

Bacterial Extracellular Enzymatic Activity in Globally Changing Aquatic Ecosystems

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Heterotrophic bacteria are key players in the processes of organic matter recycling, decomposition and mineralization in aquatic environments. Although only small and chemically simple compounds can be passively transported through bacterial membranes, substrates for bacterial utilization in aquatic environments are dominated by particulate or high-molecular-weight dissolved organic matter. Complex substrates must first be hydrolyzed outside the cell into smaller size molecules by extracellular enzymes and this process represents a limiting step in nutrient cycling. Bacterial extracellular enzymatic activity is regulated at the ecosystem level, by environmental factors and at the micro-environment level by enzyme-substrate interactions. Over the last century, changes in the atmosphere concentration of CO₂ and other greenhouse gases caused changes in climate patterns that have repercussions in ecosystem function and biodiversity. Microorganisms are generally able to respond very quickly to environmental changes because of their close contact with the surrounding environment and rapid growth. As mediators in important biogeochemical processes, namely decomposition and transformation of organic matter, release of inorganic nutrients for higher trophic levels and detoxification of xenobiotics, bacterial enzyme activities have the potential to be used as descriptors of biological responses to changing environmental conditions. The present paper reviews the currently available information on environmental regulation of bacterial extracellular enzymatic activity in aquatic environments and discusses the potential implications of direct and indirect effects global changes on heterotrophic bacterial communities and on the processes of organic matter recycling.

Keywords Extracellular Enzymatic Activity; Aquatic Bacteria; Global Changes

1. Introduction

Heterotrophic microorganisms represent a key trophic level in the processes of organic matter decomposition, nutrient cycling and carbon flow through aquatic food webs, as described by the microbial loop model [1]. The main sources of organic matter (OM) to the microbial loop are phytoplankton exudation and leakage of algal or bacterial cell material during grazing [1]. Benthic resuspension, plant/algae exudation and terrestrial or riverine inputs represent significant additional sources of OM, which can be used as carbon and energy sources [2].

Passive transport through bacterial cell wall and cell membrane is restricted to very small and chemically simple compounds. Although different in the chemical composition and in architecture, the cell wall of gram positive and gram negative bacteria only allows the transport of rather small molecules [3]. Gram positive cell wall is not as restrictive, in terms of permeability, as the outer membrane of gram negative bacteria [4] where trimeric proteins (porins) form channels between the outer membrane and the periplasmic space. The substrate uptake limit of the cell (~600 Da) is defined by the geometry of the porins [4].

Particulate organic matter (POM) and dissolved organic matter (DOM) are dominated by high-molecular-weight compounds [5-6]. In order to allow transport across the outer membrane, complex substrates must first be hydrolyzed outside the cell into smaller sized molecules [7-8]. This process is conducted by extracellular enzymes which enable heterotrophic bacteria to obtain substrates suitable for incorporation from a diverse array of complex compounds [9].

Because extracellular enzymes catalyze the rate limiting steps of nutrient cycling, i.e., the extracellular degradation of complex molecules into easily assimilable units [10-11], any factors affecting their activity or disrupting the production or availability of extracellular enzymes will impact the entire remineralisation pathway [9]. In addition, changes on the patterns of organic matter utilization by bacteria may also impose carbon cycle-mediated feedbacks on global climate [12].

2. Enzyme production and activity

Extracellular enzymes, that react outside the intracellular compartment, are mainly hydrolases (e.g., glycosidases, peptidases, esterases), that is, enzymes that cleave C-O and C-N bonds that link monomers [13]. Extracellular enzymes can also catalyze oxidative reactions, typically cleaving C-C and C-O bonds. These oxidative enzymes can be roughly divided into oxygenases and peroxidases that use molecular oxygen and hydrogen peroxide, respectively, as electron acceptors.

Extracellular enzymes can be further classified according to their physical relation with the cell as ectoenzymes and truly extracellular enzymes. Ectoenzymes are associated with viable cells [7] and include enzymes inserted in or spanning the plasma membrane, associated with the cell wall or, in gram negative bacteria, attached to the outer membrane surface or retained within the periplasmic space by the strict exclusion limit of the outer membrane [14].

Ectoenzymes ensure a close association between the hydrolysis products and the cells, and prevents that both enzyme and hydrolysis products may be easily lost to the environment [15]. Strict-sense extracellular enzymes occur in free form and catalyse reactions detached from their producers. Bacterial extracellular enzymes may be actively secreted by intact viable cells, released into the environment by viral lysis, or result from ectoenzymes that leak from the bacterial cell when the later is being predated [16]. Extracellular enzymes are proportionally more important in the decomposition of particulate or colloidal material in the dark ocean [17].

2.1. Extracellular enzymatic activity in aquatic environments

In the spectrum of enzymes studied in the aquatic environment, special attention has been given to ectoenzymes responsible for the hydrolysis of the major components of DOM. Fluorophore-labeled artificial substrates have been extensively used for sensitive assays of ectoenzyme activities in aquatic environments. Methyl coumarinyl- or methylumbelliferyl-substrates are non-toxic and yield highly fluorescent water-soluble products with optical properties significantly different from those of the substrate [18].

β -glucosidase (β -Glc) and leucine aminopeptidase (Leu-amp) are widely distributed in aquatic environments and their activity is known to be mainly associated with heterotrophic bacteria. They have been used as model ectoenzymes for studying bacterial degradation of natural polymeric compounds such as carbohydrates and proteins in the aquatic environment [19]. β -glucosidase is produced by heterotrophic bacteria in waters and sediments of both freshwater and marine environments. This enzyme exhibits a relaxed substrate specificity hydrolysing β -linked disaccharides of glucose, cellobiose and carboxymethylcellulose [20]. Leu-amp hydrolyses a large number of peptides and amino acid amides of the L-configuration, with particular affinity to L-leucyl-peptides and L-leucyl-amides [7].

Phosphatases are also widely studied enzymes in aquatic environments. Their activities can originate from bacterioplankton but also from phytoplankton and zooplankton [21]. Phosphorus acquisition, especially in P-limited areas, is dependent on the available enzymes to hydrolyse dissolved organic compounds. Phosphatases are the group of phosphohydrolases that most intensively participate in phosphate release in aquatic environments [16]. Generally the term phosphatase encloses a variety of enzymes that catalyze the hydrolysis of esters and anhydrides of phosphoric acid [22]. These enzymes are characterized by different half-saturation constants, temperature and optimum pH [23]. Alkaline phosphatase (APA) encloses a group of isoenzymes that react optimally in pH range 7.6 – 9.6 [16]. APA catalyses the hydrolysis of a variety of phosphate esters, including esters of primary and secondary alcohols, sugar alcohols, cyclic alcohols, phenols, and amines, liberating inorganic phosphate [7].

The identities of the particular members of mixed assemblages capable of producing different extracellular enzymes in natural environments is still largely unknown, since most molecular analyses of community composition focus on rRNA sequences that provide little information on the degradative capabilities of uncultured organisms [24]. Through microcosm approaches, a relation between the structure of bacterial communities, assessed by Fluorescent In Situ Hybridization (FISH), and the rates of EEA could be inferred from the covariance of several ectoenzymes with the relative abundances of the alpha-, beta- and gamma-proteobacteria, and *Cytophaga*-like bacteria [25]. The study of functional gene diversity might help to elucidate both the genetic potential for producing enzymes in microbial communities and the factors that regulate the transcription of those genes [26-28]. However, the study of functional genes present in environmental DNA only gives insights on the genetic potential for the production of extracellular enzymes but not about gene expression biochemistry of decomposition.

2.1.1. Water column

Most of the information on extracellular enzymatic activity in the sea refers to hydrolytic enzymes such as proteases, glucosidases, chitinase, lipase and phosphatases. Generally, the magnitude of activity ranges of these enzymes in seawater is in the order aminopeptidase > phosphatase > β -glucosidase > chitinase > esterase > α -glucosidase [30].

