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Acidification increases microbial polysaccharide degradation in the ocean

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Abstract. With the accumulation of anthropogenic carbon dioxide (CO_2), a proceeding decline in seawater pH has been induced that is referred to as ocean acidification. The ocean's capacity for CO_2 storage is strongly affected by biological processes, whose feedback potential is difficult to evaluate. The main source of CO_2 in the ocean is the decomposition and subsequent respiration of organic molecules by heterotrophic bacteria. However, very little is known about potential effects of ocean acidification on bacterial degradation activity. This study reveals that the degradation of polysaccharides, a major component of marine organic matter, by bacterial extracellular enzymes was significantly accelerated during experimental simulation of ocean acidification. Results were obtained from pH perturbation experiments, where rates of extracellular α- and β-glucosidase were measured and the loss of neutral and acidic sugars from phytoplankton-derived polysaccharides was determined. Our study suggests that a faster bacterial turnover of polysaccharides at lowered ocean pH has the potential to reduce carbon export and to enhance the respiratory CO_2 production in the future ocean.

1 Introduction

Organic matter in the ocean is one of the largest dynamic carbon reservoirs on Earth that interacts with atmospheric CO_2 concentrations on time scales of 1000 to 10 000 years (Hedges, 1992). Biological consumption of CO_2 during photosynthesis and the related production of organic matter are counteracted by CO_2-regenerating processes with bacterial respiration being the predominant one (Rivkin and Legendre, 2001). About 75–95% of organic matter produced by autotrophic organisms gets remineralized by heterotrophic bacterioplankton in the surface ocean (Martin et al., 1987; Boyd et al., 1999), the zone that is most strongly affected by ocean acidification (Raven et al., 2005). Equilibration of seawater with rising CO_2 in the atmosphere has already lowered the ocean pH by 0.12 units compared to pre-industrial values, which in turn has increased the concentration of hydrogen ions by 30% (Houghton et al., 2001; Sabine et al., 2004; Raven et al., 2005). Effects of ocean acidification on bacterial metabolism and activity are currently largely unexplored but of utmost importance for accurate estimates of organic matter cycling and the carbon balance in the future ocean.

Polysaccharides are a major component of marine organic matter and comprise up to 15% of sinking and suspended particulate organic carbon (Tanoue and Handa, 1987; Bhosle et al., 1992; Hernes et al., 1996) and up to 32% of dissolved organic carbon (DOC) (Pakulski and Benner, 1994). They can account for more than 50% of total phytoplankton primary production (Baines and Pace, 1991) and provide a labile energy and carbon source to heterotrophic bacterioplankton in form of structural cell components, storage glucan, and phytoplankton exudates. The bacterial degradation of high-molecular-weight organic compounds like polysaccharides is initiated by the activity of extracellular enzymes (Hoppe et al., 1988; Chróst, 1991). Thereby, macromolecules are enzymatically hydrolyzed outside of bacterial cells into units of low molecular weight that are small enough to be transported across the cytoplasmic membrane. Extracellular α- and β-glucosidase released by bacterioplankton cleave

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Table 1. Setup of culture experiments (CultExp I, CultExp II) and field assays (FieldAssay I, FieldAssay II).

Experiment	phytoplankton	bacterioplankton	CO_2 manipulation	pH PD (ΔH^+, nmol l^{-1})	pH FO (ΔH^+, nmol l^{-1})	Samplings	Incubation Mode	Incubation Period	T [°C]	Initial Bacteria[1] [$\times 10^6$ cells ml^{-1}]	Initial POC[1] [µM]
CultExp I	*E. huxleyi* (PML B92/11)	natural community North Sea	aeration	8.3	8.1 (Δ 2.93)	5	batch	30 days	14	21.4±5.3	522±168
CultExp II	*E. huxleyi* (PML B92/11)	natural community North Sea	aeration	7.9	7.7 (Δ 7.36)	8	batch	13 days	15	4.9±1.8	840±86
FieldAssay I	natural community Gulf of Biscay, 2007		dilute hydrochloric acid	8.2	7.9 (Δ 6.27)	5	batch	12 days	10	0.08[2]	14[2]
FieldAssay II	natural community Gulf of Biscay, 2006		aeration	7.9	7.6 (Δ 12.53)	1	chemostat	8 days	16	6.2±2.5	48±12

PD: present-day treatment; FO: future-ocean treatment; POC: particulate organic carbon
[1] Initial bacterial cell numbers and concentrations of POC are given as mean values ± standard deviation.
[2] For FieldAssay I, one field sample was initially subdivided into acidified and non-acidified replicates.

α- and β-glycosidic bonds in polysaccharides, respectively, and generate glucose monomers that can be assimilated by bacterioplankton and fuel its heterotrophic metabolism (Chróst, 1991).

