing biomarker methods by developing integrated standard procedures in order to improve diagnostic power and to achieve comparable data. Sampling and early sample treatment and manipulation constitute an additional uncertainty source that has been often overlooked. There is a need for standard sampling and sample processing procedures that diminish the influence of manipulation and take into account the natural variability and the specific biological processes associated to the different endpoints.
(3) Applying state-of-the-art and high-throughput technologies for early warning (biomarkers) and diagnosis of marine ecosystem health and its impairment due to chemical pollution. This may include, for example, the development and application of: (a) novel methods for conventional biomarkers; (b) alternatives to cryomethods (difficult to accomplish in field studies); (c) immunomethods suitable to be implemented in biosensors; (d) omic techniques; and (f) most recent DNA technologies.
(4) Constructing and contrasting integrative indices in order to facilitate the integration between biological and chemical data and their aggregation for regulatory purposes. Biomarkers (conventional alternative of omic-based) in-a-suite depict a toxicity profile useful for the diagnostic of the health status of sentinel populations and, therefore, for ecosystem health assessment in marine pollution monitoring. A toxicity profile is a toxicological 'fingerprint' of a sample, ranging from a pure compound (e.g. specific metals or organic compounds that constitute priority pollutants iI coastal areas) to a complex mixture, obtained by testing the sample or its extract for its activity toward a battery of biological endpoints [62]. The application of ecosystem health indices together with pollution indices allows a science-based assessment of biological effects and provides environmental stakeholders with user-friendly information [60].
(5) Examining in a systematic manner, the suitability of using objectively quantified fish behavior as an early monitoring system to identify the presence of contaminants, that is, heavy metals or cleaning agents [63] in the environment.

Proposition-3.
Integrative monitoring of pollutants, microplastics, pathogens and biotoxins in sentinel species.

Mussels have long been used for the measurement of pollutants and their biological effects in the aquatic environment since Ed Goldberg in 1976 launched the 'Mussel Watch' program. More recently, in order to seek harmonization and comparability across techniques and regions, the ICES Working Group on Biological Effects of Contaminants (WGBEC) has sought to integrate sediment, water and biota endpoints into an ecosystem framework based on the 'mussel integrated approach'; which incorporates mussel tissue chemistry, and whole organism, tissue and subcellular responses. Interestingly, early detection of molecules and cellular responses against the presence of environmental threats other than chemical pollutants (microplastics, pathogens, biotoxins) can be achieved through the same conceptual and analytical approach and the same species than in currently on-going Mussel Watch and related programs.

It is proposed to develop a biotechnological approach for the early detection of molecules and cellular responses in sentinel species (e.g. mussels) to the presence of biotoxins, pathogens and microplastics in the marine environment and its implementation together with pollution biomarkers and toxicological endpoints in environmental monitoring programs; on their own or in combination with continuous recording systems (sensors, biosensors, semipermeable membrane devices, among others). For these purposes the following research needs have been identified:

(1) Identification of molecular markers for algae toxins and biomarkers for their effects in target species, especially in sentinel species.
(2) Development of methods suitable for these biotoxin markers to be implemented together with existing chemical pollution biomarker methods.
(3) Identification of molecular markers for most relevant pathogens and biomarkers for their effects in target species, especially in sentinel species.
(4) Development of methods suitable for these pathogen markers to be implemented together with existing chemical pollution biomarker methods.
(5) Development of identification and quantification techniques and target tissues for most microplastics and biomarkers for their effects in sentinel species.
(6) Development of methods suitable for these pathogen markers to be implemented together with existing chemical pollution biomarker methods.
(7) Integration of pollution, biotoxin, pathogen and microplastic biomarkers in ecosystem health indices.

(8) Implementation of this integrative monitoring of pollutants, microplastics, pathogens and biotoxins in sentinel species with continuous recording systems (sensors, biosensors, semipermeable membrane devices, among others).

Proposition-4.
Marine litter – role of microorganisms in its mitigation in marine habitats.