The biochemical role of extracellular enzymes in the sea is similar to other aquatic environments but the hydrographic conditions in the ocean are characterized by distinct vertical and horizontal zonations [30]. Consequently, patterns of polymer hydrolysis rates change horizontally and vertically, between different water masses [17, 31-32], along estuarine gradients [25, 33] and, at a finer scale, between the surface microlayer and underlying water [34]. At the micro-scale, the marine environment is a highly diluted medium interspersed with hot spots of organic matter concentration, aggregation and decomposition [30]. Marine snow aggregates are colonized by heterotrophic microorganisms that express high levels of hydrolytic activities, making them sites of intense carbon remineralisation [35] with cell-specific β -Glc and Leu-amp activities several orders of magnitude higher than in surrounding water [36].

Temporal variation also imposes seasonal [37], diel [34] and tidal [38] patterns of hydrolytic potential, particularly in coastal ecosystems, detectable in the activity rates of particular extracellular enzymes. The differences are related with shifts in the availability of labile organic matter, leading to variations in cell-specific extracellular enzymatic expression [17].

In lacustrine environments, although β -Glc and Leu-amp frequently display the highest activity levels, phosphohydrolases also exhibit high levels of activity since the occurring amounts of readily usable orthophosphate (Pi) in most non-polluted lakes are insufficient to fulfil the phosphate requirements of microplankton [16]. Surface and deep

lake waters exhibit marked seasonal patterns of ectoenzyme production and activity, being maximal activities in surface waters detected during the late stage of phytoplankton blooms and during bloom senescence [39-40]. During summer thermal stratification, extracellular enzyme activity is particularly lower in bottom water layers, being the activity dependent on the rates of sedimentation of detritus produced in the euphotic layers above. Diurnal fluctuations of extracellular enzyme activity in lakes have also been reported [19].

In rivers, the overall activities of all the microbial extracellular enzymes are dominated by cell-bound ectoenzymes and particularly high rates of proteolytic activity are associated to flood events [41]. Although fungi are the most active group in the degradation of particulate plant-derived material, bacterial extracellular enzymes also contribute to leaf litter decomposition in freshwater streams [42].

2.1.2. Sediments

In aquatic ecosystems, dissolved enzymes and polymeric substrates may also be transported from the shallow permeable sediment to water layers above the sediment, enhancing EEA in the water column [43-44]. Extracellular enzymatic activity responds very promptly to inputs of organic matter from sedimentation events [45] and decreases with depth in the sediment column [46-47]. A clear proportionality between induction of enzyme production and supply of organic matter was demonstrated in laboratory experiments with deep-sea sediments, for enzymes degrading structural polysaccharides (β -glucosidase, chitinase) [48].

The rhizosphere, usually defined as the sediment immediately in contact with the roots or under the influence of root-derived compounds, represents a particular sediment environment where exudates from the roots of salt marsh vegetation provide bacteria with high-quality sources of carbon and energy and enhance bacterial heterotrophic activity [49-50]. A positive correlation between root biomass and EEA has been found in salt marsh sediments [51]. Rates of activity of the extracellular enzymes β -glucosidase, α -glucosidase, aminopeptidase, arylsulphatase and phosphatase were generally higher in the sediments of the vegetation banks than in control uncolonized sediments, where EEA had vertically more stable rates [52]. Sediment texture descriptors such as grain size, % of fines or water content showed significant relations with hydrolysis rates [52].

3. Environmental regulation of extracellular enzymatic activity

The prevailing conditions in the water column and in the sediment aqueous phase are unfavourable for enzymes: the substrate concentration is usually low and highly variable [53], the complexation of substrates with humic substances, colloidal organic matter and detritus can difficult the association of an enzyme with its substrate [54] and enzymes may be lost from the producer cell and become exposed to inhibitors, be denatured by physical and chemical factors or hydrolyzed by proteases [55]. This complexity of factors interacts in the regulation of bacterial extracellular enzymatic activity, modulating enzyme expression and subjecting polymer degradation kinetics to the influence of environmental physical and chemical parameters.

3.1. Biochemical regulation

Microbes should produce enzymes only when simple sources of organic C are insufficient [56]. When particular nutrients are present in limited amounts, microbes can produce enzymes to liberate them from organic matter [57]. The production of the majority of ectoenzymes by most aquatic microorganisms is repressed when the cells are grown on sources of readily utilizable dissolved organic matter. The synthesis of ectoenzymes only becomes derepressed once the concentration of readily utilizable substrates in the water falls below a critical level. By using the repression strategy for ectoenzyme synthesis, microorganisms can avoid the wasteful production of inducible enzymes [58-59]. The synthesis of many ectoenzymes in aquatic environments may also be inhibited by the accumulation of the hydrolysis end-product in the cell or in the surrounding environment [16, 60].

Control of EEA expression is sometimes very complex. Microbes may produce extracellular enzymes despite of substrate availability in the environment [7, 61-62]. In the presence of substrate, constitutive enzymes generate low concentrations of reaction products that induce additional enzyme synthesis. Once the concentration of products is sufficient to meet the demand, enzyme production becomes suppressed and returns to constitutive levels [7]. For example, APA and β -Gluc are subjected to substrate induction and catabolite repression [16].

In some cases, a clear response to nutrient availability cannot be established. For example, extracellular protease activity (assessed from Leu-amp activity) in natural and planktonic communities and semi-natural culture systems has been reported to either be reduced by the addition of dissolved inorganic nitrogen [7, 63-64], or not affected [65]. Environmental regulation might be, in such cases, the main factor governing enzyme activity. A positive correlation between aminopeptidase activity and N limitation has been observed and interpreted as an indication of the utilization of organic N-sources for bacterial growth [37].

3.2. Environmental regulation

A model of the environmental regulation of extracellular enzyme activity proposes that at the ecosystem level, enzyme production is mainly regulated by environmental factors such as temperature and that at the microenvironmental level, though still influenced by environmental factors, extracellular enzyme activity is mostly controlled by enzyme-substrate interactions such as inhibition, adsorption, stabilization and humification [66-68].

3.2.1. Temperature

One important indirect effect of temperature is its interference on the affinity of enzyme systems since, at low temperatures, the affinity of enzyme systems decreases [69-70]. The activity of protein- and polysaccharide-degrading bacterial extracellular enzymes of arctic isolates and in marine sediments increases with the temperature, showing optima well above the ambient environmental temperatures [71-74]. The membrane-bound transporters of mesophilic and psychrotolerant marine bacteria respond with decreased substrate affinities to temperatures at the lower end of their specific temperature range [75-76].

Several studies have demonstrated that the temperature sensitivity of extracellular enzymes changes seasonally [77-79], which has been explained by the synthesis of different isoenzymes (enzymes with the same function but different structure) through time, produced either by different organisms or by a single species capable of producing multiple isoenzymes [80]. There is also some evidence for biogeographical patterns in enzyme temperature sensitivity. For example, many studies have observed that enzymes from microbes inhabiting cold environments have unusually low temperature optima [81-83].

3.2.2. Salinity

Salinity seems to be only poorly correlated with the metabolic activity of bacterioplankton [84]. Analysis of the relation between the activity of Leu-amp and salinity in coastal systems has been shown to be positive in some environments [84] and negative in others [85-86]. In general, in low salinities bacterioplankton expresses higher levels of β -glucosidase (β -Gluc) activities, while in higher salinities bacterioplankton seems to be more adapted to protein degradation [33, 85].

Information on the regulatory role of salinity on sediment microbial communities is less available. Because more energy is required for the production of osmolytes and less is used for the release of extracellular enzymes, decreased extracellular enzymatic activity can be detected at higher salinity sediments [87]. However, the inverse pattern has also been reported [88-89].

3.2.3. pH

Contrarily to intracellular enzymes that act in the buffered cytoplasm of the cell, extracellular enzymes are directly affected by the pH of the extracellular environment, because changes in the concentration of hydrogen ions in the environment modify the ionization state of amino acids and the three-dimensional structure of the active site of the enzyme [90]. Deviations from the optimal pH result in a decrease in enzyme activity rates [7, 71, 91].