It is well-known that the pH is an important factor regulating the velocity of enzymatic reactions (Arrhenius, 1889; Tipton and Dixon, 1979). Changing concentrations of hydrogen ions in the enzyme's environment alter the ionization state of amino acids, and thus affect the three-dimensional protein structure of the active site. Enzymatic reactions exhibit a specific narrow range of pH, where highest reaction velocity is apparent, but already small deviations from this pH optimum result in decreased enzymatic rates. In contrast to intracellular enzymes, acting in the cell's buffered cytoplasm, extracellular enzymes directly experience the pH of the outer environment. Also the activity of extracellular enzymes in aquatic environments was shown to respond sensitive to changing pH. Rates of bacterial extracellular glucosidases of a freshwater lake and in marine sediments varied considerably when pH modifications were carried out during in vitro experiments (King, 1986; Chróst, 1991; Münster, 1991).

Today, it is not known how ocean acidification will affect the degradation activity of marine bacteria, and the microbial turnover of organic matter. Here, we investigated the effect of lowered seawater pH simulating ocean acidification on the rate of enzymatic polysaccharide hydrolysis in natural bacterioplankton communities. Our study included laboratory experiments with organic matter derived from monospecific cultures of the bloom-forming coccolithophore *Emiliania huxleyi*, as well as field assays conducted at the Bay of Biscay (North Atlantic). Degradation of polysaccharides was followed under present-day pH (7.9–8.3) and under pH lowered by 0.2–0.3 units as expected for the ocean within the next 100 years (Houghton et al., 2001; Caldeira and Wickett, 2003; Raven et al., 2005).

2 Materials and methods

2.1 Experimental setup

In current marine research, the biological response to elevated seawater pCO_2 and biogeochemical consequences are mainly investigated by perturbation experiments, in which different approaches are used to manipulate the seawater carbonate chemistry (Gattuso and Lavigne, 2009). In our experiments, reference incubations representing present-day pH conditions were compared with acidified incubations that exhibited pH values projected for the future ocean. Manipulation was carried out by both CO_2 aeration and acid addition to exclude impact of the manipulation mode. The pH was measured using a combined temperature- and pH-probe (WTW 340i) calibrated with standard buffer solutions of pH 4.006, 6.865, and 9.180 (WTW standard DIN/NBS buffers PL 4, 7, and 9). To examine the effect of acidification on the bacterial degradation of polysaccharides we conducted two culture experiments (CultExp I, II) and two field assays (FieldAssay I, II). Different setups with regard to nutrient supply, light regime, and plankton communities were applied to include variability of important abiotic and biotic factors in marine pelagic ecosystems. The experimental designs are described below and summarized in Table 1.

CultExp I: Here, incubations simulating future-ocean conditions were initially but not continuously acidified with pure CO_2 gas. Thereby, the initial seawater pH of the future-ocean treatment was adjusted to 7.8 before phytoplankton growth started. This target pH corresponded to 750 µatm CO_2 as calculated by the use of the program CO_2sys (Lewis and Wallace, 1998) after measurement of the initial total alkalinity by the Gran electrotitration method (Gran, 1952). Seawater carbonate chemistry was not experimentally modified in the present-day treatment. The pH of both present-day and future-ocean treatment increased during phytoplankton growth and declined during dark incubation and bacterial

degradation of the phytoplankton-derived organic matter. During the degradation phase, the mean pH was 8.3 and 8.1 in the present-day and the future-ocean treatment, respectively.

Organic matter was derived from biomass and exudates of the coccolithophore *Emiliania huxleyi*. Batch cultures of *E. huxleyi* (strain PML B92/11) were grown in sterile-filtered seawater enriched with 50 µmol l^{-1} nitrate and 3 µmol l^{-1} phosphate, applying a 16/8 h light/dark cycle and a photon flux density of 200 µmol photons m^{-2} s^{-1}. Culture-derived organic matter was inoculated with a natural bacterioplankton community collected at the North Sea after 27 days, when decreasing growth rates of *E. huxleyi* indicated exhaustion of inorganic nutrients. Incubations were conducted in 10 l-Nalgene bottles kept in permanent dark for 30 days after the addition of the bacterioplankton inoculum. The bottles were mixed carefully, but thoroughly twice a day and prior to samplings. Incubation at present-day and future-ocean pH was conducted in duplicate at 14 °C.

CultExp II: Permanent aeration with CO_2-air-mixtures containing 550 µatm and 900 µatm CO_2 led to constant pH values of 7.9 and 7.7 during phytoplankton growth and organic matter degradation in the present-day and future-ocean treatment, respectively.

Organic matter was derived from continuous cultures of *E. huxleyi* (strain PML B92/11) that were supplied with sterile-filtered seawater containing 30 µmol l^{-1} nitrate and 1 µmol l^{-1} phosphate at a dilution rate of 0.1 d^{-1}. A 16/8 h light/dark cycle and a photon flux density of 300 µmol photons m^{-2} s^{-1} were applied during growth of *E. huxleyi*. The cultures were grown for 12 days before the bacterial inoculum was added. After inoculation with bacteria, the flow-through was stopped and incubations were kept in permanent dark at 14 °C for 13 days.