Marine litter represents a prominent environmental, economic, human health and aesthetic problem. It poses a complex and multi-dimensional challenge with significant implications for the marine and coastal environment and human activities all over the world. Microorganisms (bacteria, archaea, and picoeukaryotes) in coastal sediments represent a key category of life with reference to understanding and mitigating the potential adverse effects of microplastics as putative mediators of the biodegradation of plastic-associated additives, contaminants, or even the plastics themselves. As such, research into the formation, structure, and activities of microplastic-associated microbial biofilms is essential in order to underpin management decisions aimed at safeguarding the ecological integrity of our seas and oceans [64]. In particular, systematic efforts addressed to provide additional insights on the microbial communities colonizing disperse microplastics under actual site conditions and on the interactions occurring between native microbes and microplastic in the same habitats are essential for predicting the actual potential of biological processes in the final degradation of microplastics in situ and for designing tailored strategies for stimulating them *in situ* efficiently and safely. Further, efforts addressed to isolate microbial consortia and/or pure cultures from biological material associated with such microplastics historically occurring in marine habitats are essential to design possible site-specific tailored bioaugmentation strategies for boosting microplastic degradation. No successful examples of microbial-based *in situ* biostimulation and bioaugmentation have been reported so far in the scientific and technical literature. Thus, new knowledge is needed in this field, in terms of both strategies/approaches to efficiently and safely introduce additives and microbes at the site and technological/engineering methods required for doing it. The effectiveness of the approach should be then investigated and validated on different sites.

Proposition-5.
The future in monitoring of harmful algal blooms (HABs).

The link between GES and seafood safety is explicit in MSFD descriptor 9, which stipulates that contaminants in seafood must not exceed relevant standards. Real-time status information of the marine environment and seafood safety is urgently needed by stakeholders (seafood and aquaculture industries, policy makers) to respond timely to rapidly occurring phenomena such as harmful algal blooms (HABs).

Thus, it is important to develop new, innovative sensors for detection of marine microalgae *in situ* for near real-time monitoring. It is important that these sensors are targeting known HAB species, but have yet to be made easily adaptable for detection of newly identified HAB species. Equally important is the development of new, innovative sensors for near real-time monitoring and in situ detection of marine biotoxins. The developed technology has to be easily adaptable to newly discovered biotoxins. Issues like maintenance, long-term stability and biofouling need also to be addressed.

Suitable data mining techniques and artificial intelligence computational (machine learning) techniques applied to the great amount of data already produced coupled to hindcast studies will improve our understanding of the processes underlying the development and spread of HABs, and possibly to identify proxies that can be used as early warning indicators. These results should enable the development of better operational model systems allowing for prediction of early warning proxies/indicators.

The development of efficient end-user interfaces for governance, farmers, fishermen, and the general public for access to, for example, simulation and forecast results, toxicity information and advice regarding HABs is important. Finally, the development of new methods and technologies for prevention and controlling of HABs, like nanotechnology, marine ecosystem models [65], natural and artificial upwelling [66,67], are important elements of an integrated management strategy for HABs.

Remediation of marine oil spills

Proposition-6.
Development of novel bioremediation agents to control oil spills.

Bioremediation is an important weathering process that takes place during marine oil spills; however, it takes at least one week before biodegradation processes start. It is important to shorten this start up period to the absolute minimum by providing technologies for example, that provide the necessary nutrients together with hydrocarbon degrading consortia and/or enhancing compounds (biosurfactants) to both accelerate and maximize bioremediation rates from the time of the first application of bioremediation agents (biostimulation and bioaugmentation agents) [68].

Furthermore, release of crude oil (continuously for a period of time) in a deep-sea environment is a new challenge facing offshore petroleum exploration and production facilities. As offshore drilling technology allows the exploration of very deep oil reservoirs, the probability of having deep sea accidents like the recent one in the Gulf of Mexico, is increasing. EU must be ready to face potential accidents in the Eastern Mediterranean Sea (south of Cyprus and south of Crete) where oil and natural gas exploratory wells are expected to be drilled in the near future. The first deep-sea use of dispersants (Corexit 9500A and 9527A) was faced with low dispersion efficiencies.

We propose the development of multifunctional agents (i.e. dispersant and biostimulant), which are expected to be a much better choice. Novel and innovative multifunctional agents should be developed that have at least two modes of action (e.g. dispersing oil and providing nutrients or microbes, or absorbent materials with encapsulated nutrients and hydrocarbon degrading microbes, or dispersants with continuous oxygen supply capabilities). Particular attention should be given to agents suitable for combating continuous deep-sea petroleum releases.

