

Marine Bacteria Affect Saccharide Enrichment in Sea Spray Aerosol during a Phytoplankton Bloom

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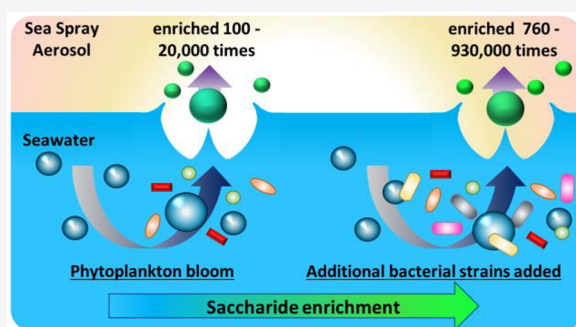
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ABSTRACT: Saccharides are a large class of organic matter in sea spray aerosol (SSA) that can impact its climate-relevant properties. In seawater, saccharides are produced, exuded, and consumed by phytoplankton and heterotrophic bacteria (HB). Herein, we add the HB strains *Alteromonas* sp. (AltSIO), *Pseudoalteromonas* (ATW7), and *Flavobacteria bacterium* (BBFL7) to marine microcosms during phytoplankton blooms and directly probe the impact of HB on the composition and size of saccharides in seawater and SSA for the first time. Enrichment factors (EFs) for saccharide SSA relative to seawater were composition- and size-dependent, ranging from 100 to 930 000 in sub-micrometer SSA and from 38 to 3700 in super-micrometer SSA. For saccharides in two similar phytoplankton blooms, lower seawater concentrations coincided with higher EFs, which may explain high saccharide enrichments in remote marine locations. Increased enzyme activity within a day of HB addition corresponded to increases in saccharide EFs in sub-micrometer SSA, by a factor of 30 for the energy-storage saccharide glucose and by a factor of 2 for the structural saccharide xylose. Meanwhile, arabinose, released in response to microorganism stress, increased by a factor of 20 in super-micrometer SSA after a lag of 2 days. Observations of HB in SSA indicate that modifications to the saccharide pool in terms of composition and size occur in both seawater and SSA, which subsequently impact their enrichment in SSA. A better understanding the chemical, biological, and physical factors that contribute to the large enrichment of saccharides in SSA will advance our ability to predict SSA composition and its impact on Earth's climate.

KEYWORDS: aerosols, sea spray aerosols, enrichment, organic, carbohydrates, heterotrophic bacteria, phytoplankton bloom, microcosm



INTRODUCTION

Oceans produce large quantities of sea spray aerosol (SSA)¹ that affect the climate by acting as cloud condensation nuclei (CCN)^{2,3} and ice nuclei (IN).^{4,5} Bubble bursting at the ocean surface generates SSA enriched in surface-active organic matter.^{6–8} Among the organic chemical compounds transferred to SSA, saccharides comprise up to 61% of the measured primary organic carbon (OC) mass.^{9–14} Prior laboratory studies show that individual saccharides contribute to variations in hygroscopicity in models of SSA and nascent SSA generated from a mesocosm.^{15,16} Further, prior field observations suggest that saccharides contribute significantly to the CCN activity of SSA.^{17–20} Still, the full extent to which saccharides affect the CCN activity of SSA is not yet understood. Identifying the chemical, physical, and biological factors that contribute to the enrichment of saccharides in SSA will improve our overall understanding of the factors controlling SSA chemical composition and properties.

Enrichment is quantified with enrichment factors (EFs) according to eq 1

$$EF_{\text{saccharide}} = \frac{[\text{saccharide}]_{\text{SSA}} / [\text{Na}^+]_{\text{SSA}}}{[\text{saccharide}]_{\text{seawater}} / [\text{Na}^+]_{\text{seawater}}} \quad (1)$$

where an EF of >1 indicates enrichment and an EF of <1 indicates depletion with respect to sodium concentrations in seawater and SSA. In laboratory studies of model systems, saccharide monomers through hexamers with negligible surface activities exhibited no enrichment (EF = 1), whereas the surface-active, acidic polysaccharide alginate showed small enrichment (EF = 1.6) in aerosol particles of <250 nm.²¹ Further, calcium and protein added separately as individual compounds increased alginate enrichment in aerosol particles up to factors of 2.4 and 5.8 in particles of <250 and 500–1000

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nm, respectively.²¹ This and prior studies demonstrate that interactions, including co-adsorption, play a role in enrichment of saccharides in SSA.^{22,23} Meanwhile, biologically active seawater exhibits total saccharide enrichments of up to 100 000 times in particulate matter with an aerodynamic diameter of less than 1.2 μm ($\text{PM}_{1,2}$).²⁴ EFs during two sequential phytoplankton blooms that varied were size-dependent (EF = 3–130 and 10–1300 for 2.5–10 and <2.5 μm sized SSA, respectively) and varied by saccharide, and maxima coincided with the highest levels of phytoplankton and heterotrophic bacteria (HB).¹⁴ The observed size dependence is consistent with prior observations of surface-active organic matter,^{11,25,26} increasingly enriched in smaller SSA particles as a result of the bubble bursting process. Surface-active components remain in the bubble film and are enriched in smaller film drops, while water-soluble and less-surface active components drain to the base of the bubble and preferentially transfer in jet drops.^{10,19,27} Isolated laboratory experiments enable examination of the relative importance of ocean microbial influence on saccharide enrichment in SSA. This study expands this understanding by focusing on the effect of HB on the enrichment of different saccharides in freshly produced SSA over the course of a phytoplankton bloom.