Similarly to the enzymatic temperature optima, the pH optima of *in situ* extracellular enzymes do not always match the ambient pH. Changes in pH induced by photosynthesis can also affect enzyme activity. Several studies have reported that, in freshwater habitats, Leu-amp has a narrow pH optimum of ca. 7.5, with low activity at pH <6-7 and rapid decline in activity above pH 8.5-9 [91-93] whereas algal activity within periphyton communities can cause pH to exceed 9 [94-99]. However, for periphytic Leu-amp, displaying higher pH optima (> 9.75), a stimulation of enzymatic activity by photosynthetic activity can also occur [60].

3.2.4. Other Factors

Trace metals and UV-B radiation are two ecologically relevant factors that control *in situ* activity of, at least, APA in sea water. APA activities in phytoplankton cultures, cell-free enzyme preparations and field collected samples where bacterioplankton is represented, are inhibited by environmentally occurring concentrations of free copper ions [100]. This trace metal has been speculated to inhibit or totally block the direct utilization of selected DOP compounds by natural microbial assemblages [100]. Marine APA activity is also sensitive to environmental levels of UV. Photodegradation of APA activity may limit the ability of the cells to obtain inorganic phosphate from the ambient DOM pool, enhancing the effects of P limitation in well-lighted, near surface habitats [101]. Experimental irradiation of natural marine bacterioplankton assemblages also caused a significant reduction in lipase and Leu-amp activities [102].

4. Global changes and extracellular enzymatic activities

Changes in the composition of the atmosphere and the relative increase of the so called “greenhouse gases”, has altered the global radiative balance by decreasing the long-wave radiation flux leaving the troposphere [103]. This change is thought to be responsible for climate effects that range from global warming to changes in winds, clouds, sea level, precipitation, storm frequency and intensity, long-term climate models, ecosystem function and biodiversity [104].

Microorganisms are generally highly responsive to environmental changes because their large surface in relation to their small volume facilitates close contact with the surrounding environment. Furthermore, with relatively short generation times, microbial communities could be among the fastest components of an ecosystem to respond to changing environmental conditions [105-106].

4.1. Warming and changing weather patterns

Without the interference of other environment factors, increases in temperature should, within limits, result in increased enzymatic activity [107]. However, adding to the direct effects of temperature on the rates of biological processes, warming is also expected to affect pelagic ecosystems by changing the patterns of vertical mixing and nutrient allocation in the water column [70]. This might cause a shift in the spectrum of extracellular enzymatic activities acting in surface and in deeper ocean layers. Other environmental factors, such as nutrient concentration or primary productivity which co-vary with temperature, may themselves have a larger effect on bacterial activity [108]. Enhanced photosynthetic rates of polar phytoplankton have been observed in response to increasing temperatures [109-111] and, since heterotrophic bacteria are the major potential users of most of phytoplankton primary productivity [112], warming could thus result in the increase in the relative proportion of primary production processed via the dissolved pool and a stimulation of polysaccharide degrading extracellular enzymes [113-114].

Climate change-related increase in the frequency of extreme weather events might have even greater effects on microbes and their activity than overall changes in temperature. Extreme meteorological events, such as typhoons, mix the stratified water column and considerably change the structure of phyto- and bacterioplankton communities. However, the later seem to recover more rapidly than phytoplankton [115] and shifts in the patterns of organic matter recycling and of the extracellular enzymes initiating the degradation of polymeric material will most probably be mediated by primary production. Dust deposition from desert storms occurs at wide spatial scales and, by representing a significant source of mineral nutrients and organic carbon to aquatic ecosystems, has a positive effect on bacterial growth and abundance [116]. However, the input of metals such as arsenic and copper, associated with desert dust [117] might also have an inhibitory effect on some extracellular enzymes. Polar ice melting was found to affect the spectra of bacterial extracellular enzymes and to increase the relative importance of polysaccharide hydrolysis [32].

4.2. UV Radiation

Trends of increased UV radiation levels are predicted to persist for sometime because the effects of global warming on the stratosphere may delay the recovery of ozone layer [108]. UV-B (λ 280-320 nm) is the most biological significant wavelength range within the ultraviolet spectrum, causing both indirect (mediated by reactive oxygen species) and direct damage (UV-mediated photoproduct generation) because of the strong absorption of wavelengths below 320 nm by DNA. Several bacterial extracellular enzymes have shown decreased activity upon UV-B exposure [118-121], which sustains the hypothesis of direct photolysis of enzymes by UVR [119].

UV-B radiation can also affect bacterial extracellular enzymatic activity indirectly by enhancing the release of dissolved organic carbon by algae [122] therefore compensating for the UV-B inhibition of bacterial activity at the cellular level [123]. However, other authors have reported reduction and alterations in the molecular composition of photosynthetic extracellular release upon irradiation of phytoplankton [124-127], resulting in phytoplankton exudates more refractory to bacterial utilization [128-130] which would induce a shift in the spectrum of active enzymes. Algal exudates can also act as photosensitizers, initiating secondary photochemical reactions that lead to bacterial enzyme inactivation [131]. Alternatively, UV radiation can alter the bioavailability of organic matter rendering it more susceptible to microbial degradation, effectively substituting extracellular enzymes in the processing of DOM [119, 121]. However, reports of decreased availability of organic matter for bacterial metabolism by photochemical transformation indicate that the overall effect depends on the quality of DOM [132-133].

UV exposure is likely to have multi-level effects, rather than being limited to affected individuals or single processes [121, 134]. However, the complex relationship between UV radiation, DOM nature and bacterial activity [130] limits the accurate prediction of future trends in bacterial extracellular enzymatic activity and mineralization rates in a context of increased UV radiation.

4.3. CO₂ and ocean acidification

Carbon dioxide reacts with seawater and is hydrated to carbonic acid (H₂CO₃), which subsequently dissociates to bicarbonate (HCO₃⁻), carbonate ions (CO₃²⁻) and protons (H⁺), in a process that is designated as the carbonate system.

The sum of all dissolved carbon forms composes the dissolved inorganic carbon (DIC) pool. A small fraction of DIC (<1%) remains in the form of dissolved CO₂, while the rest is converted into HCO₃⁻ (~90%) and CO₃²⁻ (~9%) with the consequent release of H⁺ and progressive decrease of pH [135].

CO₂ concentrations remain fairly constant in large water masses, like open oceans, but can vary considerably in coastal ecosystems depending on the rates of heterotrophic activity and photosynthesis and on the limitations to gas exchange with the atmosphere [136]. Ocean acidification can impact the physiological responses at the organism level, impose changes in community structure and shifts in biogeochemical cycling, but most studies have been mainly focused on phytoplankton assuming that bacterial responses in terms of extracellular enzymatic activity are expected to be exerted by phytoplankton-bacterioplankton interactions.

Little is known about the direct consequences of ocean acidification on bacterial extracellular enzymatic activity. Changing pH was shown to affect the functioning of permeases in cultures of isolated bacterial strains [137], suggesting that changing ocean pH may affect the coupling between polymer hydrolysis and monomer uptake. Mesocosm studies have shown that hydrolytic ectoenzymatic activity (β and α -glucosidases) was highest in the enhanced pCO₂ conditions [138]. Experimental ocean acidification experiments demonstrated a relative enhancement of extracellular glucosidase implying that, in more acidic conditions, polymer hydrolysis may be diverted towards polysaccharide degradation, making simple sugars more available for bacterial growth [139]. A possible consequence of increased glucose availability at lowered seawater pH would be the stimulation of bacterial competition for inorganic nutrients in order to keep a balanced growth and this could indirectly affect primary production in the ocean. By negatively affecting primary producers in acidified seawater, in relation to heterotrophs [140], a polysaccharide-base DOM hydrolysis could inhibit the vertical transport of particulate organic carbon to the deeper ocean layers, referred to as “biological carbon pump” [141], resulting in a positive feedback on CO₂ emissions. Elevated CO₂ was found to increase the activities of polysaccharide degrading enzymes in soils [142-143], suggesting that changes in the patterns of EEA of rhizosphere bacteria in aquatic ecosystems can also be expected.