FieldAssay I: A field sample collected at the Bay of Biscay (47°07′83″ N, 6°92′01″ E, North Atlantic) in May 2007 was subdivided into incubations at present-day and future-ocean pH. Incubations of the future-ocean treatment were acidified with 0.1 M hydrochloric acid. The pH was lowered by 0.3 units to 7.9 by acid addition. Due to low concentrations of organic matter and consequently low bacterial degradation activity the pH remained constant until the end of dark incubation although no further acid addition was carried out.

The surface samples included the in situ assemblages of phyto- and bacterioplankton. The phytoplankton community was dominated by coccolithophores. Organic matter degradation was conducted in 10 l-Nalgene bottles in permanent dark. Incubations were run in triplicate close to in situ temperature at present-day and at future-ocean pH for 12 days.

FieldAssay II: Like in FieldAssay I, surface samples were collected at the Bay of Biscay (47°05′34″ N, 7°16′63″ E, June 2006). Aeration with CO_2-air-mixtures of 380 µatm and 750 µatm CO_2 generated constant pH values of 7.9 and 7.6 in the present-day and future-ocean treatment, respectively.

The surface sample was subdivided into duplicate incubations at present-day and future-ocean pH and incubated in a chemostat system. A 16/8 h light/dark cycle and a photon flux density of 200 µmol photons m^{-2} s^{-1} were applied during the whole incubation time of 8 days. Incubations were supplied with 20 µmol l^{-1} nitrate and 1.8 µmol l^{-1} phosphate in filtered seawater of the sampling site. A flow rate of 0.13 d^{-1} was applied. Hence, in contrast to the other experiments described above, autotrophic production and bacterial degradation of organic matter occurred simultaneously at steady state. The chemostat was run for 8 days prior to sampling.

2.2 Analytical methods

The analysis of polysaccharides was conducted by High Performance Anion Exchange Chromatography (HPAEC) coupled with Pulsed Amperometric Detection (PAD) on a Dionex ICS 3000. Concentrations of dissolved and particulate combined glucose, galactose, arabinose, mannose, xylose, fucose, rhamnose, glucuronic acid, and galacturonic acid were detected. The sum concentration is referred to as total polysaccharides. Only polysaccharides >1 kDa were analyzed, since this fraction requires cleavage by extracellular glucosidases prior to bacterial metabolization. Polysaccharides <1 kDa, oligosaccharides, and monosaccharides were separated prior to analysis by the use of a 1 kDa dialysis membrane during desalination of the seawater sample. After that, samples were hydrolyzed with hydrochloric acid at a final concentration of 0.8 M for 20 h at 100 °C.

Samples for particulate organic carbon (POC) were filtered onto precombusted glass fibre filters (GF/F, Whatman). Filters were acidified with 0.2 M hydrochloric acid to remove all particulate inorganic carbon. After drying, concentrations of POC were determined with an elemental analyzer (EuroEA, Euro Vector).

Activities of extracellular enzymes were determined by the use of fluorogenic substrate analogues (Hoppe, 1983). The activities of α-glucosidase and β-glucosidase were estimated from the enzymatic hydrolysis of 4-methylumbelliferyl-α-glucopyranoside and 4-methylumbelliferyl-β-glucopyranoside, respectively.
Samples were incubated at in situ temperature for 3 to 5 h with fluorogenic substrates added to a final concentration of 1 µmol l^{-1} in all experiments. The concentration of 1 µmol l^{-1} substrate analogue corresponds to the magnitude of natural polysaccharide concentration in the ocean (Myklestad and Børsheim, 2007). The fluorescence emitted by 4-methylumbelliferone (MUF) molecules was detected at 355 nm excitation and 460 nm emission wavelength, using a plate reader (FLUOstar OPTIMA, BMG Labtech, and Fluoroskan Ascent, Thermo Labsystems) or a cuvette fluorometer (F-2000, Hitachi). Calibration was carried out with solutions of MUF. In order to consider pH effects on the fluorescence intensity of MUF, standard solutions