Saccharides are an important class of molecules in the ocean that comprise ~20% of the measured mass of dissolved organic matter (DOM).²⁸ They span a large size range: from microgels several micrometers in length down to dissolved small polymers and monomers.²⁹ The abundance and chemical composition of saccharides in seawater tightly links to phytoplankton and HB abundance, speciation, and environmental conditions.^{30,31} Phytoplankton release saccharides actively or by passive diffusion out of the cell membrane.³¹ Polysaccharides are also released through lysis of old or dying cells.³² Overall, polysaccharide release varies widely between phytoplankton but may comprise up to 90% of the mass of DOM released by specific diatoms^{33,34} and include arabinose, fucose, galactose, glucose, mannose, rhamnose, and xylose.^{31,35,36} The size and composition of saccharides produced by phytoplankton varies by species, stage of phytoplankton growth, and environmental conditions, including nutrient and HB abundance.^{30,31,37,38} Glucose largely comprises polysaccharides that are used for energy storage,^{32,39,40} while other monomers, including galactose and xylose, form parts of cell walls.^{32,39,41} Polysaccharides are a major food source for HB,⁴² and HB can modify saccharides through carbohydrate-active enzymes (CAZymes; <http://www.cazy.org/>)^{43,44} as well as incorporate small molecules that are cleaved.^{32,45} Saccharides chemically modified and released by HB are distinct from those released by phytoplankton,^{30–32} such as the incorporation of significant amounts of uronic acids (up to 50% by mass) that are more resistant to break down.^{31,46} Thus, the saccharide abundance and composition in seawater is impacted by phytoplankton and HB populations. Further, changes in the abundance and composition of organic compounds in the seawater as a result of the growth and degradation of marine microbes impact the transfer of organic compounds to SSA and, consequently, impact its chemical composition and properties, as demonstrated by laboratory mesocosm experiments.^{4,14,47–50}

Prior mesocosm studies induced phytoplankton blooms to assess how phytoplankton and HB together affect the chemical composition of SSA. To improve the overall understanding of how specifically HB affect the chemical composition of SSA,

the 2018 Biological Effects on Air Sea Transfer (BEAST) microcosm experiment added HB to study their effect on SSA composition. During phytoplankton blooms, SSA was generated in marine aerosol reference tanks (MARTs)⁵¹ with and without three strains of marine HB added after the concentration of chlorophyll *a* (chl *a*) began decreasing. Our aim is to quantify the effect of HB on the enrichment of saccharides in the SSA. The production of gaseous organo-sulfur species and their reliance on the biological and chemical factors in the seawater during this experiment are discussed elsewhere.⁵² Here, we examine seawater phytoplankton via chl *a* concentrations and HB abundance by counts, the size-resolved concentrations of saccharides in the seawater and SSA, and saccharide EFs. We also measure five dominate marine enzyme classes; while these classes do not act on saccharides, we use their summed enzyme activities to infer a trend for CAZymes. In doing so, we gain insight into how the addition of three specific HB strains affect the concentration of individual saccharides in seawater and the resulting impact on saccharide enrichment in SSA. Our experimental design compares two phytoplankton blooms (numbered 2 and 3) with additions of HB increasing bacterial abundance 0.01 and 25% of the natural level, respectively.

■ EXPERIMENTAL SECTION

Microcosm Experiment. In the 2018 Biological Effects on Air Sea Transfer at the Scripps Institution of Oceanography (SIO) in La Jolla, CA, U.S.A., three separate phytoplankton blooms were studied. This paper focuses on the two phytoplankton blooms that occurred between August 4–15 and 22–30, referred to as “microcosm 2” and “microcosm 3”, respectively. The seawater sampling, cleaning procedures, and MART parameters used in this study were previously described in detail elsewhere.⁵³ On day 1 of each microcosm, seawater was collected at the Ellen Browning Scripps Memorial Pier, filtered with a 50 μm mesh, and transferred to a 2400 L outdoor tank.

To induce a phytoplankton bloom, f/2 algae growth medium (Proline, Aquatic Eco-Systems, Apopka, FL, U.S.A.) and sodium metasilicate nutrients were added. A phytoplankton bloom developed in natural sunlight, and then 120 L was transferred to each of three MARTs to generate SSA.⁵¹ To monitor biological activity in the water, *in vivo* chl *a* was tracked via fluorescence (Turner Designs Aquafluor). While chl *a* increased, the MARTs were filled with 120 L of the water from the outdoor tank each morning and returned to the outdoor tank at the end of the day. This transfer provided a common source of water (true biological replicates) across the multiple MARTs that was exposed to natural sunlight and reduced potential inhibition of phytoplankton caused by MART plunging and light limitation. The MART headspace was purged with zero air (Sabio Instruments 1001)⁵⁴ before resuming SSA generation and sampling for the day.

Once chl *a* in the outdoor tank decreased, the water was left in the MARTs and no longer remixed with the outdoor tank. The control tank was left unperturbed, while to the HB tank, three strains of HB were added: *Alteromonas* sp. (AltSIO), *Pseudoalteromonas* (ATW7), and *Flavobacterium bacterium* (BBFL7) at 10^8 cells per strain per MART. These strains were isolated in the Azam laboratory at SIO from water samples collected from the same location. They were selected for this experiment as a small group of native strains known to express significant levels of enzymes. The γ -proteobacteria,

Pseudoalteromonas species ATW7, and the sphingobacteria, *Flavobacteria* BBFL7, were chosen for their intense protease activities that are effective at the dissolution of particulate silica from diatom frustules.⁵⁵ The γ -proteobacteria, *Alteromonas* strain AltSIO, was chosen for its comprehensive processing of dissolved organic material.⁵⁶

Seawater and SSA Sampling/Collection. Seawater samples were collected daily from the spigot on the side of each MART at a depth of 8 in. SSA was collected at ambient relative humidity (RH, 65–89%) by a five-stage Sioutas Personal Cascade Impactor (PCIS, SKC model 225-370, with 50% cutoff aerodynamic diameters of 2.5, 1.0, 0.5, 0.25, and 0.25 μm) at 9 L min^{-1} using clean air as described above. Samples were collected on substrates consisting of 25 mm polytetrafluoroethylene (PTFE) substrate (Zefluor, 0.5 μm , PALL Life Sciences) for the top four stages and pre-baked 37 mm quartz fiber filter (QFF, PALL Life Sciences) for the after filter. For each microcosm, three sets of samples were collected for 3–5 days each to try to capture three distinct periods of the phytoplankton bloom. At 1 h before sampling each day, filters were pulled from cold storage, thawed, impacted for 2–3 h, and refrozen at $-20\text{ }^\circ\text{C}$. Field blanks were obtained before the start of the first and third set for both microcosms. All samples were kept frozen ($-20\text{ }^\circ\text{C}$) prior to analysis. For HB counts, SSA was collected via impingement into liquid without bubbling (SS110A Universal Spot Sampler in Liquid Spot Sampler configuration, Aerosol Devices, Inc.).