4.4. Pollution and bioremediation

The accumulation of pollutants resulting from anthropogenic activities has become a major problem that made the necessity of new technologies for environmental decontamination more urgent. Bioremediation approaches involving microbial degradative capacities are regarded as effective and environmental friendly alternatives [144]. The principle of bioremediation techniques is the use of organisms, from bacteria to plants, or their derivatives, in the degradation of pollutants [145]. Microorganisms have an enormous catabolic potential and molecular tools have been used to characterize relevant groups or strains and their involvement in pollutant degradation processes [145-146]. Nonetheless, the application of this knowledge into effective microbial bioremediation protocols is still on a preliminary phase [147].

Because enzymes are simpler systems than the whole microorganism, in the past years enzymatic bioremediation has been seen as a possible alternative [148-149]. Some advantages in using enzymes instead of microorganisms or chemicals have been pointed out. The degradation of pollutants does not generate toxic or bio-hazardous products, the enzymes are themselves biodegradable by the indigenous microorganisms and the efficiency of the process can be improved by recombinant-DNA technology [148, 150].

The role of bacterial extracellular enzymes in the degradation of organic matter and their broad range of substrates makes them suitable candidates for remediation of pollutants from contaminated environments [151]. Bacterial hydrolases are a class of enzymes that are able to degrade several pollutants, including recalcitrant plastic polymers. For example, an extracellular esterase involved in the degradation of polyester polyurethanes was isolated from *Comamonas acidovorans* TB-35 [152].

Specific or extreme environments, such as the surface microlayer, are potential natural reservoirs of extracellular hydrolases with unusual properties, worth to explore for biotechnological and bioremediation applications. Higher rates of peptide and polysaccharide hydrolysis were found in the surface microlayer in relation to subsurface waters [153-154]. High rates of polymer degradation have also been found in the rhizospheres of salt marsh vegetation [51-52].

Although enzyme technology is appealing, there are some intrinsic limitations to their environmental application. Enzymes cannot reproduce themselves like microorganisms, so they cannot increase their populations and respond to an increase in the substrate amount. Also, they do not possess the same adaptability as microorganisms. Even though enzymes can persist and hydrolyse polymers in a wide range of environments, they are not able to adjust their kinetic parameters to function in environments different than their natural conditions [155]. Nonetheless, there is obvious potential in the application of bacterial extracellular enzymes in emerging bioremediation techniques.

5. Future perspectives

Since the oceanic uptake of atmospheric CO₂ is strongly affected by biological processes, marine research currently undertakes strong efforts to explore the feedback potential provided by marine biota and biogeochemical cycles to climate change. Extracellular hydrolysis and further decomposition and mineralization of organic carbon by heterotrophic bacteria influence the flux of atmospheric CO₂ to the ocean, but the combined effects of different drivers

of global change have yet hardly been assessed, mostly because rigorous temporal and spatial sampling schemes are required and the results of microcosm experiments are often difficult to extrapolate to the natural environment. Therefore, the scientific knowledge is still insufficient to predict consequences of global change on extracellular enzymatic activity, the marine environment and on the subsequent processes of carbon cycle.

A fairly reliable approach to study the responses of microorganisms to global environmental change would be the analysis of existing environments where such shifts naturally occur. For example, the effects of enhanced CO₂ and pH could be addressed by studying zones with values close to the upper and lower limits of the ranges of natural variation of these parameters, such as heterotrophic systems where respiration is much higher than primary production, or polar waters which have lower calcium carbonate saturation rates [156]. Freshwater lakes and estuarine waters are less buffered than the oceans and exhibit daily to seasonal changes in pH. Furthermore, coastal and estuarine environments also show high spatial variability in pH, over short time scales.

UV-exposed environments, such as high altitude Andean lakes [157] have been proposed as representative of wider variety of bacterial adaptative strategies. For example, it has been hypothesized that the importance of *Actinobacteria* in the microbial community of lakes of different UV-transparency in the Tyrolean Alps is related to their higher UV resistance [158]. Likewise, the bacteria from the surface microlayer are more exposed to solar radiation and have enhanced resistance to solar radiation [159].

Understanding how microbial communities will adjust to multiple climate change drivers is important to make accurate predictions of ecosystem response to changing climate scenarios. Multifactorial experiments of simulated climate conditions have addressed the combined effects of atmospheric CO₂, temperature and precipitation on soil microbial community composition [160]. Similar experimental designs could be extremely valuable for the characterization of the effects of multiple climate factors on bacterial extracellular enzymatic activities in the aquatic environment and ultimately on the patterns of organic matter recycling in a context of global environmental changes.

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References

- [1] Azam F, Fenchel T, Field JG, Gray JS, Meyer-Reil LA, Thingstad F. The Ecological Role of Water-Column Microbes in the Sea. *Marine Ecology Progress Series*. 1983;10:257-263.
- [2] Williams C, Jochem F. Ectoenzyme kinetics in Florida Bay: Implications for bacterial carbon source and nutrient status. *Hydrobiologia*. 2006;569:113-127.
- [3] Decad GM, Nikaido H. Outer membrane of gram-negative bacteria. XII. Molecular-sieving function of cell wall. *Journal of Bacteriology*. 1976;128:325-336.
- [4] Ghuysen J, Hackenbeck R. 1994. Bacterial cell wall. Elsevier Science Ltd.
- [5] Amon RMW, Benner R. Rapid cycling of high-molecular-weight dissolved organic matter in the ocean. *Nature*. 1994;369:549-552.
- [6] Amon RMW, Benner R. Bacterial Utilization of Different Size Classes of Dissolved Organic Matter. *Limnology and Oceanography*. 1996;41:41-51.
- [7] Chróst RJ. Environmental control of the synthesis and activity of aquatic microbial ectoenzymes, In: Chróst RJ eds. *Microbial enzymes in aquatic environments* New York: Springer-Verlag; 1991.
- [8] Weiss M, Abele U, Weckesser J, Welte W, Schiltz E, Schulz G. Molecular architecture and electrostatic properties of a bacterial porin. *Science*. 1991;254:1627-1630.
- [9] Arnosti C. Microbial extracellular enzymes and their role in dissolved organic matter cycling, In: Sinsabaugh RL eds. *Aquatic ecosystems: interactivity of dissolved organic matter* San Diego: Academic Press; 2003.315-342.
- [10] Hoppe HG. Microbial extracellular enzyme activity: a new key parameter in aquatic ecology, In: Chróst RJ eds. *Microbial enzymes in aquatic environments*. New York, Berlin, Heidelberg, Paris, Tokyo, Hong Kong, Barcelona: Springer Verlag; 1991.
- [11] Meyer-Reil LA. Ecological aspects of enzymatic activity in marine sediments, In: Chróst RJ eds. *Microbial enzymes in aquatic environments* New York, Berlin, Heidelberg, Paris, Tokyo, Hong Kong, Barcelona: Springer Verlag; 1991.
- [12] Bardgett RD, Freeman C, Ostle NJ. Microbial contributions to climate change through carbon cycle feedbacks. *ISME J*. 2008;2:805-814.
- [13] Sinsabaugh RS. Enzymic analysis of microbial pattern and process. *Biology and Fertility of Soils*. 1994;17:69-74.
- [14] Skujins J. History of abiotic soil enzyme research, In: Burns RG eds. *Soil Enzymes* New York: Academic Press; 1978.
- [15] Karp-Boss L, Boss E, Jumars PA. Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. *Oceanography and Marine Biology: an Annual Review*. 1996;34:71-107.
- [16] Chróst RJ, Siuda W. Ecology of Microbial Enzymes in Lake Ecosystems, In: Burns RC and Dick RP eds. *Microbial Enzymes in the Environment Activity, Ecology, and Applications* New York: Marcel Dekker, Inc; 2002.35-72.
- [17] Baltar F, Aristegui J, Gasol J, Sintes E, Van Aken H, Herndl G. High dissolved extracellular enzymatic activity in the deep central Atlantic Ocean. *Aquatic Microbial Ecology*. 2010;58:287-302.
- [18] Hoppe H. Use of fluorogenic model substrates for extracellular enzyme activity (EEA) measurement of bacteria. *Handbook of methods in aquatic microbial ecology*. 1993:423-431.
- [19] Chróst RJ. Significance of bacterial ectoenzymes in aquatic environments. *Hydrobiologia*. 1992;243-244:61-70.
- [20] Barman TE. Enzyme handbook, Berlin, New York: Springer-Verlag; 1969.928.