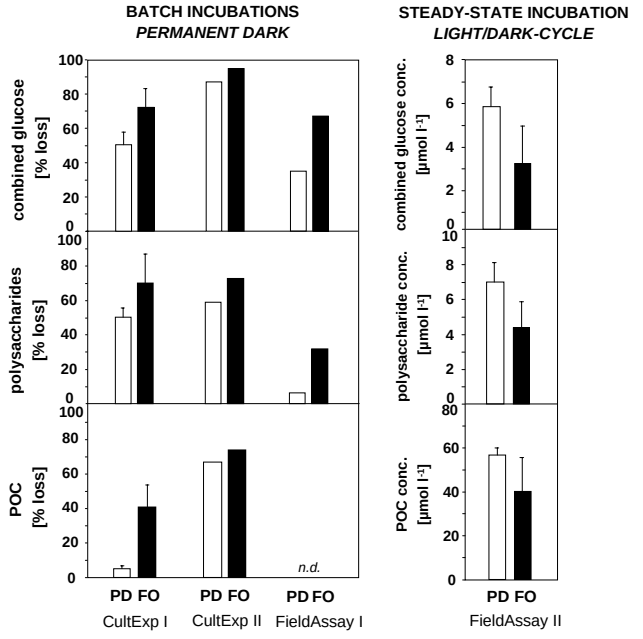

Fig. 1. Degradation of polysaccharides and organic carbon at present-day and future-ocean pH. Combined glucose (>1 kDa), polysaccharides (>1 kDa), and Particulate Organic Carbon (POC) were determined in reference incubations (open bars, PD: present-day pH) and at lowered seawater pH (solid bars, FO: future-ocean pH). In FieldAssay I, samples from replicate incubations were pooled for analysis. For the experiments CultExp I, CultExp II, and FieldAssay I, losses were calculated by subtracting the residual from the initial concentration (n.d.: no loss detectable). Significance of differences between the pH treatments was assessed by means of paired *t*-tests (combined glucose: p=0.026; polysaccharides: p=0.005). Data of CultExp I, CultExp II, and FieldAssay I were compiled for statistical tests. For the chemostat experiment FieldAssay II concentrations under steady state conditions are given. Error bars denote the standard deviation from replicate incubations.

were adjusted to pH 7.6, 7.8, 8.0, 8.2, and 8.3, buffered with 1% 3-(N-Morpholino)-propanesulfonic acid. The activities of α-glucosidase purified from *Bacillus staerothermophilus* (Sigma) was calculated from the turnover time of 50 µmol l^{-1} 4-methylumbelliferyl-α-glucopyranoside in 1 mM n-2-hydroxyethylpiperazone-n-2-ethanesulfonic acid adjusted to pH 7.55, 7.70, 7.90, and 8.10. Fluorescence was measured in time intervals of 5 min for 2 h using a plate reader (FLUOstar OPTIMA, BMG Labtech).

Bacterial cell numbers were determined by flow cytometry (FACSCalibur, Becton Dickinson) in both culture experiments and in FieldAssay I. Nucleic acid was stained with SybrGreen I (Invitrogen). Bacterial abundances were estimated after visual inspection and manual gating of the bacterial subpopulation in the side scatter vs. green fluorescence – cell cytogram. Yellow-green fluorescent latex beads (diameter 0.94 µm, Polyscience) were used to normalize the counted events to a reference volume. TruCount beads (Becton Dickinson) were used for daily intercalibration and absolute volume calculation (Gasol and del Giorgio, 2000). In FieldAssay II, bacterial cells were counted by epifluorescence microscopy. For this purpose, samples were filtered onto black 0.2 µm polycarbonate filters and stained with 4′,6-diamidino-2-phenylindole (DAPI) (Porter and Feig, 1980). Bacterial abundances were calculated from cell counts of 10 randomly selected fields per filter that contained at least 100 cells each.

2.3 Data analysis

The losses of polysaccharides and POC were calculated by subtracting the final from the initial concentrations. Data on polysaccharide loss (n=4) and glucosidase activity (n=5) of CultExp I, CultExp II, and FieldAssay I were compiled for statistical analysis. Differences between reference and acidified treatment were tested by means of paired *t*-test. Statistical significance was accepted for $p<0.05$. Linear regression was performed using the software package SigmaPlot 9.0 (SysStat).

3 Results

The bacterial degradation of polysaccharides in CultExp I, CultExp II, and FieldAssay I was assessed from the loss of polysaccharides during dark incubation. The loss of total polysaccharides, including dissolved and particulate sugars >1 kDa, was significantly higher at lowered pH than in the reference incubations representing present-day conditions (p=0.005) (Fig. 1). At the end of the degradation experiments, the loss of combined glucose, the dominating sugar in polysaccharides, was up to 32% higher in future-ocean treatments, and the loss of total polysaccharides, including seven neutral sugars and two uronic acids, was higher by 26%. In CultExp I and CultExp II, experiments conducted with organic matter freshly produced by *E. huxleyi*, the higher loss of polysaccharides at lowered pH coincided with a higher loss of POC (Fig. 1). In FieldAssay II, a natural plankton community was sampled from surface waters at the Bay of Biscay and incubated in a chemostat system (Table 1). Because a light/dark cycle was applied and a low but constant nutrient supply was provided during this experiment, concentrations of polysaccharides and POC are the net result of phytoplankton production and of simultaneous bacterial degradation. Nevertheless, final concentrations of combined glucose, total polysaccharides, and POC were reduced by 46%, 37%, and 29% respectively, in acidified incubations compared to the present-day reference. It has been shown before that production of polysaccharides by marine phytoplankton increases with pCO_2 as a result of higher photosynthesis rates (Engel, 2002; Rost et al., 2003). Hence, lower concentrations of polysaccharides under elevated pCO_2 point to an