Sample Preparation and Analysis of HB. HB abundances were obtained by flow cytometry at The Scripps Research Institute (TSRI) Flow Core Facility. All samples were prepared by pipetting samples into cryogenic vials and preserved using 10% electron-microscopy-grade glutaraldehyde. Samples were then incubated at $4\text{ }^\circ\text{C}$ for 10 min, followed by flash freezing in liquid nitrogen and then storing at $-80\text{ }^\circ\text{C}$. Samples analyzed via flow cytometry (ZES Cell Analyzer, Bio-Rad) for HB were first diluted (1:10) in $1\times$ TE buffer (pH 8) and then were stained with SYBR Green I at room temperature for 10 min (at a 10:4 dilution of the commercial stock) in the dark.⁵⁷ HB populations were discriminated on the basis of their signature in the FL1 (488 nm laser, green fluorescence) versus SSC-specific cyto-grams.^{58,59}

Sample Preparation and Analysis of Enzyme Activities. Enzyme activities were measured on seawater samples using fluorogenic substrate analogues at saturating concentrations (24 μM).⁶⁰ Leucine protease, serine protease, oleate lipase, stearate lipase, and alkaline phosphatase activities were measured with L-leucine-7-amino-4-methylcoumarin hydrochloride, L-serine-7-amido-4-methylcoumarin hydrochloride, 4-methylumbelliferone oleate, 4-methylumbelliferone stearate, and 4-methylumbelliferone phosphate, respectively. Fluorescence of the enzymatic release of the fluorophores 4-methylumbelliferone and 7-amino-4-methylcoumarin was measured with a BioTek Synergy H1 multimode microplate reader at excitation/emission wavelengths of 360(40)/460(40) nm. Aliquots of seawater were pipetted into the wells of a 96-well microtiter plate, with each well containing one fluorogenic substrate. Fluorescence was measured initially and after 45 min of incubation in the dark at *in situ* temperature.

Sample Preparation for Analysis of Saccharides and Sodium. Aliquots of seawater samples were subjected to ultrafiltration accomplished using pre-cleaned [rinsed with ultrapure (UP, 18.2 M Ω cm, Thermo Barnstead EasyPure II)

water, ethanol, and UP water immediately before use] commercially available centrifuge tubes containing filter units centrifuged in series (Allegra X-30R centrifuge with a SX4400 swinging bucket rotor). The filtration size cuts were 200 nm [polyether sulfone (PES) PALL Corporation Microseep Advance], 6 nm (100 kDa, PES, Sartorius Vivaspin 6), and 2 nm (3 kDa, PES, Millipore Sigma Amicon Ultra-4) that were chosen to yield truly DOM ($<2\text{ nm}$), two fractions of colloidal DOM (2–6 and 6–200 nm), and particulate organic matter ($>200\text{ nm}$). Size cuts based on molecular weights are approximately determined on the basis of the minimum radius.⁶¹

The extraction and hydrolysis techniques employed here were previously described.¹⁴ Briefly, low mass loadings necessitated that filters were combined into two extractions that enabled analysis of saccharides in sub-micrometer ($<1\text{ }\mu\text{m}$) and super-micrometer ($>1\text{ }\mu\text{m}$) SSA. PTFE substrates were pre-wet with 50 μL of acetone and then extracted into UP water by 30/40/30 min of shaking/sonication/shaking. A 4.0 mL extraction volume was used for sub-micrometer SSA samples, while 3.0 mL was used for super-micrometer SSA. Unfiltered and ultrafiltered seawater as well as SSA extracts were hydrolyzed with 0.1 M trifluoroacetic acid (TFA) at $100\text{ }^\circ\text{C}$ for 12 h and then filtered with a 0.45 μm filter (polypropylene, Whatman).¹⁴

Quantification of Saccharides and Sodium. SSA extracts as well as size-fractionated seawater samples (total, <200 , <6 , and $<2\text{ nm}$) were analyzed via high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD, ICS 5000, Dionex) following a previously described method.¹⁴ Briefly, saccharides were separated using a 0.480 mL min^{-1} isocratic flow of 27.5 mM sodium hydroxide (Fisher) with a Dionex AminoTrap guard column and CarboPac PA20 analytical column. Nine saccharides were identified and quantified using standards, rhamnose, mannose, ribose (Across Organics), fucose (Alfa Aesar), galactose (Fisher), arabinose, xylose, fructose (Sigma-Aldrich), and glucose (TCI), with seven-point calibration curves. Size-resolved concentrations of saccharides in seawater were calculated. Sodium in SSA extracts and filtered seawater was quantified using ion-exchange chromatography with conductivity detection (ICS 5000, Dionex) following a previously described method.⁶²

RESULTS AND DISCUSSION

Biological Activity in Microcosm 3. During the peak of the phytoplankton bloom in microcosm 3, chl *a* concentrations peaked at $12.6\text{ }\mu\text{g L}^{-1}$ on day 3 (Figure 1), with HB counts peaking 2 days afterward. On the basis of the concentration of chl *a*, the three SSA periods sampled were designated “pre-bloom”, “phytoplankton bloom”, and “bloom decline” (Figure 1). On day 5, three bacterial strains of HB, AltSIO, ATW7, and BBFL7 were added to the “HB tank”. This addition initially increased counts of HB by 25% (to a maximum of 2.3×10^7 cells mL^{-1}) compared to the control. However, this tank subsequently crashed, decreasing HB abundance by $>90\%$ on day 7. In contrast, HB levels in the control declined gradually by 50% by day 9.

Summed enzyme activity represents the sum of the measured activities of several dominant marine enzyme classes: leucine protease, serine protease (preferentially hydrolyzes proteins at leucine and serine, respectively), oleate lipase, stearate lipase (preferentially hydrolyzes the oleate and stearate

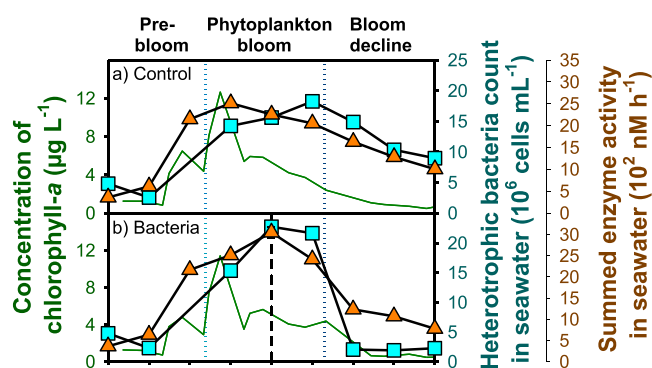


Figure 1. Temporal variation during microcosm 3 in the seawater of the (a) control tank and (b) HB tank for chl *a* (solid green line), HB abundance (blue square), and summed enzyme activity (orange triangle). The black dashed line indicates when the HB isolate strains were added.

