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Detection of Active Microbial Enzymes in Nascent Sea Spray Aerosol: Implications for Atmospheric Chemistry and Climate

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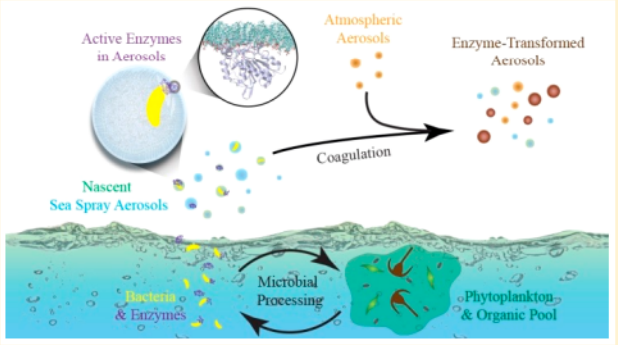
Supporting Information

ABSTRACT: The oceans cover nearly three-quarters of the Earth's surface and produce vast quantities of sea spray aerosols (SSA). Studies have shown that due to ocean biology SSA particles are comprised of much more than just sea salt and often include proteins, lipids, sugars, viruses, and bacteria. In this study, we show for the first time that a diverse array of microbial enzymes (protease, lipases, and alkaline phosphatase) are transferred from the ocean into the atmosphere and often become even more active with measured activities in SSA particles that are 1−2 orders of magnitude higher than those in bulk seawater. We hypothesize that these enzymatic reactions are enhanced in the interfacial environment of droplets and aerosols that can dynamically modify surface chemical species and properties. Simulations reveal that enzyme-containing SSA particles can rapidly coagulate with other preexisting aerosols, thus transferring the impact of enzyme reactions to a broad range of marine aerosols. These biotic reaction pathways are expected to profoundly change the composition of marine aerosols, particularly at the interface, and thus will impact cloud properties in marine environments. Future studies are needed to determine how photochemistry, changing ocean conditions in a warming climate, and other external factors will influence the activities of these enzymes and their impact on the composition of the marine atmosphere.



INTRODUCTION

Sea spray aerosols (SSA) represent one of the most abundant particle types in the atmosphere.[1−4] SSA influence atmospheric optical properties, the number and properties of cloud condensation and ice nuclei, and the overall composition of the atmosphere.[5] SSA particles are produced when bubbles burst at the ocean surface[2] and include mixtures of sea salt and biologically derived organic compounds.[6,7] These particles contain viable marine bacteria and viruses, gels, vesicles, carbohydrates, proteins, and lipids that enrich SSA.[8−18] Studies are just beginning to probe the processes that regulate the transfer of different biological compounds from the ocean to the atmosphere.

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In the upper ocean, bacteria are abundant (10^{12} m^{-3}) and play a major role in controlling the composition, structure, and abundance of organic matter. Because biologically derived organic compounds occur predominantly in the form of large polymeric molecules or particulate organic matter, a critical biochemical strategy of bacteria is to express multiple cell surface-bound enzymes that can hydrolyze organic compounds into forms accessible for cellular uptake such as monomers and a few amino acids to a single amino acid.[19,20] In low-diffusion environments, such as algal or detrital particles, bacteria can release enzymes (free enzymes) into the surrounding microenvironment, thus maximizing substrate utilization.[21,22] Bacterial cell surface enzymes are ubiquitous in upper ocean seawater (SW) samples, including the sea surface microlayer (SSML), over a broad range of ocean environments.[15,16,19,20] Protease, lipase, phosphatase, glucosidase, nuclease, and chitinase constitute the adaptive molecular toolkit that marine bacteria use to utilize the very diverse (10^{12}–10^{15} different compounds) and complex organic matter pool.[23] In the ocean, it has been estimated that there are ~20000 diverse molecular formulas[17] in the organic matter pool that bacteria can metabolize by their enzymatic repertoire.[18,24] Thus, the constant recycling of the organic pools by bacteria has fundamental consequences for the creation and maintenance of the vast array of organic compounds.