- [21] Vidal M, Duarte CM, Agusti S, M. GJ, Vaque D. Alkaline phosphatase activities in the central Atlantic Ocean indicate large areas with phosphorus deficiency. *Marine Ecology Progress Series*. 2003;262:43-53.
- [22] Feder J. The phosphatases, In: Griffith EJ, Benton A, Spencer JM, and Mitchell DT eds. *Environmental phosphorus handbook* New York: J. Wiley & Sons; 1973.475-508.
- [23] Hoppe HG. Phosphatase activity in the sea. *Hydrobiologia*. 2003;493:187-200.
- [24] Tringe S, Von Mering C, Kobayashi A, Salamov A, Chen K, Chang H, Podar M, Short J, Mathur E, Detter J. Comparative metagenomics of microbial communities. *Science*. 2005;308:554.
- [25] Kirchman DL, Dittel AI, Findlay SEG, Fischer D. Changes in bacterial activity and community structure in response to dissolved organic matter in the Hudson River, New York. *Aquatic Microbial Ecology*. 2004;35:243-257.
- [26] LeCleir GR, Buchan A, Hollibaugh JT. Chitinase gene sequences retrieved from diverse aquatic habitats reveal environment-specific distributions. *Applied and Environmental Microbiology*. 2004;70:6977-83.
- [27] Blackwood CB, Waldrop MP, Zak DR, Sinsabaugh RL. Molecular analysis of fungal communities and laccase genes in decomposing litter reveals differences among forest types but no impact of nitrogen deposition. *Environmental Microbiology*. 2007;9:1306-1316.
- [28] Kunst F, Rapoport G. Salt stress is an environmental signal affecting degradative enzyme synthesis in *Bacillus subtilis*. *Journal of Bacteriology*. 1995;177:2403.
- [29] Luis P, Kellner H, Martin F, Buscot F. A molecular method to evaluate basidiomycete laccase gene expression in forest soils. *Geoderma*. 2005;128:18-27.
- [30] Hoppe H, Arnosti C, Herndl G. Ecological significance of bacterial enzymes in the marine environment. *Enzymes in the Environment: Activity, Ecology, and Applications*. 2002:73-107.
- [31] Hoppe H, Ullrich S. Profiles of ectoenzymes in the Indian Ocean: phenomena of phosphatase activity in the mesopelagic zone. *Aquatic Microbial Ecology*. 1999;19:139-148.
- [32] Sala M, Arrieta J, Boras J, Duarte C, Vaqué D. The impact of ice melting on bacterioplankton in the Arctic Ocean. *Polar Biology*. in press:1-12.
- [33] Cunha MA, Almeida MA, Alcântara F. Patterns of ectoenzymatic and heterotrophic bacterial activities along a salinity gradient in a shallow tidal estuary. *Marine Ecology Progress Series*. 2000;204:1-12.
- [34] Santos AL, Mendes C, Gomes NCM, Henriques IS, Correia A, Almeida A, Cunha A. Short-term variability of abundance, diversity and activity of estuarine bacterioneuston and bacterioplankton. *Journal of Plankton Research*. 2009;31:1545-1555.
- [35] Ziervogel K, Steen A, Arnosti C. Changes in the spectrum and rates of extracellular enzyme activities in seawater following aggregate formation. *Spectrum*. 2009;6:11293-11316.
- [36] Grossart H, Hietanen S, Ploug H. Microbial dynamics on diatom aggregates in resund, Denmark. *Marine Ecology Progress Series*. 2003;249:69-78.
- [37] Cunha A, Almeida A. Inorganic nutrient regulation of bacterioplankton heterotrophic activity in an estuarine system (Ria de Aveiro, Portugal). *Hydrobiologia*. 2009;628:81-93.
- [38] Cunha M, Almeida M, Alcântara F. Short-term responses of the natural planktonic bacterial community to the changing water properties in an estuarine environment: Ectoenzymatic activity, glucose incorporation, and biomass production. *Microbial Ecology*. 2001;42:69-79.
- [39] Chróst RJ. Microbial ectoenzymes in aquatic environments, In: Overbeck J and Chrost RJ eds. *Aquatic microbial Ecology: biochemical and molecular approaches* New York: Springer-Verlag; 1990.47-79.
- [40] Boon PI. Organic matter degradation and nutrient regeneration in Australian freshwaters. I: Spatial and temporal variation, and relation with environmental conditions. *Archiv für Hydrobiologie*. 1990;117:405-436.
- [41] Karrasch B, Bormki G, Herzprung P, Winkler M, Baborowski M. Extracellular enzyme activity in the river Elbe during a spring flood event. *Acta hydrochimica et hydrobiologica*. 2003;31:307-318.
- [42] Smart K, Jackson C. Fine Scale Patterns in Microbial Extracellular Enzyme Activity during Leaf Litter Decomposition in a Stream and its Floodplain. *Microbial Ecology*. 2009;58:591-598.
- [43] Cunha MA, Almeida MA, Alcántara F. Ectoenzymatic activity and glucose heterotrophic metabolism in a shallow estuary (Ria de Aveiro, Portugal): influence of bed sediments and salt marshes. *Acta Oecologica-International Journal of Ecology*. 2003;24:S97-S107.
- [44] Arnosti C, Ziervogel K, Ocampo L, Ghobrial S. Enzyme activities in the water column and in shallow permeable sediments from the northeastern Gulf of Mexico. *Estuarine, Coastal and Shelf Science*. 2009;84:202-208.
- [45] Meyer-Reil LA. Seasonal and spatial distribution of extracellular enzymatic activities and microbial incorporation of dissolved organic substrates in marine sediments. *Applied and Environmental Microbiology*. 1987;53:1748-55.
- [46] Fabiano M, Danovaro R. Enzymatic Activity, Bacterial Distribution, and Organic Matter Composition in Sediments of the Ross Sea (Antarctica). *Applied and Environmental Microbiology*. 1998;64:3838-3845.
- [47] Santos L, Almeida A, Coelho F, Cunha A, Lima E, Loureiro N, Matos P, Salvador S, Pinheiro L. Distribution, activity, and diversity of microbial communities from sediments of the mud volcano Mercator (Gulf of Cadiz). *Geological processes on deep-water European margins*.
- [48] Boetius A, Lochte K. Regulation of microbial enzymatic degradation of organic matter in deep-sea sediments. *Marine Ecology Progress Series*. 1994;104:299-299.
- [49] Bagwell C, Piceno Y, Ashburne-Lucas A, Lovell C. Physiological diversity of the rhizosphere diazotroph assemblages of selected salt marsh grasses. *Applied and Environmental Microbiology*. 1998;64:4276.
- [50] Berg G, Smalla K. Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*. 2009;68:1-13.
- [51] Reboreda R, Caçador I. Enzymatic activity in the rhizosphere of *Spartina maritima*: Potential contribution for phytoremediation of metals. *Marine Environmental Research*. 2008;65:77-84.
- [52] Oliveira V, Santos AL, Coelho F, Gomes NCM, Silva H, Almeida A, Cunha A. Effects of Monospecific Banks of Salt Marsh Vegetation on Sediment Bacterial Communities. *Microbial Ecology*. in press.