lipids, respectively), and alkaline phosphatase (dephosphorylates compounds). Measured summed enzyme activity was overall similar between the two tanks; however, the added HB increased enzyme activity by 26% (to a maximum of 3.1×10^3 nM h⁻¹) compared to the control. Like HB, the summed enzyme activity decreased at a faster rate compared to the control, although not as fast as the decline in the HB population. The enzymes measured do not process saccharides; rather CAZymes, such as α -glucosidase and β -glucosidase, that preferentially cleave glucose linkages to break down complex saccharides affect the temporal composition of saccharides in the seawater and SSA. While CAZymes were not measured directly, they were expected to trend with the measured summed enzyme activity and, on the basis of prior literature, could contribute an additional 6–35% on top of this measured activity.^{45,63} In the seawater, 60–80% of heterotrophic marine isolates can express CAZymes.^{43,44} Thus, the control tank provides a measure of saccharides during the natural progression of phytoplankton and HB in an unperturbed phytoplankton bloom, while the HB tank provides insight into changes in the concentration and transfer of saccharides as a result of elevated levels of HB and enzymatic activity, followed by rapid declines in these levels.

Enrichment of Saccharides in SSA. Saccharides were enriched in SSA generated via a plunging waterfall via MARTs in the microcosms studied here, with EF greater in sub-micrometer SSA compared to super-micrometer SSA (Table 1). The observation of increasing enrichment with decreasing SSA size is consistent with prior studies of saccharides^{14,24} and more broadly surface-active organic matter.^{11,25,26} Overall, the enrichment of saccharides in the bacteria tank is consistently higher than the control tank; differences in EF among HB and control tanks are discussed in the context of specific classes of saccharides in the following sections.

Energy-Storage Saccharides. Phytoplankton store energy primarily in glucose-containing polysaccharides that span oligomers (<2 nm) to polymers (>200 nm) in the seawater.^{32,39,40,68} During microcosm 3, glucose was the dominant saccharide observed in seawater during the phytoplankton bloom (panels a and b of Figure 2 and Table S1 of the Supporting Information). The peak concentration of glucose coincided with the maximum level of chl *a* because phytoplankton rapidly produce labile glucose-containing polysaccharides for energy storage.^{32,39,40} Glucose found in seawater particles with sizes ranging from <2, 2–6, and to a lesser extent >200 nm increased during the phytoplankton bloom. Subsequently, glucose declines as phytoplankton lyse and HB use these polysaccharides.⁴² In the HB tank, glucose in 2–6 nm seawater particles increased 1.8 times more than the control tank at the peak of the bloom. Glucose in 2–6 nm seawater particles also remained at a higher relative concentration in the HB tank compared to the control, suggesting that the CAZymes released by added HB, which we assume to be present, preferentially consumed or modified glucose in larger particles.^{31,56}

Maximum concentrations of glucose in SSA coincided with peak concentrations of glucose and chl *a* in the seawater (panels c and d of Figure 2). In the control tank, concentrations peaked in super-micrometer SSA, whereas in the HB tank, concentrations peaked in sub-micrometer SSA. This difference suggests that CAZymes from the added HB strains increased the amount of glucose-containing polymers in the seawater, which then transferred preferentially to sub-micrometer SSA, and/or HB modified their surface activity (i.e., through incorporation of uronic acids that can bind to

Table 1. Range of EFs for Saccharides in Sub-micrometer (<1 μ m) and Super-micrometer (>1 μ m) SSA across Microcosms 2 and 3

analyte	EF in SSA (range)				microbial role
	control		HB		
	sub-micrometer	super-micrometer	sub-micrometer	super-micrometer	
Energy-Storage Saccharides					
glucose	700–20000	93–780	8300–420000	280–940	energy storage ^{a,b,c}
fructose	1700–14000	150–880	1800–500000	280–3700	energy storage ^{b,d}
Structural Saccharides					
galactose	270–1900	160–470	1200–20000	77–680	cell wall ^{a,b,e}
xylose	430–1700	110–400	880–42000	380–2600	cell wall, stress ^{a,b,e,f}
mannose	BDL ^g –1200	37–270	54000–80000	300–1700	cell wall, HB ^{a,b}
arabinose	730–1200	150–820	760–50000	200–2300	stress, HB ^{a,b}
rhamnose	1900–2400	190–710	BDL–8600	250–3400	stress, HB ^{a,b}
fucose	BDL–100	85–430	BDL	140–830	stress ^f
ribose	2700–4900	300–1200	5600–930000	1700–2200	DNA, HB ^{h,i}

^aFrom ref 32. ^bFrom ref 39. ^cFrom ref 40. ^dFrom ref 64. ^eFrom ref 41. ^fFrom ref 65. ^gBDL indicates below the detection limit. ^hFrom ref 66. ⁱFrom ref 67.