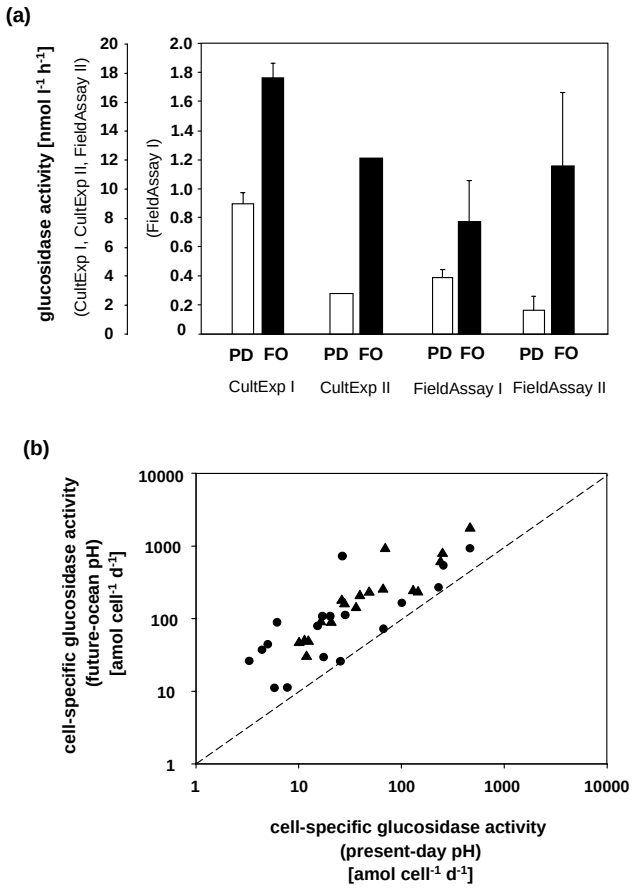

Fig. 2. Extracellular glucosidase activity at present-day and future-ocean pH. **(a)** Extracellular glucosidase activity in reference incubations (open bars, PD: present-day pH) and at lowerd pH (solid bars, FO: future-ocean pH) in culture experiments and field assays. Glucosidase activity at future-ocean pH was significantly higher than at present-day pH (paired t-test, $p<0.01$). Glucosidase rates of CultExp I, CultExp II, FieldAssay I, and FieldAssay II were compiled for the statistical test. **(b)** Log-log plot of cell-specific α- and β-glucosidase activity (circles and triangles, respectively) at present-day versus future-ocean pH.

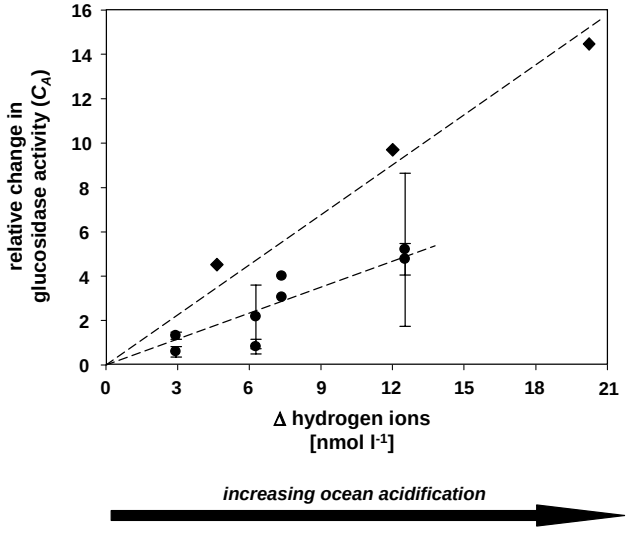

Fig. 3. Changing activity of extracellular glucosidases in response to rising hydrogen ion concentrations. Relative changes in glucosidase activity (C_A) of natural bacterioplankton communities (circles) and of *Bacillus stearothermophilus* (diamonds) were calculated according to $C_A=(A_{FO}-A_{PD})/A_{PD}$, where A_{PD} and A_{FO} are the glucosidase activities at present-day and future-ocean pH, respectively. The increase in hydrogen ion concentration (Δ hydrogen ions) induced by experimental acidification was calculated from the difference in pH between the present-day and the future-ocean treatment. Dashed lines represent linear regressions (marine glucosidase activity: $r^2=0.80$, $p<0.01$; α-glucosidase$_{B.\ stearothermophilus}$: $r^2=0.96$, $p<0.01$).

accelerated bacterial degradation that counter-steered phytoplankton production (Fig. 1).

The degradation of marine organic matter is driven by the hydrolytic activity of extracellular enzymes, which are predominately produced by bacteria. In our experiments, activities of extracellular α- and β-glucosidase were determined to assess rates of enzymatic polysaccharide hydrolysis. Extracellular glucosidase activity was significantly higher at future-ocean pH than at present-day pH ($p<0.01$) in all experiments (Fig. 2a). Higher enzymatic activities were not induced by differences in bacterial cell abundances, since bacterial cell numbers of all experiments did not reveal significant differences between the two pH treatments ($p=0.38$; data not shown). Hence, also cell-specific glucosidase rates at lowered seawater pH clearly exceeded those at present-day pH (Fig. 2b).