Furthermore, develop novel formulations by combining growth supplements (oxygen, nutrients and micro-nutrients) with microbial consortia, including dispersing porous sorbent and oxygen releasing gel-substrates. Alternatively, one could synthesize mesoporous silica nanostructured materials, which (i) slowly release biochemical, (ii) stimulate the growth of microbes and (iii) favor sorption to oil. Particular attention should be paid to the development of multifunctional agents for use in deep-sea marine environments to promote besides dispersion, enhanced biodegradation of the resulting 'micro-droplet cloud'.

Proposition-7.
Exploitation of marine bacteria producing biosurfactants.

Large numbers of microbial strains producing biosurfactants have already been isolated from diverse environments, including marine environments [69]. A range of major international companies is actively investigating potential applications for the biosurfactants with interests in a wide range of market niches and potential applications.

The following are priorities for further investigation of microbial biosurfactants, principally glycolipids, from marine environments:

(1) Limited searches for new producer organisms, since many strains are already known but not fully investigated. One target ecological niche might be arctic and Antarctic seas and ice where psychrophilic strains may yield biosurfactants with different functionalities applicable at low temperature environments.
(2) Investigating microorganisms capable of producing biosurfactants within biofilms as tools for bioremediation of marine environments for both pristine areas and polluted sites, with emphasis on coastal intertidal and littoral environments.
(3) Detailed investigations of the structure and functionality of marine microbial biosurfactants to identify those with unique characteristics, which could be produced *ex situ* by fermentation for applications in remediation and elsewhere in manufacturing industry.
(4) Definition of biosurfactant production conditions for optimized yield, downstream processing and raw material consumption.
(5) Examination of the genetic and metabolic control of biosurfactant production by target marine microorganisms.
(6) Laboratory and field investigations of the potential use of marine microbial biosurfactant producers as an augmentation tool for *in situ* hydrocarbon pollutant remediation.

Microbial biodiversity of pollutant degraders in the marine environment

Proposition-8.
In search of more powerful hydrocarbon degraders.

Deep-sea spills represent new modes of contamination with a potential very negative impact on the deep-sea ecosystems. Following the deepwater horizon disaster, there are now concerns for deep sea drilling for oil exploration all over the world. Concerns have been raised for the increasing trends in deep sea explorations. For instance, in the Mediterranean Sea that is a close basin with a very high biological diversity [70] or in delicate extreme environments like the Arctic where a lot of novel explorations are starting following the ice melt due to global warming [71], the availability of microbial resources adapted to the deep sea and increased knowledge on bacterial physiology and response to the extreme treatment conditions (like growth rate, HC degradation rate, microbial diversity dynamics, degradation pathway activation, biosurfactant production, among others) are urgent needs.

It is thus proposed to intensify the quest of novel degraders adapted to extremely polluted terrestrial sites like many producing or refining sites in the North Africa and Middle East. A recent survey of the bioremediation studies worldwide has shown that this is a neglected area for the study of the microbial communities with oil degradation potential [72]. The high oil pollution levels of certain of these sites, coupled with the extreme conditions of temperature and water availability that occur in summer, makes these sites a potential source of novel microbes with improved physiologies adapted to extreme conditions. Indeed, there is the need of exploring the range of environmental conditions where oil-degrading microbes can operate, being the different polluted sites located in very different latitudes and climates worldwide. A well-suited North–South cooperation might lead to addressing the clean-up of these polluted sites and the better management of large oil producing regions.

Proposition-9.
Exploitation of marine bacteria in decontamination of sediments contaminated with chlorinated pollutants.

Sediments are the final sink of a variety of chlorinated hydrophobic compounds, like polychorinated dioxins, furans and biphenyls as well as polychlorinated aliphatic compounds. New knowledge is needed to develop strategies/approaches suitable to efficiently and safely stimulate microbial reductive dechlorination processes, as well as technological/engineering methods for the biostimulants application to contaminated sediments. Bioaugmentation with dehalorespiring bacteria has been also proposed as a potentially effective strategy for the enhancement of reductive dechlorination processes in impacted sediments [73]. The effectiveness of the approach should be however investigated on sediments from different sites. In addition, more research is needed to develop cost effective methods to grow rapidly large amounts of dechlorinating cultures for bioaugmentation.

Aquaculture – environmental and sustainability issues

Proposition-10.
Treatment of saline wastewater from the aquaculture industry.