The ocean and atmosphere exchange significant quantities of biological particles. It has been estimated that on a daily basis, on the order of 10^6 bacterial and archaeal cells per square meter of ocean surface can travel thousands of kilometers before reentering the ocean.[25−27] It has been shown that *Emiliania huxleyi* viruses emitted in marine aerosol can infect coccolithophore blooms after traveling for hundreds of kilometers.[28] Recent work has shown a taxon-specific aerosolization pattern for marine bacteria and viruses, with certain bacteria having a greater propensity for aerosolization.[29] The selective transfer of certain microbes was associated with hydrophobic functional molecules on the cell surface. While studying the effect of microbial action on organic species in the ocean before they are ejected in SSA, we have shown that microbial processing of the dissolved organic matter pool in SW controls the timing of the release of different classes of organic species that are transferred into the atmosphere.[30] Consequently, microbial interactions play a fundamental role in controlling the transfer of biologically derived molecules between the ocean and atmosphere.[31]

Despite our improving understanding of diverse and active microbial communities and their potential impact in the atmosphere,[12,14,32,33] this study shows for the first time the direct transfer of active marine microbial enzymes in nascent SSA. We used model simulations to investigate whether these active enzymes can impact aerosols in the marine atmosphere after they are ejected. Thus, ocean microbes not only control the organic composition of the ocean but also have the potential to profoundly influence the composition of the marine atmosphere, which can continue to evolve over time through previously unexplored biotic pathways.

■ MATERIALS AND METHODS

Aerosol Sampling in MART Microcosm Bloom Experiments and over the Coastal Ocean. Surface SW was collected off the end of the Ellen Browning Scripps Memorial Pier, La Jolla, California (Scripps Pier, 32° 52.02′ N, 117° 15.43′ W), with an acid clean carboy. The SW was prefiltered through 50 μm acid-washed Nitex and brought into the laboratory, and the Marine Aerosol Reference Tank (MART)[34] was filled. Three MART experiments were set up in November 2013 (MART-A), December 2013 (MART-B), and January 2014 (MART-C) (Table S1). Nutrients[35] were added to stimulate a phytoplankton bloom under constant light (100 μE m^{-2} s^{-1}, 5700 K). Detailed methods can be found in ref 36; study-specific details such as experiment duration and sampling techniques for SSML (by glass plate dipping) and SSA (by impinger) can be found in the Supporting Information and Table S1.

The MART is a breaking wave analogue that produces SSA using a pulsed-plunging waterfall that creates the full bubble spectrum of a breaking wave.[34,36] The tank headspace was purged with particle free air (Sabio Instruments) to constantly maintain a positive pressure to minimize room contamination. Water was plunged on the day after the seawater collection day (day 1), and daily plunging was resumed after *in vivo* Chl *a* fluorescence increased exponentially. SSA was collected by impinging the air into a 40 mL precombusted glass vial that was filled with 0.2 μm filtered autoclaved seawater (FASW). The impinging method preferentially collects >200 nm particles.[36] The same batch of FASW was used for the three MART experiments and stored in the dark at 4 °C. The SSA-impinged solution was immediately aliquoted undiluted for bacterial and viral abundance analyses and for enzymatic activity assays after a 1:1 dilution in FASW.

Aerosol size measurements were conducted after the sample had been passed through silica gel diffusion driers to obtain a relative humidity of <10%. Number size distributions of SSA particles were measured using a scanning mobility particle sizer (SMPS, TSI 3936, 0.3/3.0 SLPM sample/sheathe flow) for particles with mobility diameters (d_m) between 0.013 and 0.7 μm and an aerodynamic particle sizer (APS, TSI 3321, 1 SLPM sample flow) for particles with aerodynamic diameters (d_a) between 0.6 and 20 μm (see Table S1).

Marine aerosols were sampled from the Scripps Pier, to probe for the presence of active enzymes (in 2017, June 8, 9, and 10 and July 1, 7, and 14), using the same aerosol impingers used in MART studies. Each day, two precombusted glass impingers were set out, each pulling 0.75 SLPM (1 SLPM on June 8) and kept protected from direct sunlight to prevent photodegradation. The wind direction and speed obtained from the National Oceanic and Atmospheric Administration's National Data Buoy Center Station LJPC1 during the sampling periods ranged from 231° to 356° (ocean direction) and from 0.6 to 3 m/s, respectively,[37] suggesting the prevalence of the sea spray aerosol component. One impinger sampled ambient aerosol through a stainless steel inlet extending ~0.75 m from the pier. The other impinger served as a field blank by sampling air through a HEPA filter that removed all particles and, thus, all sources of airborne enzymes.