In our experiments, seawater pCO_2 was increased to simulate future-ocean conditions. Elevated pCO_2 levels corresponded to different pH values in the four experiments, ranging from 7.6 to 8.1. From the difference in pH between present-day and future-ocean treatment the increase in proton concentration induced by experimental manipulation was calculated for the four experiments (Table 1). This allowed us to relate the difference in glucosidase activity between present-day and future-ocean treatment to the increase in hydrogen ion concentration induced by acidification. The synthesis of all experiments revealed that the observed increase in glucosidase activity was directly proportional to the increasing acidity of seawater ($r^2=0.80$, $p<0.01$) (Fig. 3). Changes in glucosidase activities as inferred from our experiments reflect a community response of bacterioplankton to simulated acidification. In addition, we tested the response of purified α-glucosidase that was isolated from *Bacillus stearothermophilus* to decreasing seawater pH. *B. stearothermophilus* is a generalist bacterium that is widely distributed in ocean sediments and at marine vents (Sharp et al., 1992; Maugeri et al., 2002). Exposed to the same range of acidification, the increase of this specific α-glucosidase activity

was in the same order of magnitude as that of the natural glucosidase assemblages (Fig. 3). This similarity in pH sensitivity of natural glucosidase assemblages and of an isolated bacterial α-glucosidase (Fig. 3), together with increased cell-specific glucosidase rates at lowered seawater pH (Fig. 2) strongly suggest that the velocity of enzymatic polysaccharide hydrolysis in our experiments was directly affected by changes in seawater pH.

4 Discussion

The pH is known as an important regulating factor for bacterial extracellular enzyme activity in aquatic environments (Chróst, 1991), but potential impacts of ocean acidification on bacterial degradation activity are only poorly investigated. In previous experimental studies, large pH ranges with large intervals were applied to characterize enzyme assemblages of selected aquatic ecosystems biochemically (King, 1986; Chróst, 1991; Münster, 1991). Results from these studies are not sufficient to answer questions concerning effects of current and expected future ocean acidification that is characterized by rather small pH changes on large spatial scales. So far, potential effects of ocean acidification on marine bacterioplankton were tested only by two studies that investigated bacterial growth and activity during the development of phytoplankton blooms under different seawater pCO_2 in mesocosms (Grossart et al., 2006; Allgaier et al., 2008). Grossart et al. (2006) showed higher rates of bacterial extracellular protease activity and a higher cell-specific bacterial biomass production during the phytoplankton bloom at elevated pCO_2. In contrast, Allgaier et al. (2008) did not find differences in bacterial biomass production and carbon demand between the different pCO_2 treatments. The experimental design of both mesocosm studies, however, did not allow to distinguish direct pCO_2- and pH-effects on bacterioplankton activity from effects induced by CO_2-related changes in algal organic matter production (Engel et al., 2004; Egge et al., 2009). Primary production and phytoplankton exudation were highest in mesocosms of elevated pCO_2, so that the supply of labile substrates was enhanced and likely affected bacterial activity. Our experiments suggest that small pH decreases of 0.2 to 0.3 units, corresponding to the near-future seawater pCO_2, had a direct effect on the physicochemical control of natural extracellular glucosidase assemblages in marine pelagic ecosystems (Figs. 2 and 3). Extracellular glucosidase activity increased directly in response to rising proton concentration in our experiments (Fig. 3). The experimental results also show that rates of polysaccharide hydrolysis by marine glucosidase assemblages are not at their maximum at present-day seawater pH. This interpretation is in good accordance with previous studies conducted in aquatic environments, where optima for extracellular enzymes in freshwater and marine sediments did not correspond to in situ pH values (King, 1986; Münster, 1991). Hence, ocean acidification may shift seawater pH closer towards the optimum value of marine glucosidase activity.

4.1 Effects of acidification on polysaccharide and carbon degradation

Polysaccharides are a major component of reactive organic matter in the ocean as indicated by sharply declining concentrations in the subsurface layer (Kaiser and Benner, 2009). The hydrolytic activity of extracellular enzymes accomplishes the initial step in bacterial organic matter degradation (Chróst, 1991; Hoppe, 1991), and drives the solubilization of organic particles (Smith et al., 1992; Hoppe et al., 1993). Higher rates of extracellular glucosidases at lowered seawater pH significantly accelerated the degradation of polysaccharides in our simulation experiments (Figs. 1–3). Therefore, experimental results strongly suggest that the impact of ocean acidification on the reaction velocity of extracellular enzymes will be strong enough to affect early stages in the diagenetic processing of organic matter. In culture experiments, acidification did not only accelerate polysaccharide degradation but also enhanced the loss of POC (Fig. 1), which can be explained by the large polysaccharide fraction of freshly produced particulate organic matter. In the ocean, high polysaccharide yields in organic matter often coincide with high production of algal biomass and exudates during phytoplankton blooms (Baines and Pace, 1991; Handa et al., 1992; Engel et al., 2002). Hence, when bacterial activity increases during the decline of phytoplankton blooms, lowered seawater pH might exert substantial influence on the overall turnover of organic carbon in the ocean. Since the penetration depth of anthropogenic CO_2 in the ocean and related changes in seawater pH extend up to several hundred meters depth (Caldeira and Wickett, 2003; Sabine et al., 2004), freshly produced organic particles sinking out of the surface ocean may also become subject to an accelerated degradation in the twilight zone (100–1000 m depth), where intense bacterial activity strongly attenuates material fluxes to the deep ocean (Martin et al., 1987; Smith et al., 1992).