Fish and shellfish farms could discharge high volumes of saline wastewater containing dissolved metabolites primarily in the form of dissolved inorganic nitrogen and phosphorus [74]. These effluents are rich mainly in nitrogen ammonia and can lead to eutrophication and reduced oxygen levels, due to oxidation of ammonia to nitrate. The development of land-based marine recirculating aquaculture systems offers the possibility to treat the effluents, before discharge [75]. Hence, it is important to develop novel wastewater treatment systems that can cope with salinity at seawater levels and can successfully remove excess ammonia and phosphorous at various organic loadings.

It is proposed to investigate two alternative approaches:

(1) Use of immobilized microbial systems employing marine degraders, and
(2) Use of constructed wetlands with halophytes that can easily cope with the high salinity stress.

Proposition-11.
Integrated waste recycling – marine polyculture systems.

As an alternative or better complementary to wastewater treatment one should exploit the advantages of marine polyculture systems. Integrated waste recycling using the principles of polyculture complies with the strategy to maintain the nutrient state of inshore and coastal waters with respect to the EU Water Framework Directive. Future research should thus focus on:

(1) The need for a better understanding of temporal, spatial and trophic interactions in both freshwater and marine polyculture systems to enable more realistic budgets for example, energy, nutrient and CO_2 flow.
(2) Biotechnological tools will be of great importance for the system requirements and thus the bio-economy in closed recirculation systems, while in open systems the choice of high value species will rely on the downstream processes needed for a given product portfolio (e.g. food, feed, bioenergy and health-products) enabled by new biotechnological tools.
(3) Determination of each species' capacity to assimilate waste nutrients released from fish farms and their effects on biomass growth and food value to enable realistic estimates for productivity.

Proposition-12.
Macro- and microalgae as future sustainable feed resources.

Growth of the aquaculture industry depends on a corresponding growth in the feed production and a sustainable feed production must avoid exploiting of natural fish stocks and the use of arable. Unexploited feed sources like macro- and microalgae should be investigated as a future feedstock. Cultivation of macroalgae in seawater can produce large biomasses without input of extra energy, proteins, nutrients or pesticides. Future research should consider the following issues:

(1) A bottleneck for economic viable exploitation of macro- and microalgae is efficient, industrial scale cultivation technology. The technologies for macro- and microalgae cultivation have very little in common, but in general, basic knowledge about the premises for an optimized production of biomass and synthesis of targeted feed components is crucial. It is important to focus on the development of cultivation systems for predictable and optimized biomass production as well as for conditioning of the composition and quality of the biomass.
(2) Macroalgal biomass is attractive for extractions of bulk components such as proteins and sugars. The extracted proteins can be used directly in the feed, while the sugars may be fermented into proteins.
(3) As the algal biomass starts deteriorating shortly after being harvested, the development of relevant pre-treatment technology is important in order to avoid spoilage of the valuable components.
(4) To increase the bio-availability of nutritious components from macro- and microalgae suitable biotechnological methods must be developed and optimized for extraction, fractionation and enzymatic modifications.

Proposition-13.
Application of cultivated copepods (live-feed organisms).

Copepods constitute a vital tropho-dynamic link between primary producers and fish in the marine food chain. They have naturally high levels of essential omega-3 fatty acids and important micro- and macro-nutrients essential for a normal larval development. There are several thousand different copepod species in the sea, many of them vital as feed for newly hatched marine fish larvae. Cultivation technology for high quality copepods is essential for production of test organisms for studying effects of for example, climate change and pollution on the marine environment. Through development of standardized cultivation protocols for several species of marine copepods, the knowledge of their biology will be broadened and as a consequence one will be better equipped with the tools needed to establish intensive copepod cultures.

Future research should consider the following key points:

(1) Ecotoxicological tests based on the use of marine and estuarine native organisms have proved to be an important tool in assessing environmental quality. Intensively produced marine copepods can be used as test organisms in toxicity tests. Standardized protocols for ecotoxicological tests, as well as culturing protocols for relevant species of copepods must be established for this to be successful. The advantage of using intensively produced copepods as test organisms is that the risk of pre-trial exposures to toxic compounds can be eliminated.
(2) As many species of copepods have a short generation time, they are good candidates for studying long-term environmental effects of climatic changes such as ocean acidification on several generations of a population in a short period of time.
(3) Advanced cultivation equipment for continuous production of copepods does not exist. Automated production systems in copepod production must be developed.