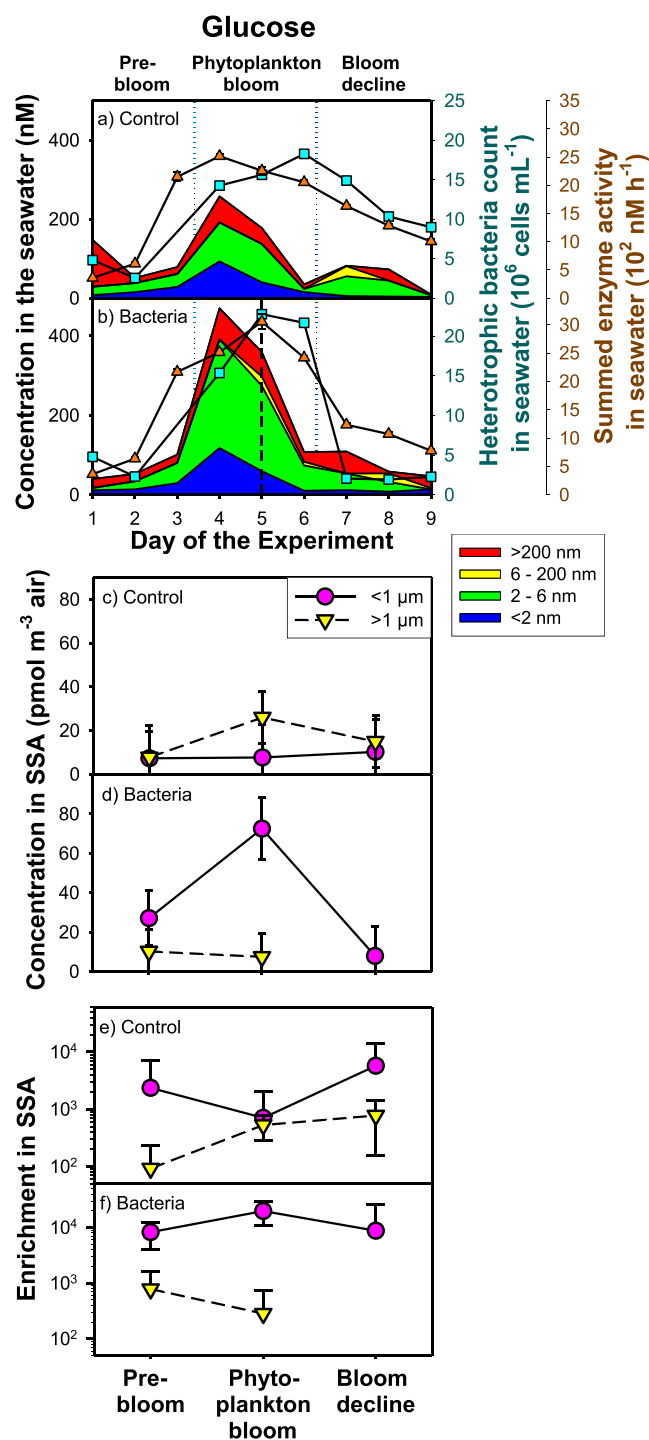


Figure 2. (a and b) Glucose concentrations, HB cell abundance (blue square), and summed enzyme activity (orange triangle) in the seawater of microcosm 3, with the black dashed line marking the addition of HB. For the three time periods studied, (c and d) glucose concentrations and (e and f) EFs in sub- and super-micrometer SSA.

divalent cations and increase enrichment^{21,23,46}). HB can influence the quantity and composition of polysaccharides produced by phytoplankton through CAZymes that hydrolyze larger polysaccharides (i.e., >200 nm) into smaller sizes^{64,68,69} as well as directly modify and release distinct polysaccharides.^{30,46,70} Through some combination of these processes, the added HB increased the concentration of glucose-containing energy-storage polysaccharides in sub-micrometer SSA. In

addition to glucose, energy-storage polysaccharides from specific phytoplankton species may also include fructose, galactose, mannose, fucose, xylose, and arabinose.⁶⁸

EFs help elucidate whether the trends observed in SSA scale with the trends observed in seawater. EFs of glucose are similar throughout the microcosm; however, the two tanks exhibit different trends in EFs during the phytoplankton bloom (panels e and f of Figure 2). The EF of glucose in sub-micrometer SSA of the HB tank increased in comparison to the control as a result of the bacterial influence that increased the concentration of glucose in sub-micrometer SSA. In the HB tank, an increase of glucose in 2–6 nm seawater particles (panels a and b of Figure 2) coincides with the increase in glucose concentrations and enrichment in SSA. Together, these data show that glucose in 2–6 nm seawater particles, which could be whole polymers or aggregated gels, are selectively transferred to SSA. This increase in enrichment implies that higher HB abundance and enzyme activity in seawater generates highly enriched glucose-containing saccharides in SSA. This finding agrees with Jayarathne et al.,¹⁴ who observed that saccharides were more enriched in SSA during a phytoplankton bloom with higher HB abundance. The EF of glucose in sub-micrometer SSA during the bloom decline returns to observed pre-bloom levels, coincident with a large decrease in HB abundance in seawater. Together, these results provide new insight into the size-dependent transfer of saccharides to SSA, in which marine-derived glucose-containing particles 2–6 nm are enriched; we hypothesize that CAZymes released by HB actively modify this fraction of glucose-containing saccharides, causing them to preferentially transfer to sub-micrometer SSA and leading to increases in their enrichment.

Structural Saccharides. Monomers that comprise structural polysaccharides form cellular components, such as cell walls (i.e., xylose, galactose, and mannose)^{32,39,41,46,68} and DNA (ribose),^{66,67} while others are released as exopolymeric substances when nutrients become limited (xylose and fucose)⁶⁵ and by HB and stressed phytoplankton (arabinose and rhamnose).^{32,39} Among these structural saccharides, xylose, galactose, and fucose exhibited similar trends in seawater and SSA (panels a and b of Figure 3 and Figure S1 of the Supporting Information). Xylose is discussed in detail because it was detected most frequently.

Xylose comprises portions of polysaccharide-containing cell walls^{32,39,41} and stress-related exopolysaccharides.⁶⁵ During microcosm 3, the concentration of xylose in the seawater peaked after chl *a*, when phytoplankton death outpaced growth. In the control tank (Figure 3a), xylose peaked during the bloom decline period, suggesting decaying phytoplankton as the likely origin. In the HB tank (Figure 3b), the concentration of xylose peaked, coincident with the addition of HB, suggesting the release of xylose, particularly in particles of 6–200 nm, by stressed phytoplankton or CAZymes associated with HB. Because the phytoplankton–HB relationship ranges from cooperative to antagonistic, phytoplankton modify the quantity of exudates that they release based in part on HB abundance and composition.^{31,71,72} Further, some HB release enzymes that can lyse cell walls^{31,73} that could release xylose-containing saccharides. The concentration of xylose in the seawater decreased more slowly than glucose, reflecting slower modification of structural saccharides by CAZymes and utilization by HB.^{32,39,41}