- [53] Unanue M, Ayo B, Agis M, Slezak D, Herndl G, Iriberry J. Ecto enzymatic activity and uptake of monomers in marine bacterioplankton described by a biphasic kinetic model. *Microbial Ecology*. 1999;37:36-48.
- [54] Busto MD, Perez-Mateos M. Extraction of humic- β -glucosidase fractions from soil. *Biology and Fertility of Soils*. 1995;20:77-82.
- [55] Rao M, Sannino F, Nocerino G, Puglisi E, Gianfreda L. Effect of air-drying treatment on enzymatic activities of soils affected by anthropogenic activities. *Biology and Fertility of Soils*. 2003;38:327-332.
- [56] A.L. Koch. The macroeconomics of bacterial growth. In: M. Fletcher and G.D. Floodgate, eds, *Bacteria in their Natural Environments*, Academic Press, London 1985:1 - 42.
- [57] Harder W, Dijkhuizen L. Physiological Responses to Nutrient Limitation. *Annual Review of Microbiology*. 1983;37:1-23.
- [58] Siuda W. Phosphatases and their role in organic phosphorus transformation in natural waters. A review. *Polskie Archiwum Hydrobiologii* 1984;31 207-233.
- [59] Hoppe HG, Kim SJ, Gocke K. Microbial Decomposition in Aquatic Environments: Combined Process of Extracellular Enzyme Activity and Substrate Uptake. *Applied and Environmental Microbiology*. 1988;54:784-790.
- [60] Francoeur SN, Wetzel RG. Regulation of periphytic leucine-aminopeptidase activity. *Aquatic Microbial Ecology*. 2003;31:249-258.
- [61] Koroljova-Skorobogat'ko OV, Stepanova EV, Gavrilova VP, Morozova OV, Lubimova NV, Dzchafarova AN, Jaropolov AI, Makower A. Purification and characterization of the constitutive form of laccase from the basidiomycete *Coriolus hirsutus* and effect of inducers on laccase synthesis. *Biotechnology and Applied Biochemistry*. 1998;28 (Pt 1):47-54.
- [62] Klonowska A, Gaudin C, Fournel A, Asso M, Le Petit J, Giorgi M, Tron T. Characterization of a low redox potential laccase from the basidiomycete C30. *European Journal of Biochemistry*. 2002;269:6119-25.
- [63] Francoeur SN, Wetzel RG, Neely RK. New spatially explicit method for detecting extracellular protease activity in biofilms. *Applied and Environmental Microbiology*. 2001;67:4329-34.
- [64] Sala MM, Karner M, Arin L, Marrasé C. Measurement of ectoenzyme activities as an indication of inorganic nutrient imbalance in microbial communities. *Aquatic Microbial Ecology*. 2001;23:301-311.
- [65] Nausch M, Nausch G. Stimulation of peptidase activity in nutrient gradients in the Baltic Sea. *Soil Biology and Biochemistry*. 2000;32:1973-1983.
- [66] Insam H, Parkinson D, Domsch KH. Influence of macroclimate on soil microbial biomass. *Soil Biology and Biochemistry*. 1989;21:211-221.
- [67] Insam H. Are the soil microbial biomass and basal respiration governed by the climatic regime? *Soil Biology and Biochemistry*. 1990;22:525-532.
- [68] Sinsabaugh RL, Antibus RK, Linkins AE. An enzymatic approach to the analysis of microbial activity during plant litter lytic enzyme activities using proteins and dipeptide derivatives as substrates. *Agriculture, Ecosystems & Environment*. 1991;34:43-54.
- [69] Zweifel UL. Factors Controlling Accumulation of Labile Dissolved Organic Carbon in the Gulf of Riga. *Estuarine, Coastal and Shelf Science*. 1999;48:357-370.
- [70] Pomeroy LR, Wiebe WJ. Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. *Aquatic Microbial Ecology*. 2001;23:187-204.
- [71] King GM. Characterization of β -Glucosidase Activity in Intertidal Marine Sediments. *Applied and Environmental Microbiology*. 1986;51:373-380.
- [72] Helmke E, Weyland H. Effect of temperature on extracellular enzymes occurring in permanently cold marine environments. *Kieler Meeresforschung, Sonderheft*. 1991;8:198-204.
- [73] Christian JR, Karl DM. Bacterial ectoenzymes in marine waters: activity ratios and temperature responses in three oceanographic provinces. *Limnology and Oceanography*. 1995;40:1042-1049.
- [74] Arnosti C, Jørgensen BB, Sagemann J, Thamdrup B. Temperature dependence of microbial degradation of organic matter in marine sediments: polysaccharide hydrolysis, oxygen consumption, and sulfate reduction. *Marine Ecology Progress Series*. 1998;165:59-70.
- [75] Wiebe WJ, Sheldon WM, Pomeroy LR. Evidence for an enhanced substrate requirement by marine mesophilic bacterial isolates at minimal growth temperatures. *Microbial Ecology*. 1993;25:151-159.
- [76] Nedwell DB, Rutter M. Influence of temperature on growth rate and competition between two psychrotolerant Antarctic bacteria: low temperature diminishes affinity for substrate uptake. *Applied and Environmental Microbiology*. 1994;60:1984-92.
- [77] Fenner N, Freeman C, Reynolds B. Observations of a seasonally shifting thermal optimum in peatland carbon-cycling processes; implications for the global carbon cycle and soil enzyme methodologies. *Soil Biology and Biochemistry*. 2005;37:1814-1821.
- [78] Koch O, Tschirko D, Kandeler E. Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. *Global Biogeochemical Cycles*. 2007;21:GB4017.
- [79] Trasar-Cepeda C, Gil-Sotres F, Leirós MC. Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. *Soil Biology and Biochemistry*. 2007;39:311-319.
- [80] Loveland J, Gutshall K, Kasmir J, Prema P, Brenchley JE. Characterization of psychrotrophic microorganisms producing beta-galactosidase activities. *Applied and Environmental Microbiology*. 1994;60:12-8.
- [81] Huston AL, Krieger-Brockett BB, Deming JW. Remarkably low temperature optima for extracellular enzyme activity from Arctic bacteria and sea ice. *Environmental Microbiology*. 2000;2:383-388.
- [82] Coker JA, Sheridan PP, Loveland-Curtze J, Gutshall KR, Auman AJ, Brenchley JE. Biochemical Characterization of a β -Galactosidase with a Low Temperature Optimum Obtained from an Antarctic Arthrobacter Isolate. *Journal of Bacteriology*. 2003;185:5473-5482.
- [83] Feller G. Molecular adaptations to cold in psychrophilic enzymes. *Cellular and Molecular Life Sciences*. 2003;60:648-662.
- [84] Murrell MC, Hollibaugh JT, Silver MW, Wong PS. Bacterioplankton dynamics in northern San Francisco Bay: role of particle association and seasonal freshwater flow. *Limnology and oceanography*. 1999;44:295-308.