Industrial development – challenges and opportunities

Proposition-14.
New modes of research and industry cooperation.

As already mentioned earlier, in order to boost its leadership in the marine biotechnology sector, EU needs innovative strategies leaning on its strong assets and new models of Research and Industry cooperation. The few items that should be prioritized are:

(1) *Harnessing inventions:* A representative number of European Countries (with Germany clearly at the top of the list) are top ranked as to the national patents ownership related to the marine biotech sector. Nevertheless, the research sector in those countries has been particularly active in patenting, while industrial patenting is still rather limited. Among

various reasons, the unfavorable cost-benefit ratio related to intellectual property rights (IPR) explains often the choice. It is proposed to optimize (EU) IPR solutions.

(2) *Seeking a competitive edge in the world market:* The biotechnology industries in the United States and Western Europe tend to focus on high-cost solutions related to health biotechnology. The market for the targeted R&D products is therefore geographically restricted and fully driven by multi-national companies. Added value can come from a more diversified demand, including the emerging maritime sectors (i.e. aquaculture, off-shore plants, eco-compatible maritime transport) and the new industrial needs emerging in developing countries (agriculture, bioprocessing, disease-, insect-resistant crops, disease diagnostics, etc.). Technology itself could benefit of inputs from the marine biotechnology research sector (i.e. new microarrays, biosensors, new materials, etc.). It is proposed to utilize cross-sector efforts to achieve a greater market diversification.

(3) *Fostering natural resources and industrial competencies:* In spite of its large and highly diversified marine environments, EU lacks knowledge on the potential and geographic distribution of its marine genetic resources. Coordinated mapping efforts should be promoted to create baseline knowledge for targeted bioprospecting exercises. Special international (access and benefit sharing) agreements should be developed for EU to better benefit of its advantageous geopolitical position (i.e. to gain preferential access to high-potential, neighboring maritime zones such as the Mediterranean extreme marine environments). Among the *in kind* options, 'not-for-profit industry modes for developing countries should be tested. There is a clear need of new political and structural (including logistic) instruments that closely link private companies, foreign investment groups, and university research centers so as to transfer knowledge more efficiently. Some success stories (i.e. marine/maritime national hubs; Brazilian national commitment to natural resources-based innovation) could be used to pave the way.

(4) *Making private investors step up:* The importance of the private sector cannot be discounted when considering the biotech industry. Actions to promote entrepreneurship are the way forward. In order to optimize those actions, test studies should focus on local response, government action, resources, health and education, and private-sector support. Government actions could include: *(i)* facilities for public research institutions for being partially converted to private companies (with both researchers and professors at the helm); *(ii)* strategies to lower industrial costs (i.e. international agreements with neighboring developing Countries to facilitate delocalization of scaling-up steps), among others. It is proposed to promote test case studies to optimize strategies promoting entrepreneurships and to develop new programs for the new generation.

Concluding remarks

Environmental biotechnology can and should play an important role in addressing challenges relating to the marine environment. The 14 propositions cover the following research topics:

(1) Biosensors for hydrocarbon contamination: the next step.
(2) Early warning bio-tools to assess ecosystem health status (Biomarkers).
(3) Integrative monitoring of pollutants, microplastics, pathogens and biotoxins in sentinel species.
(4) Marine Litter–role of microorganisms in its mitigation in marine habitats.
(5) The future in monitoring of harmful algal blooms (HABs).
(6) Development of novel bioremediation agents to control oil spills.
(7) Exploitation of marine bacteria producing biosurfactants.
(8) In search of more powerful hydrocarbon degraders.
(9) Exploitation of marine bacteria in decontamination of sediments contaminated with chlorinated pollutants.
(10) Treatment of saline wastewater from the aquaculture industry.
(11) Integrated waste recycling–marine polyculture systems.
(12) Macro- and microalgae as sources for new feed components.
(13) Application of cultured copepods – live-feed organisms.
(14) New modes of research and industry cooperation.

In the context of Horizon 2020, environmental biotechnology focus areas related to the marine environment are part of Societal Challenge 2 (food security, sustainable agriculture and forestry, marine and maritime and inland water research, and the bioeconomy) and in particular of ''Blue Growth'', the evolution of ''The Ocean of Tomorrow'' joint initiative in the new framework program.

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