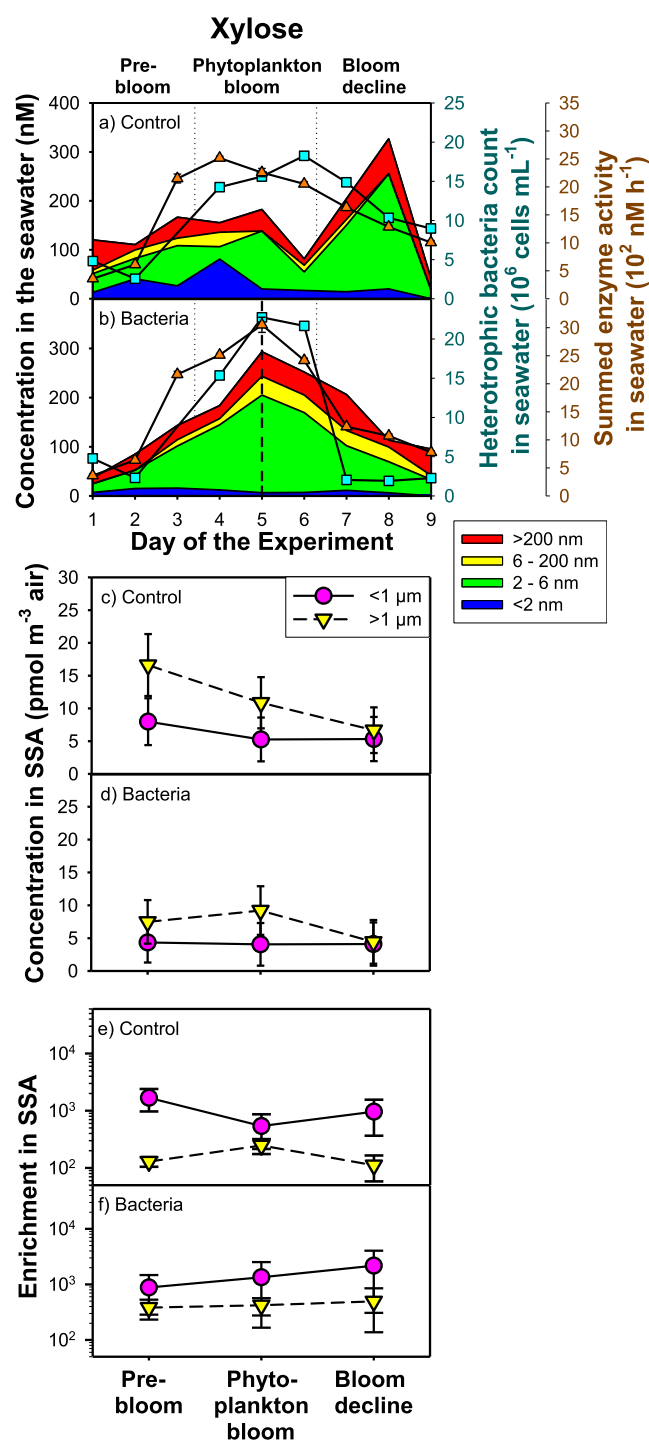


Figure 3. (a and b) Xylose concentrations, HB cell abundance (blue square), and summed enzyme activity (orange triangle) in the seawater of microcosm 3, with the black dashed line marking the addition of HB. For the three time periods studied, (c and d) xylose concentrations and (e and f) EFs in sub- and super-micrometer SSA.

The concentrations of xylose in SSA exhibited small changes in concentration during the microcosm experiment, despite the addition of bacteria (panels c and d of Figure 3 and Table S2 of the Supporting Information). This observation contrasts that of glucose, suggesting that the concentration of xylose-containing particles of >2 nm in SSA are less sensitive to HB in seawater relative to energy-storage saccharides. Likewise, the relative change in the EF of xylose in SSA as a result of added

HB strains was small compared to energy-storage saccharides (panels e and f of Figure 3 and Table S3 of the Supporting Information). During the phytoplankton bloom, the EF of xylose in sub-micrometer SSA trended downward in the control tank and upward in the HB tank. This upward trend coincided with increased xylose-containing particles of 2–6 nm, suggesting that HB induced earlier release of these polysaccharides into the seawater compared to the control tank. However, this release resulted in a small increase in the EF of xylose in sub-micrometer SSA. In comparison to glucose, the small increase in the EF of xylose suggests slower processing of xylose-containing polysaccharides by CAZymes that led to less enrichment in sub-micrometer SSA compared to energy-storage saccharides.

Additionally, EF of xylose in super-micrometer SSA trended downward in the control tank and upward in the HB tank, suggesting that CAZymes released by HB slowly modified xylose-containing saccharides in the seawater that increased their transfer to super-micrometer SSA. Prior studies observed time lag between elevated chl *a* in the seawater and elevated transfer of organic compounds to SSA.⁵³ This study suggests at least a 2 day lag before bacterial modification of organic matter impacts SSA.

HB and stressed phytoplankton produce arabinose- and rhamnose-containing extracellular polysaccharides.^{32,39} Arabinose and rhamnose exhibit similar trends in seawater and SSA (panels a and b of Figure 4 and panels e and f of Figure S1 of the Supporting Information); the more frequently detected arabinose is discussed in detail. The concentration of arabinose in seawater peaked after peak chl *a* (panels a and b of Figure 4). In the control tank, the concentration peaked during the bloom decline, where HB abundance was high and phytoplankton were likely stressed. In the HB tank, the concentration peaked during the phytoplankton bloom, coinciding with added HB strains and peak summed enzyme activity. The HB added likely increased phytoplankton stress, and both HB and stressed phytoplankton released arabinose-containing polysaccharides.^{30–32,42} As the abundance of HB in seawater rapidly dropped during the bloom decline of the HB tank, the concentration of arabinose in the seawater also decreased and shifted to particles of >200 nm, suggesting that the remaining arabinose-containing polysaccharides were released by phytoplankton and HB as exopolymers and particulate detritus as they senesced and perished.^{32,39} This more rapid decrease compared to that of xylose-containing saccharides suggests that arabinose-containing saccharides were more labile. These measurements reaffirm a strong link between arabinose-containing polysaccharides and HB abundance.^{14,32,39,65,74}

The concentration of arabinose in super-micrometer SSA exhibited similar trends in both tanks, despite different trends in the seawater (panels c and d of Figure 4 and Table S2 of the Supporting Information), suggesting that, similar to xylose, the concentrations of arabinose in SSA were affected less by HB added in comparison to energy-storage saccharides. The EF of arabinose in super-micrometer SSA of both tanks trended upward during the phytoplankton bloom (panels e and f of Figure 4 and Table S3 of the Supporting Information). During the bloom decline, the EF of arabinose in super-micrometer SSA trended downward in the control tank and upward in the HB tank. Further, the increased EF of arabinose in super-micrometer SSA during the bloom decline of the HB tank coincided with elevated concentrations of arabinose in