- [85] Hoppe HG, Gocke K, Alcântara F. Shifts between autotrophic and heterotrophic processes in a tidal lagoon (Ria de Aveiro, Portugal). *Arch. Hydrobiol. Spec. Issues Adv. Limnol.* 1996;48:39-52.
- [86] Patel AB, Fukami K, Nishijima T. Regulation of seasonal variability of aminopeptidase activities in surface and bottom waters of Uranouchi Inlet, Japan. *Aquatic Microbial Ecology.* 2000;21:139-149.
- [87] Hobbie JE, Daley RJ, Jasper S. *Applied and Environmental Microbiology.* 1977;33:1225-1228.
- [88] Taylor GT, Way J, Yu Y, Scranton MI. Ectohydrolase activity in surface waters of the Hudson River and western Long Island Sound estuaries. *Marine Ecology Progress Series.* 2003;263:1-15.
- [89] Rejmánková E, Sirová D. Wetland macrophyte decomposition under different nutrient conditions: Relationships between decomposition rate, enzyme activities and microbial biomass. *Soil Biology and Biochemistry.* 2007;39:526-538.
- [90] Tipton KF, Dixon HBF. Effects of pH on enzymes, In: Daniel LP eds. *Methods in Enzymology* vol. Volume 63. Academic Press; 1979.183-234.
- [91] Münster U. Extracellular enzyme activity in eutrophic and polyhumic lakes. In: Chróst, R.J., Editor, 1991. *Microbial Enzymes in Aquatic Environments*, Springer, New York, pp. 96–122. 1991.
- [92] Halemejkó GZ, Chróst RJ. Enzymatic hydrolysis of proteinaceous particulate and dissolved material in a eutrophic lake. *Archiv für Hydrobiologie Beiheft Ergebnisse der Limnologie.* 1986;107:1-21.
- [93] Mallet C, Debroas D. Relations between organic matter and bacterial proteolytic activity in sediment surface layers of a eutrophic lake (Lake Aydat, Puy de Dôme, France). *Archiv für Hydrobiologie.* 1999;145:39-56.
- [94] Joergensen BB, Revsbech NP, Cohen Y. Photosynthesis and structure of benthic microbial mats: microelectrode and SEM studies of four cyanobacterial communities. *Limnology and Oceanography.* 1983; 28: 1075-1093.
- [95] Revsbech NP, Joergensen BB, Blackburn TH. Microelectrode studies of the photosynthesis and O₂, H₂S, and pH profiles of a microbial mat. *Limnology and Oceanography.* 1983; 28: 1062-1074.
- [96] Carlton RG, Wetzel RG. Phosphorus flux from lake sediments: Effect of epipellic algal oxygen production. *Limnology and Oceanography.* 1988;33:562-570.
- [97] Woodruff SL, House WA, Callow ME, Leadbeater BSC. The effects of biofilms on chemical processes in surficial sediments. *Freshwater Biology.* 1999;41:73-89.
- [98] Iwan Jones J, Eaton JW, Hardwick K. The influence of periphyton on boundary layer conditions: a pH microelectrode investigation. *Aquatic Botany.* 2000;67:191-206.
- [99] Espeland EM, Wetzel RG. Effects of Photosynthesis on Bacterial Phosphatase Production in Biofilms. *Microbial Ecology.* 2001;42:328-337.
- [100] Reuter JG. Alkaline phosphatase inhibition by copper: Implications to phosphorus nutrition and use as a biochemical marker of toxicity. *Limnology and Oceanography.* 1983;28:743-748.
- [101] Garde K, Gustavson K. The impact of UV-B radiation on alkaline phosphatase activity in phosphorus-depleted marine ecosystems. *Journal of Experimental Marine Biology and Ecology.* 1999;238:93-105.
- [102] Müller-Niklas G, Heissenberger A, Puskaric S, Herndl G. Ultraviolet-B radiation and bacterial metabolism in coastal waters. *Aquatic Microbial Ecology.* 1995;9:111-116.
- [103] Houghton JT, Jenkins GJ, Ephraums JJ. Climate change. The IPCC scientific assessment, Cambridge: Cambridge University Press; 1990.
- [104] Easterling D, Meehl G, Parmesan C, Changnon S, Karl T, Mearns L. Climate extremes: observations, modeling, and impacts. *Science.* 2000;289:2068.
- [105] Wolters V, Silver WL, Bignell DE, Coleman DC, Lavelle P, Van Der Putten WH, De Ruiter P, Rusek J, Wall DH, Wardle DA, Brussard L, Dangerfield JM, Brown VK, Giller KE, Hooper DU, Sala O, Tiedje J, Van Veen JA. Effects of Global Changes on Above- and Belowground Biodiversity in Terrestrial Ecosystems: Implications for Ecosystem Functioning. *BioScience.* 2008;50:1089-1098.
- [106] Prosser JI, Bohannan BJM, Curtis TP, Ellis RJ, Firestone MK, Freckleton RP, Green JL, Green LE, Killham K, Lennon JJ, Osborn AM, Solan M, van der Gast CJ, Young JPW. The role of ecological theory in microbial ecology. *Nature Review Microbiology* 2007;5:384-392.
- [107] Price PB, Sowers T. Temperature dependence of metabolic rates for microbial growth, maintenance, and survival. *Proceedings of the National Academy of Sciences USA* 2004;101:4631-6.
- [108] Lomas MW, Glibert PM, Shiah F-K, Smith EM. Microbial processes and temperature in Chesapeake Bay: current relationships and potential impacts of regional warming. *Global Change Biology.* 2002;8:51-70.
- [109] Neori A, Holm-Hansen O. Effect of temperature on rate of photosynthesis in Antarctic phytoplankton. *Polar Biology.* 1982;1:33-38.
- [110] Jacques G. Some ecophysiological aspects of the Antarctic phytoplankton. *Polar Biology.* 1983;2:27-33.
- [111] Reay DS, Priddle J, Nedwell DB, Whitehouse MJ, Ellis-Evans JC, Deubert C, Connelly DP. Regulation by low temperature of phytoplankton growth and nutrient uptake in the Southern Ocean. *Marine Ecology Progress Series.* 2001;219:51-64.
- [112] Amon RMW, Fitznar H, Benner R. Linkages among the bioreactivity, chemical composition, and diagenetic state of marine dissolved organic matter. *Limnology and oceanography.* 2001;46:287-297.
- [113] Karl DM, Björkman KM, Dore JE, Fujieki L, Hebel DV, Houlihan T, Letelier RM, Tupas LM. Ecological nitrogen-to-phosphorus stoichiometry at station ALOHA. *Deep Sea Research Part II: Topical Studies in Oceanography.* 2001;48:1529-1566.
- [114] Wohlers J, Engel A, Zöllner E, Breithaupt P, Jürgens K, Hoppe H-G, Sommer U, Riebesell U. Changes in biogenic carbon flow in response to sea surface warming. *Proceedings of the National Academy of Sciences USA.* 2009;106:7067-7072.
- [115] Jones S, Chiu C, Kratz T, Wu J, Shade A, McMahon K. Typhoons initiate predictable change in aquatic bacterial communities. *Limnology and Oceanography.* 2008;53:1319-1326.
- [116] Reche I, Ortega-Retuerta E, Romera O, Pulido-Villena E, Morales-Baquero R, Casamayor E. Effect of Saharan dust inputs on bacterial activity and community composition in Mediterranean lakes and reservoirs. *Limnology and Oceanography.* 2009;54:869-879.