Enzymatic Activity within SW, SSML, and SSA Fractions. The enzymatic activities for total, <1 μm (bacterial), and <0.2 μm (free) fractions were measured using fluorogenic substrate analogues at saturating concentrations (20 μM).[19] Alkaline phosphatase (APase, AP), protease (leucine, P), and lipase (oleate, O, and stearate, S) were measured with 4-methylumbelliferone phosphate, L-leucine-7-amino-4-methylcoumarin, 4-methylumbelliferone oleate, and 4-methylumbelliferone stearate, respectively. Fluorescence resulting from enzymatic release of the free fluorophores [4-methylumbelliferone (MUF) and 7-amido-4-

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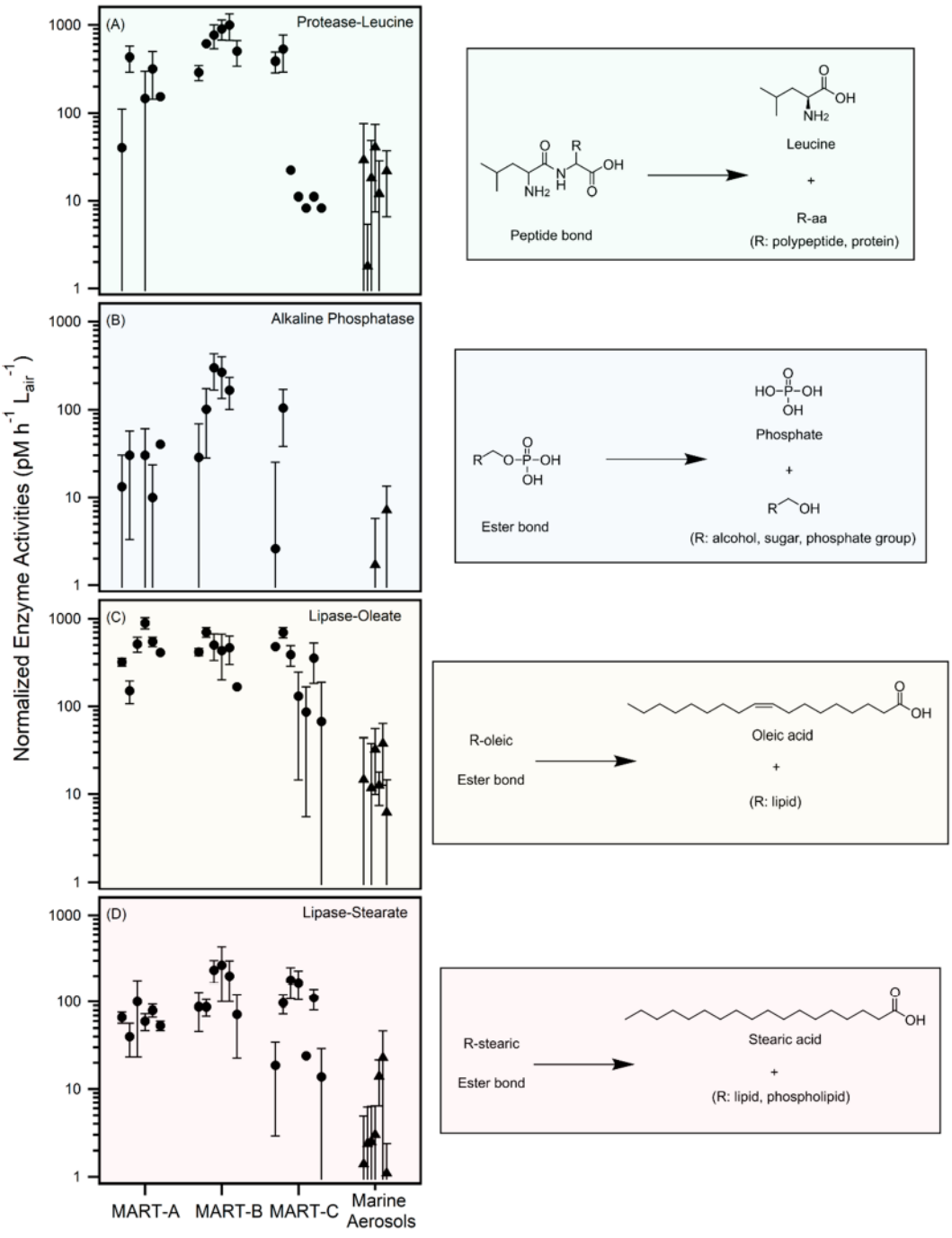