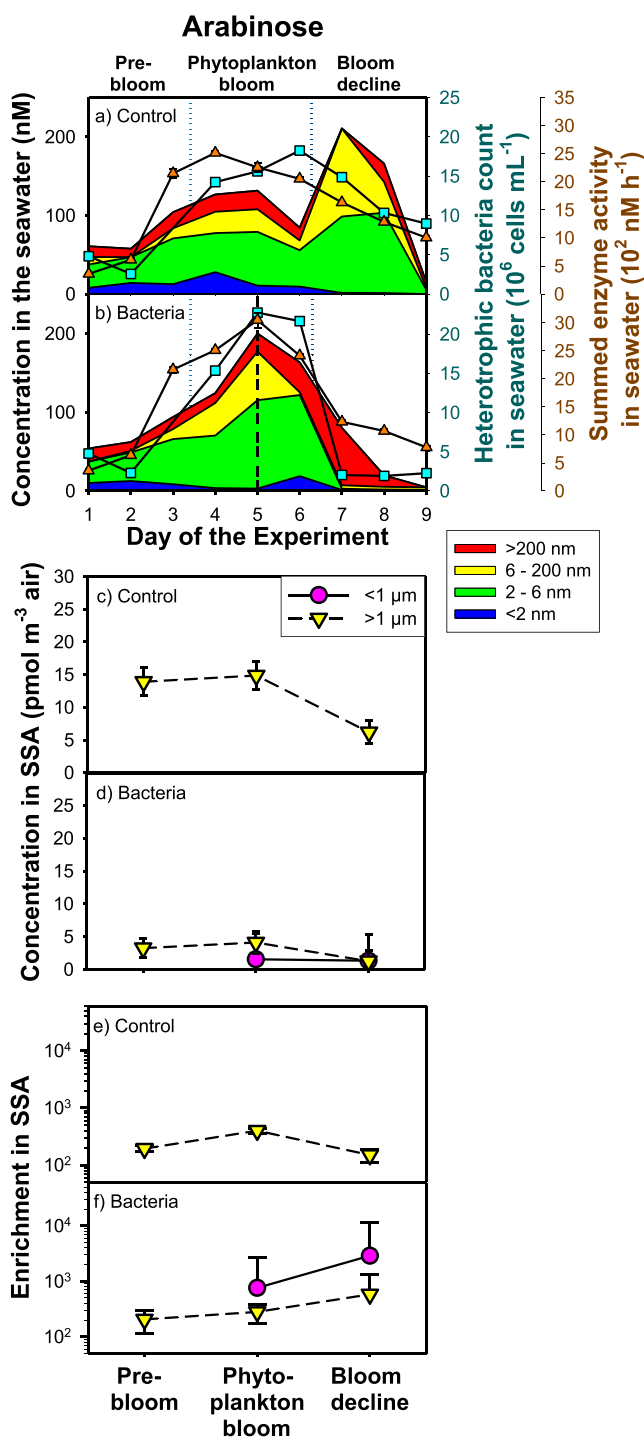


Figure 4. (a and b) Arabinose concentrations, HB cell abundance (blue square), and summed enzyme activity (orange triangle) in the seawater of microcosm 3, with the black dashed line marking the addition of HB. For the three time periods studied, (c and d) arabinose concentration and (e and f) EFs in sub- and super-micrometer SSA. Excluded data points were below the detection limit.

seawater particles of >200 nm, whereas, in the control tank, the decreased EF of arabinose in super-micrometer SSA coincided with an elevated concentration of arabinose in seawater particles of 2–6 nm. This comparison suggests that the rapid decline of the HB population following the addition of HB released arabinose-containing particles of >200 nm that were enriched in super-micrometer SSA.

Comparison of Energy-Storage Saccharides to Structural Saccharides. Energy-storage and structural polysaccharides trended differently in seawater during the phytoplankton bloom. In this study, a larger fraction of energy-storage saccharides in the seawater are <2 nm, which is consistent with the synthesis of smaller oligomers and quick break down observed in prior work.^{32,39,40} In contrast, a larger fraction of structural saccharides in the seawater comprise particles of >6 nm in this study, which is consistent with larger exopolymeric particles and cellular components.^{32,39,40,74}

The concentrations of structural saccharides in super-micrometer SSA were higher than in sub-micrometer SSA compared to energy-storage saccharides that exhibited less difference in concentrations between the two size fractions (Table S2 of the Supporting Information). This observation agrees with prior studies that suggest that these structural saccharides are transferred to larger SSA particles via jet drops.^{14,27} In super-micrometer SSA, the EFs of saccharides ranged from 10^1 to 10^3 (Table 1). In comparison, saccharides in sub-micrometer SSA ranged from 10^2 to 10^4 (Table 1); the higher EFs of sub-micrometer SSA are likely driven by film drop production that incorporates more surface-active organic matter.^{6–8,75} Comparing the trends in EF of glucose and xylose (panels e and f of Figures 2 and 3, respectively) highlights that CAZymes from the added HB increased the transfer and enrichment of glucose more than structural saccharides. CAZymes released from HB likely took longer to produce and/or modify these less labile structural compounds, as evident by the lag in enrichment of both xylose and arabinose and in agreement with prior works that highlight glucose as an important substrate for bacterial enzymatic processes.⁴²

Comparison of Microcosms 2 and 3 and Prior Studies. Results from microcosm 3, discussed in detail above, are compared and contrasted here to microcosm 2. Experimentally, microcosms 2 and 3 were run under identical experimental conditions, with the following exceptions: (1) the initial microbe populations differed across seawater collected on different days, which resulted in microcosm 2 having lower concentrations of phytoplankton and HB (Figure S2 of the Supporting Information), and (2) the addition of three bacterial strains to microcosm 3 increased bacterial abundance substantially (by 25%), whereas in microcosm 2, this HB addition was negligible (0.01%). Temporally, the phytoplankton and HB trended similarly between both microcosms, and in both cases, the levels of these microorganisms were within the range of natural seawater (Table S1 of the Supporting Information).^{11,14,53,76}

In both tanks of microcosm 2, the general trends in the concentrations and EFs of saccharides in sub-micrometer SSA matched those of the control tank of microcosm 3 (Tables S4 and S5 of the Supporting Information). First, the concentration and EF of glucose-containing saccharides increase as the phytoplankton bloom declines. The magnitude of the increase in the EF of glucose was not as great in microcosm 2, likely as a result of the substantially smaller addition of HB. The sub-micrometer enrichment of xylose-containing saccharides also increases into the bloom decline; however, this increase is much less pronounced than for glucose. The size distribution of saccharides were also similar across the two microcosms (Figures S1 and S3 of the Supporting Information), indicating that, for this study, the majority of saccharides from two different seawater samples likely underwent similar production and processing by the

phytoplankton and HB. The similarity in these trends across both microcosms supports that processing of saccharides by CAZymes contributes to increases in their enrichment in sub-micrometer SSA. In both microcosms, this increase in enrichment is more prominent for glucose compared to xylose, suggesting that HB impact the enrichment of energy-storage saccharides to a greater extent than structural saccharides.