4.2 Effects of increasing glucosidase activity at lowered seawater pH on bacterial carbon acquisition and growth

The activity of extracellular enzymes largely determines the supply of low molecular weight substrates for direct bacterial uptake (Chróst, 1991). Among the great diversity of organic carbon compounds in the ocean, free glucose monomers must be considered as main carbon and energy source for bacterial growth (Rich et al., 1996). Concentrations of glucose monosaccharides in the ocean are usually below 100 nmol l^{-1}, but high glucose uptake rate constants reveal high fluxes and underscore the importance of glucose as substrate for the bacterial metabolism (Rich et al., 1996;

Skoog et al., 2002). The enhanced enzymatic hydrolysis of polysaccharides induced by lowered seawater pH in our experiments increased the availability of glucose for bacterial uptake and thus improved the bacterial carbon supply. The fate of glucose monomers taken up by bacterioplankton depends on the nutrient availability and the physiological state of the bacterial cell. Up to 60% of glucose consumed by marine bacterioplankton gets remineralized by respiration in nutrient-poor regions (Rich et al., 1996; Bianchi et al., 1998). The proportion of respired glucose is significantly lower in nutrient-rich areas, where appropriate nitrogen and phosphorous sources fulfil bacterial growth demands (Bianchi et al., 1998). In order to balance an increased availability of labile carbon, bacteria are able to utilize inorganic nitrogen (Kirchman et al., 1990; Kirchman, 2000). In particular actively growing marine bacteria act as sink for inorganic nitrogen, when an easily utilizable carbon source like glucose is available (Goldman and Dennett, 1991). The increased bacterial consumption of inorganic nutrients in response to increasing labile carbon availability changes the partitioning of inorganic nutrients between bacterioplankton and phytoplankton and leads to lower phytoplankton biomass production (Thingstad et al., 2008). Hence, it must be assumed that also increased glucose availability resulting from enhanced glucosidase activity at lowered seawater pH can stimulate bacterial competition for mineral nutrients and can mediate secondary effects on autotrophic production in the ocean.

With respect to ocean acidification, the acclimation and adaptation potential of organisms on the species- and community-level must be taken into account. However, the capability of single species and natural assemblages, including bacterioplankton, to adapt to changing seawater carbonate chemistry is poorly investigated. Therefore, the impact that potential acclimation and adaptation of key species and marine communities would have on biogeochemical processes in the future ocean is unclear. This study is focused on polysaccharide degradation in marine systems that is driven by highly diverse bacterial communities. Experiments included natural glucosidase assemblages derived from bacterioplankton communities of different oceanic sites. Therefore, experimental results suggest that increased glucosidase activity at lowered seawater pH does not depend on the abundance of some specific bacterial strains, but reflects a community response to lowered seawater pH. Furthermore, the accelerated enzymatic polysaccharide hydrolysis represents a biochemical pH effect. The elevated proton concentration in acidified incubations likely interacted with the three-dimensional protein structure of extracellular glucosidases. Acidification shifted the in situ pH to a value more favourable for marine glucosidase activity and led to an improved supply of labile substrates to bacterioplankton. Hence, higher glucosidase rates at lowered seawater pH are a chemical acidification effect on natural enzyme assemblages that is beneficial for the bacterial metabolism. Therefore, one might expect that acclimation or adaptation of bacterioplankton communities will not counter enhanced polysaccharide degradation in the future ocean.