Figure 1. SSA enzyme activities (left) for MART-induced phytoplankton bloom experiments (MART-A, -B, and -C, circles) and marine aerosols from the coastal Pacific Ocean (triangles) normalized to liters of air sampled. Error bars represent 1σ. Examples of chemical reaction schematics with substrates and products (right). Protease-leucine cleaves the peptide bonds, thus producing leucine from a polypeptide/protein. Alkaline phosphatase cleaves the ester bond, thus releasing orthophosphate from a phosphate-containing molecule (phosphate monoester, phosphate sugar, polyphosphate). Lipase-oleate cleaves oleic acid from lipids. Lipase-stearate cleaves steric acid from lipids and phospholipids.

methylcoumarin (AMC)) was measured by a multimode reader (SpectraMax M3, Molecular Devices) on 96-well microtiter plates incubated in the dark at *in situ* temperature for 1 h. Fluorescence was measured immediately after adding substrates and again at the end of the incubation,[38] upon 355 nm excitation and 460 nm emission. As marine bacteria in the ocean express the same enzyme classes, we tested for their presence and activity in the SSA.

Enzyme activities were measured as the concentration of substrate hydrolyzed per unit time of incubation. Replicates for each sample−substrate combination were analyzed in three to six wells. Standard curves of the free fluorophores AMC and MUF were used to relate fluorescence to free fluorophore concentration. Enzyme activity for aerosol samples is reported as the difference between the sample and a blank generated by sampling particle free air. We tested a range of substrate concentrations (from 20 to 100 μM) over the course of 24 h to determine the saturating concentrations. In the MART-C experiment, the seawater sample was filtered onto a 1 μm autoclaved polycarbonate filter and then sequentially onto a

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0.2 μm low-binding protein Acrodisc syringe filter[39,40] to determine the bacterial fraction and the free fraction.

Particle Coagulation Simulations. Particle Brownian coagulation simulations of SSA with background particles were performed, using the stochastic, particle-resolved aerosol model PartMC. Details of the simulation model can be found elsewhere.[41] In brief, the particle-resolved approach allows for the tracking of the full aerosol mixing state as the population is undergoing Brownian coagulation. Two initially externally mixed particle distributions were used as inputs for the simulation, and mixed particles were formed as the particles evolved over the course of the 24 h simulation. One of the input distributions was the dry nascent SSA particle distribution previously reported,[42] whereas the other, termed "background", was adapted from the idealized urban plume distribution reported in ref 41. The concentrations in the Aitken and accumulation modes of the background aerosol distribution were scaled to three different total background concentrations to quantify the formation of mixed particles under different atmospherically relevant conditions. A summary of parameters used for the background distributions can be found in Table S6.

■ RESULTS AND DISCUSSION

Herein, the relevance and impact of enzymatically active SSA particles within the conceptual framework of ocean−atmosphere chemical interactions were explored using (1) laboratory microcosm incubation experiments in the MART (Table S1)[34,36] designed to study the impacts of ocean biology on SSA chemistry, (2) ambient measurements validating the relevance of active enzymes in coastal marine aerosol, and (3) model simulations. The simulations provide an estimate of the potential influence of these ocean-derived enzymes on preexisting marine aerosols over a range of relevant marine atmosphere concentrations.

Discovery of Active Microbial Enzymes in Nascent SSA. *Laboratory Microcosm Experiments.* In three separate seawater MART microcosm experiments, diatom-dominated phytoplankton blooms formed and then decayed over multiple days [SI MART dynamics (Figure S1)]. In SW, bacteria responded to the phytoplankton bloom by growing at the expense of newly synthesized organic matter and algal detritus thus expressing specific enzymes (Figure S1). During the phytoplankton blooms, intense protease, lipase, alkaline phosphatase, and chitinase activities were measured in the SW and SSML (Figure S2A−E). In SW and SSML, protease and alkaline phosphatase trends reflected the Chl *a* trend [$p < 0.01$; $p = 0.05$ (Table S5)], thus suggesting a tight coupling between bacterial degradation of proteins and phosphorus-rich compounds with phytoplankton dynamics. Enzymatic activities in SSML were greater than those in SW by factors ranging from 1 to 7.7 times, thus suporting the idea of SSML as a hot spot of degradation processes. Within these dynamic biological scenarios, we demonstrate for the first time that specific bacterial enzymes present in SW and in SSML (Figure S2) were selectively transferred into nascent SSA (Figure 1 and Figure S3). Here, lipase (oleate and stearate), protease, and alkaline phosphatase become more active than in SW and SSML (Table S2 vs Figure S3) by 1−2 orders of magnitude. To compare the activities in SSA versus those in SW and SSML, we have expressed the hydrolysis rate of the substrate for SSA in femtomoles per liter of air per hour by normalizing SSA enzymatic rates to the total volume of SSA sampled and for SW and SSML in attomoles per cell per hour by normalizing the enzymatic rates to the bacterial cell abundance (cells per liter) in SW and SSML, respectively (Table S2 vs Figure S3).