- [117] Griffin D. Atmospheric movement of microorganisms in clouds of desert dust and implications for human health. *Clinical Microbiology Reviews*. 2007;20:459.
- [118] Wetzel RG, P.G. H, TS.. B. Natural photolysis by ultraviolet irradiance of the recalcitrant dissolved organic matter to simple substrates for rapid bacterial metabolism. *Limnology and Oceanography*. 1995;40:1369-1380.
- [119] Lindell M, Edling H. Influence of light on bacterioplankton in a tropical lake. *Hydrobiologia*. 1996;323:67-73.
- [120] Espeland EM, Wetzel RG. Complexation, Stabilization, and UV Photolysis of Extracellular and Surface-Bound Glucosidase and Alkaline Phosphatase: Implications for Biofilm Microbiota. *Microbial Ecology*. 2001;42:572-585.
- [121] Herndl GJ, Muller-Niklas G, Frick J. Major role of ultraviolet-B in controlling bacterioplankton growth in the surface layer of the ocean. *Nature*. 1993;361:717-719.
- [122] Ridal JJ, Moore RM. Resistance to UV and persulphate oxidation of dissolved organic carbon produced by selected marine phytoplankton. *Marine Chemistry*. 1993;42:167-188.
- [123] Chatila K, Demers S, Mostajir B, Gosselin M, Chanut JP, Monfort P, Bird D. The Responses of a Natural Bacterioplankton Community to Different Levels of Ultraviolet-B Radiation: A Food Web Perspective. *Microbial Ecology*. 2001;41:56-68.
- [124] Goes JI, Handa N, Taguchi S, Hama T, Saito H. Metabolism of neutral monosaccharide constituents of storage and structural carbohydrates in natural assemblages of marine phytoplankton exposed to ultraviolet radiation. *Limnology and Oceanography*. 1996;41:1478-1489.
- [125] Kirchman DL, Suzuki Y, Garside C, Ducklow HW. High turnover rates of dissolved organic carbon during a spring phytoplankton bloom. *Nature*. 1991;352:612-614.
- [126] Lignell R, Heiskanen AS, Kuosa H, Gundersen K, Kuuppo-Leinikki P, Pajuniemi R, Uitto A. Fate of a phytoplankton spring bloom, sedimentation and carbon flow in the plankton food web in the northern Baltic. *Marine Ecology Progress Series*. 1993;239-252.
- [127] Norman B, Zeveifel UL, C.S. Hopkinson J, Fry B. Production and utilization of dissolved organic carbon during an experimental diatom bloom. *Limnology and Oceanography* 1995;40:898-907.
- [128] Thomas DN, Lara RJ. Photodegradation of algal dissolved organic carbon. *Marine Ecology Progress Series*. 1995;116:309-310.
- [129] Naganuma T, Konishi S, Inoue T, Nakane T, Sukizaki S. Photodegradation or photoalteration? Microbial assay of the effect of UV-B on dissolved organic matter. *Marine Ecology Progress Series*. 1996;135:309-310.
- [130] Pausz C, Herndl GJ. Role of ultraviolet radiation on phytoplankton extracellular release and its subsequent utilization by marine bacterioplankton. *Aquatic Microbial Ecology*. 1999;18:85-93.
- [131] Scully N, Tranvik LJ, Cooper WJ. Photochemical effects on the interaction of enzymes and dissolved organic matter in natural waters. *Limnology and Oceanography*. 2003;48:1818-1824.
- [132] Benner R, Biddanda B. Photochemical transformations of surface and deep marine dissolved organic matter: effects on bacterial growth. *Limnology and Oceanography*. 1998;43:1371-1378.
- [133] Tranvik LJ, Bertilsson S. Contrasting effects of solar UV radiation on dissolved organic sources for bacterial growth. *Ecology Letters*. 2001;4:458-463.
- [134] Lyons MM, Aas P, Pakulski JD, Van Waasbergen L, Miller RV, Mitchell DL, Jeffrey WH. DNA damage induced by ultraviolet radiation in coral-reef microbial communities. *Marine Biology*. 1998;130:537-543.
- [135] Riebesell U. Effects of CO₂ Enrichment on Marine Phytoplankton. *Journal of Oceanography*. 2004;60:719-729.
- [136] Guinotte J, Fabry V. Ocean acidification and its potential effects on marine ecosystems. *Annals of the New York Academy of Sciences*. 2008;1134:320-342.
- [137] Page MG, Rosenbusch JP, Yamato I. The effects of pH on proton sugar symport activity of the lactose permease purified from *Escherichia coli*. *Journal of Biological Chemistry*. 1988;263:15897-905.
- [138] Grossart HP, Allgaier M, Passow U, Riebesell U. Testing the effect of CO₂ concentration on the dynamics of marine heterotrophic bacterioplankton. *Limnology and Oceanography*. 2006;51:1-11.
- [139] Piontek J, Lunau M, Händel N, Borchard C, Wurst M, Engel A. Acidification increases microbial polysaccharide degradation in the ocean. *Biogeosciences Discuss*. 2009;6:11377-11400.
- [140] Karl DM, Laws EA, Morris P, Williams PJ, Emerson S. Global carbon cycle (communication arising): Metabolic balance of the open sea. *Nature*. 2003;426:32-32.
- [141] Volk T, Hoffert MI. Ocean carbon pumps: Analysis of relative strengths and efficiencies in ocean-driven atmospheric CO₂ changes. In: Sundquist E and Broecker S eds. *The carbon cycle and atmospheric CO₂: natural variations Archean to Present*, : Geophysical Monograph 32, American Geophysical Union; 1985.
- [142] Dhillon SS, Roy J, Abrams M. Assessing the impact of elevated CO₂ on soil microbial activity in a Mediterranean model ecosystem. *Plant and Soil*. 1995;187:333-342.
- [143] Mayr C, Miller M, Insam H. Elevated CO₂ alters community-level physiological profiles and enzyme activities in alpine grassland. *Journal of Microbiological Methods*. 1999;36:35-43.
- [144] Singh S, Kang SH, Mulchandani A, Chen W. Bioremediation: environmental clean-up through pathway engineering. *Current Opinion in Biotechnology*. 2008;19:437-444.
- [145] Wood TK. Molecular approaches in bioremediation. *Current Opinion in Biotechnology*. 2008;19:572-8.
- [146] Watanabe K. Microorganisms relevant to bioremediation. *Current Opinion in Biotechnology*. 2001;12:237-241.
- [147] Chauhan A, Fazlurrahman, Oakeshott J, Jain R. Bacterial metabolism of polycyclic aromatic hydrocarbons: strategies for bioremediation. *Indian Journal of Microbiology*. 2008;48:95-113.
- [148] Alcalde M, Ferrer M, Plou FJ, Ballesteros A. Environmental biocatalysis: from remediation with enzymes to novel green processes. *Trends in Biotechnology*. 2006;24:281-287.
- [149] Sutherland TD, Horne I, Weir KM, Coppin CW, Williams MR, Selleck M, Russell RJ, Oakeshott JG. Enzymatic bioremediation: From enzyme discovery to applications. *Clinical and Experimental Pharmacology and Physiology*. 2004;31:817-821.

- [150] Ahuja SK, Ferreira GM, Moreira AR. Utilization of Enzymes for Environmental Applications. *Critical Reviews in Biotechnology*. 2004;24:125-154.
- [151] Gianfreda L, Rao MA. Potential of extra cellular enzymes in remediation of polluted soils: a review. *Enzyme and Microbial Technology*. 2004;35:339-354.
- [152] Nakajima-Kambe T, Shigeno-Akutsu Y, Nomura N, Onuma F, Nakahara T. Microbial degradation of polyurethane, polyester polyurethanes and polyether polyurethanes. *Applied Microbiology and Biotechnology*. 1999;51:134-40.
- [153] Kuznetsova M, Lee C. Enhanced extracellular enzymatic peptide hydrolysis in the sea-surface microlayer. *Mar Chem*. 2001;73:319-322.
- [154] Coelho F. Bacterioneuston da Ria de Aveiro: abundância, actividade e degradação de hidrocarbonetos aromáticos policíclicos. MSc. Dissertation. University of Aveiro. 2008 Aveiro .
- [155] Ruggaber TP, Talley JW. Enhancing Bioremediation with Enzymatic Processes: A Review. *Practice Periodical of Hazardous, Toxic, and Radioactive Waste Management*. 2006;10:73-85.
- [156] Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RA, Gnanadesikan A, Gruber N, Ishida A, Joos F, Key RM, Lindsay K, Maier-Reimer E, Matear R, Monfray P, Mouchet A, Najjar RG, Plattner G-K, Rodgers KB, Sabine CL, Sarmiento JL, Schlitzer R, Slater RD, Totterdell IJ, Weirig M-F, Yamanaka Y, Yool A. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*. 2005;437:681-686.
- [157] Fernandez Zenoff V, Sineriz F, Farias ME. Diverse responses to UV-B radiation and repair mechanisms of bacteria isolated from high-altitude aquatic environments. *Applied and Environmental Microbiology*. 2006;72:7857-63.
- [158] Warnecke F, Sommaruga R, Sekar R, Hofer JS, Pernthaler J. Abundances, identity, and growth state of actinobacteria in mountain lakes of different UV transparency. *Applied and Environmental Microbiology*. 2005;71:5551-9.
- [159] Agogue H, Joux F, Obernosterer I, Lebaron P. Resistance of marine bacterioneuston to solar radiation. *Applied and Environmental Microbiology*. 2005;71:5282.
- [160] Castro H, Classen A, Austin E, Norby R, Schadt C. Soil microbial community responses to multiple experimental climate change drivers. *Applied and Environmental Microbiology*. 2010;76:999.