4.3 Implications for carbon cycling in the future ocean

Heterotrophic bacteria are the main producers of CO_2 in the ocean, drive organic matter turnover, and sustain food webs (Pomeroy, 1974; Azam, 1998; Karl et al., 2003). Despite these key roles in biogeochemical cycles and ecosystem functioning, the effects of current and future changes in seawater carbonate chemistry on marine bacteria are largely unknown. Here, we showed that a decrease in seawater pH as expected for the near future increases enzymatic hydrolysis rates of polysaccharides and accelerates the bacterial degradation of organic carbon. Extrapolating results of CO_2-perturbation experiments to large scales bears considerable uncertainties. Simulation experiments investigate acidification effects on biological processes isolated from complex natural systems. Nevertheless, implications of experimental studies are essential to identify feedback mechanisms of marine biological processes to rising CO_2. If our results are representative for the ocean, ocean acidification will accelerate the degradation of polysaccharides and organic carbon on large spatial scales and may affect the vertical carbon export. The export of organic carbon from the surface to the deeper ocean, referred to as biological carbon pump (Volk and Hoffert, 1985), sustains a vertical gradient of dissolved inorganic carbon that in turn drives the ocean's uptake of atmospheric CO_2. In the ocean, the flux of sinking organic carbon is strongly reduced by the activity of extracellular enzymes solubilising organic particles in the surface layer and in the mesopelagic zone (Smith et al., 1992). Therefore, an enhanced degradation of particulate polysaccharides at lowered seawater pH may reduce the sinking flux of organic carbon, as it accelerates the dissolution of organic particles and favours the bacterial uptake of DOC. Large-scale implications are supported by field observations from the Sargasso Sea over the last decade. Here, a doubling of the mesopelagic POC flux attenuation was determined between 1996 and 2007, when ocean acidification progressed. The increased loss of organic matter in the mesopelagic zone of the Sargasso Sea is attributed to changes in metabolic activity that, however, could not be specified (Lomas et al., 2009). Based on our findings, it can be assumed that the amount of exported polysaccharide-derived carbon was curtailed due to increasing extracellular glucosidase activity at decreasing seawater pH. Thus, increased rates of enzymatic organic matter hydrolysis could at least partly explain the increased POC flux attenuation observed at the Sargasso Sea. A large proportion of organic matter in the ocean is produced in dissolved form. DOC includes high yields of polysaccharides and can be exported to the deeper ocean by convective mixing (Carlson et al., 1994; Goldberg et al., 2009). Enhanced bacterial glucosidase activity in the future ocean may also reduce the export of DOC due to an accelerated hydrolysis of

dissolved polysaccharides and a rapid bacterial assimilation of the labile hydrolysates.

In addition to effects on carbon export, the enhanced enzymatic polysaccharide degradation at lowered seawater pH may also increase the respiratory production of CO_2 in the future ocean. Higher rates of polysaccharide hydrolysis would improve the glucose availability for heterotrophic bacteria and may increase their respiratory activity. Increased bacterial respiration would establish independently from enhanced autotrophic production and therefore has the potential to disturb the metabolic balance of the sea for the benefit of net heterotrophy (Karl et al., 2003).

Both less export of polysaccharides and increased respiratory CO_2 production at lowered seawater pH have the potential to reduce the ocean's ability to absorb CO_2 from the atmosphere. In the future ocean, the accelerated organic carbon turnover by heterotrophic bacterioplankton will interact with other CO_2- and pH-effects on the marine biota. For instance, the draw-down of inorganic carbon by phytoplankton was shown to increase under elevated seawater CO_2 (Engel, 2002; Rost et al., 2003; Engel et al., 2004; Riebesell et al., 2007). Hence, the accelerated bacterial carbon turnover may coincide with an enhanced autotrophic production of organic matter in the future ocean. Elevated seawater pCO_2 led to a higher phytoplankton production of transparent exopolymer particles that are rich in polysaccharides. Therefore, organic matter produced at high seawater CO_2 should be particularly prone to increased glucosidase activity at lowered pH. This interaction between phytoplankton production and bacterial degradation at changing seawater chemistry is an example for the complexity of acidification effects in the ocean. Complex interactions and unexplored effects of changing seawater carbonate chemistry on important biological processes make it impossible to predict the prevailing feedback of the marine biota to rising CO_2 to at the current state of knowledge.

5 Outlook

In the face of rapidly changing marine ecosystems, a better understanding of acidification effects on the metabolism and physiology of marine organisms and on biogeochemical cycles becomes a matter of urgency. Here, bacterial extracellular enzymes, which play a decisive role in the turnover of marine organic matter (Azam, 1998; Azam and Malfatti, 2007), were identified as pH-sensitive keystone. Since enzymes catalyze biochemical reactions in all life forms, it can be assumed that effects of decreasing pH on enzymatic activities will impact a variety of biological processes in the future ocean and evoke consequences of unprecedented complexity. For example, it has been shown that enzymes in muscle tissues of fish will respond to ocean acidification (Michaelidis et al., 2007), same as enzymes involved in growth and carbon acquisition of phytoplankton species (Hansen et al., 2007). Like in this study, results were obtained from manipulative laboratory experiments that provide a valuable tool to investigate potential consequences of lowered seawater pH on specific biological and biogeochemical processes. As an alternative scientific approach, natural pH gradients in the ocean, for example induced by marine CO_2 vents, can be used to investigate ocean acidification on the ecosystem level and can serve as validation for findings from in vitro perturbation experiments (Hall-Spencer et al., 2008). With regard to acidification effects on enzymatic reactions, in situ studies along natural pH gradients could provide insights into the interaction of different enzymatic reactions at changing rate and could evaluate potential effects on ecosystem processes.

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