MART SSA enzyme activities evolved over the course of the blooms in terms of both the magnitude and the patterns, defined as the specific combination of detected enzymes within SSA during a given period (Figure S3). Furthermore, SSA possessed unique enzymatic activity signatures that were different from those in SW and SSML (Figure S2F−H). It is important to note that enzyme assays used here do not provide information about specific structural enzyme diversity within the broad categories (e.g., protease is different than a lipase and an alkaline phosphatase); rather, they assess enzyme activity associated with different compound classes. These differences can likely be attributed to the selective transfer of bacterial species to SSA as recently reported by Michaud.[29] Alternatively, and complementarily, the enzymes present in SSA could be due to changes in the bacterial abundances and community composition of seawater as marine bacterial isolates possess a unique enzymatic signature[20] to degrade the marine organic matter pools. We propose that the increased rates of SSA enzymatic activities are due to their confinement at interfaces enriched with organic compounds as well as enhanced transfer efficiencies. Because it is known that marine bacteria have the potential to express a vast suite of enzymes that can also present biphasic kinetics,[43−46] we envision that other enzymes, such as α- and β-glucosidase, serine- and lysine-aminopeptidase, and lipase butyrate, will also be transferred in nascent SSA, thus creating an even more complex array of reactions.

Cell-Bound and Free Enzymes Are Present in SSA. In MART-C, we have tested whether any active free enzymes were present in SSA. We found that most of the enzyme activity in SSA was in the cell-bound bacterial size fraction (<1 μm) and the free fraction (<0.2 μm) never exceeded 21% of the total activity for protease and lipase (Table S3, in MART-C). The cell-bound enzyme activity is linked to bacterial abundance in SSA and their enzymatic degradative ability. On the other hand, the free enzymes can come from the bacteria themselves or from dying cells that are emitting their internal cytosol. It is important to note that free enzymes such as free protease and lipase can process organic compounds even when bacteria are absent, especially in submicrometer SSA particles that are typically rich in long chain fatty acids[30,31] and proteins.[47] In the ocean, free enzyme activities can last for months,[16,21] suggesting long-term changes in the organic matter pools.

The discovery of the presence of free enzymes in SSA is consistent with recent studies showing different physical production mechanisms involving jet and film drops[48] that selectively transfer different proportions of enzymes at the interface relative to bulk seawater.[49] Furthermore, active enzymes present in SSA will degrade the larger organic molecules into smaller subunits with different solubilities and affinities for interfaces.[50] In terms of size, enzymes can range from picometers to several nanometers compared to tens of nanometers to micrometers for SSA.[2,51] Our findings suggest dynamic changes in biologically derived organic matter pools in SSA, including lipids, proteins, and phosphorus-rich organic molecules in a post-ejection scenario. Future studies will probe the selectivity mechanisms that influence the ejection of free

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enzymes versus cell surface-bound enzymes and their impact on the composition of SSA in different particle sizes.

Coastal Ocean Site. To test the atmospheric relevance of these laboratory findings, we measured the enzyme activities of proteases, alkaline phosphatase, lipases, and β-glucosidase in ambient aerosols at a coastal location (Figure 1 and Table S4). Enzyme activities presented comparable rates [1−100 pM h^{-1} (L of air)$^{-1}$] at the lower end of activities measured in the MART microcosm experiments. This is to be expected given the lower relative abundance of nascent SSA (10−100 times in comparison with the MART system[52]), compared to the presence of additional nonmarine sources in ambient air,[53] and the intense phytoplankton bloom [up to 10 times in Chl *a* units (Table S4)] occurred in the MART. However, lipase-butyrate exhibited much higher enzyme activity of ≤1000 pM h^{-1} (L of air)$^{-1}$, which leads to rapid turnover of lipids in ambient aerosols. The measured activities in ambient SSA varied over the course of hours, which can be attributed to changes in ocean biology and other environmental factors, including meteorological conditions and ultraviolet intensity.[54] Future studies will explore how different enzymatic reaction pathways are influenced by the biogeochemical state of the ocean, ocean−atmosphere exchange processes, and environmental conditions.

Simulating the Atmospheric Impact of Active Enzymes in SSA. When nascent SSA particles with active enzymes coagulate with other marine aerosols, these enzyme reactions will have even broader impacts on the composition of the marine atmosphere. Under the assumption that the SSA particles will remain enzymatically active due to the presence of cell-bound or free enzymes that are distributed across all particles, we investigated the potential impact of enzyme reactions using a stochastic, particle-resolved aerosol model to simulate coagulation time scales under three atmospherically relevant background conditions: 100 (low), 1000 (medium), and 6100 (high) particles cm^{-3}.[41] Background ambient particle size distributions from Riemer et al. and previously reported size distributions for nascent SSA (section T5 of the Supporting Information)[41,42] were used as inputs for the model. The model predicts that after only 24 h, up to 18% of background marine aerosols will coagulate with at least one enzyme-containing SSA particle (Figure 2A). Importantly, further size-resolved analysis reveals that >70% of marine aerosols in the size range (>500 nm) most important for heterogeneous reactions (i.e., largest surface area)[55−57] will coagulate with at least one SSA after only 24 h (Figure 2B; further discussion in section T5 of the Supporting Information). This enzyme distribution process is extremely important as larger supermicrometer particles have been shown to have significant concentrations of surfactants on their surfaces.[57] The enzyme reactions reported here have the potential to rapidly transform these surface-active molecules, thus changing the surface properties and heterogeneous reactivity of SSA particles after they are ejected into the atmosphere. These results set the stage for future studies focusing on how ocean-derived active enzymes can efficiently transform the composition, reactivity, and water uptake properties of marine aerosols.

Marine Microbial Structuring of SSA Chemistry and Fate. The discovery of active microbial enzymes being ejected in SSA extends the aerosol bioreactor hypothesis proposed by Dobson et al. nearly two decades ago.[58] These results build upon previous findings showing how bacteria can influence the organic matter pool in seawater before being transferred to SSA (pre-ejection scenario).[30] We hypothesize herein that active enzymes can dynamically influence the composition of marine aerosols after ejection from the ocean. With this perspective, we foresee fundamental roles for enzyme-mediated biotransformation in the atmosphere: (1) catalyzing the chemical transformations of SSA and background marine aerosols over time, (2) providing potential new interfacial reaction pathways, and (3) changing the surface activities of organic compounds, including lipids, fatty acids, alcohols, and proteins, which can alter cloud droplet, ice nucleation, and heterogeneous reaction processes of SSA. This study illuminates a previously unidentified chemical link between microbial activities in the oceans and the composition of the atmosphere.

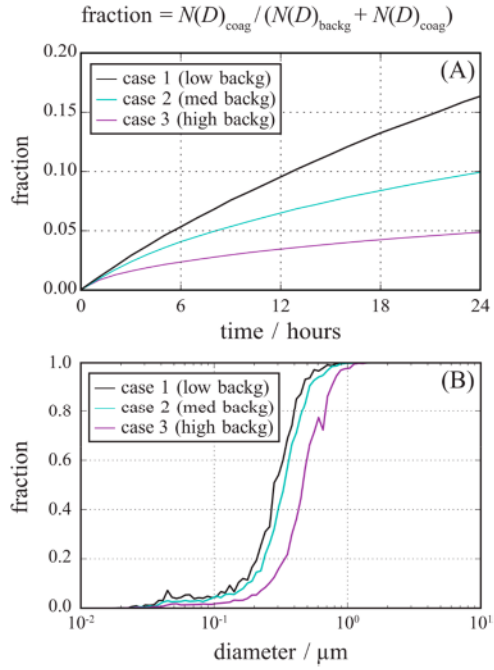

Figure 2. Coagulation simulation results of nascent SSA with background particles at three background particle concentrations (cases 1−3, 100, 1000, and 6100 cm^{-3}, respectively). Calculated fractions of (A) enzyme-containing particles (i.e., fraction of SSA background coagulated particles to the sum of coagulated and background particles) over time and (B) calculated enzyme-containing particles as a function of particle diameter after 24 h. Abbreviations: N, number of particles; D, particle diameter; Coag, coagulated SSA background particles; backg, background particles.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.estlett.8b00699.

Additional details about the methods and supporting discussion, Figures S1−S4, Tables S1−S6, and references (PDF)

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Notes

The authors declare no competing financial interest.

The data utilized to generate the figures and conclusions in this paper are hosted by the UCSD Library Digital Collections (https://doi.org/10.6075/J0251GDB).

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