In contrast to microcosm 3, microcosm 2 had lower concentrations of saccharides in the seawater (Table S1 of the Supporting Information) and higher concentrations in SSA, yielding higher EFs. The greater enrichment of saccharides in microcosm 2 may result from a steep concentration gradient in organic matter at the seawater surface, which occurs in cases of low seawater concentrations of surface-active material.^{27,75} Between the two microcosms, the trends in the EFs of saccharides in super-micrometer SSA were more variable than for sub-micrometer SSA, particularly in the control tank. For example, the EF of xylose-containing saccharides in super-micrometer SSA of the control tank decreases during the bloom decline of microcosm 2, opposite of the trend for the same period of microcosm 3. The greater variability in super-micrometer particles likely arises in part from the inclusion of marine microbes in SSA (that are too large to be in sub-micrometer SSA),^{10,77} which imparts greater chemical heterogeneity. This heterogeneity is exacerbated by varying microbial populations in different microcosm experiments and after addition of HB.

The EFs observed in both microcosms of this study are 1–2 orders of magnitude higher than those observed in a 2014 mesocosm study (where EF ranged from 3 to 130 for particles of 2.5–10 μm and from 10 to 1300 for particles of <2.5 μm) that also sourced seawater from the Scripps Pier and observed similar concentrations of saccharides in seawater.¹⁴ The increase in EFs observed here derives from the smaller particle size range studied and the increase in the saccharide enrichment with a decreasing particle size.¹⁴ Specifically, the size cutoffs of <2.5 and 2.5–10 μm used in 2014 yield smaller EFs than the sub- and super-micrometer cutoffs used here. Additional factors that contribute to differences in EF across studies include (a) differing microbial populations in each experiment, (b) stress on marine microbes by the MART pump, which likely impacts their speciation, abundance, and behavior,^{51,54} (c) the development of phytoplankton blooms in natural sunlight with a diel cycle in this study compared to 2014 when phytoplankton blooms were developed under 24 h of artificial light,⁷⁸ and (d) minor differences in the size distribution of SSA generated by a paddle in a wave flume and the plunging waterfall in MART.⁵¹

Ribose is released during the enzymatic breakdown of nucleic acids,^{64,67} which are a large component of HB cells released during cell lysis.^{67,79} During the bloom decline of microcosm 2, ribose exhibited an EF of 900 000 that coincided with HB added. Jayarathne et al.¹⁴ observed that ribose was also associated with high HB counts; however, it was not enriched in SSA more than other saccharides.¹⁴ Recently, a study off the coast of Northern Ireland reported an EF of 30 000 for DNA in <1.2 μm marine aerosol.²⁴ Together, these data suggest very high enrichments of DNA and nucleic acids in SSA. However, because of the limited data points in this study, further investigation is needed.

HB Transfer to SSA. Measurements in microcosm 2 demonstrate that HB are transferred to SSA, which is important as a result of the ability of these HB to modify

the composition and size of saccharides in the atmosphere. The HB counts were consistently higher (even before HB added) in SSA generated from the HB tank compared to the control (Figure 5). Absolute differences in the seawater (10^5 –

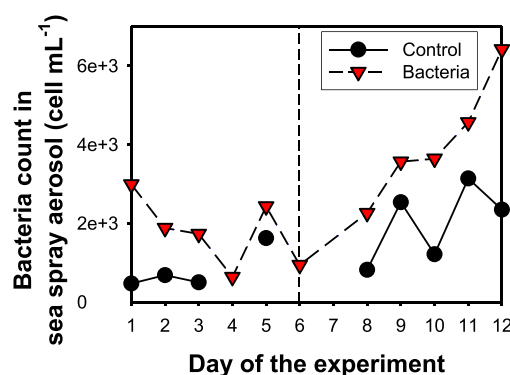


Figure 5. HB abundance in total suspended SSA particles during microcosm 2. Control tank samples from days 4, 6, and 7 were below the detection limit.

10^7 cells) overshadow absolute differences in the SSA (10^2 – 10^3 cells); however, even on days where the HB count is slightly higher in the seawater of the control tank compared to the HB tank (i.e., days 10–12), the HB counts in the SSA of the HB tank were always higher than the control. The transfer of HB to SSA is selective,^{24,80} suggesting that the two tanks exhibited different microbial control after HB addition in microcosm 2. Marine aerosol can contain whole HB,^{81,82} these cells could rupture, directly releasing their contents into SSA. Further, HB in aerosol may be active,⁸³ and active microbial enzymes were detected in SSA,⁸⁴ which could cause continued processing of saccharides and other organic matter in SSA. While HB counts in the SSA were not obtained for microcosm 3, the direct transfer of bacteria to SSA and subsequent release and processing of saccharides in SSA likely occurs there as well. These processes in the SSA likely affect the composition of saccharides in SSA and, thus, their enrichment.

Implications. This work reports that specific HB strains added to a phytoplankton bloom led to increased enzymatic activity that modified the transfer and enrichment of saccharides into SSA. The magnitude of this effect depends upon the saccharide (e.g., up to 4 orders of magnitude in sub-micrometer SSA during a single collection period), reflecting the diverse composition of saccharides in marine microbes and functions of saccharides into cellular and extracellular materials. As a complement to prior work that demonstrates high enrichments of total saccharides in SSA,²⁴ this work demonstrates that individual saccharide enrichments can vary widely. The large addition of HB to microcosm 3 triggered a large increase in enzyme activity that substantially increased the enrichment of energy-storage saccharides (i.e., glucose) and ribose (i.e., from RNA) in SSA. This HB addition also increased the enrichment of structural and stress-related saccharides, such as xylose and arabinose, respectively, however, with substantially lower EF. This result reflects chemical and/or physical selectivity in the enrichment of some saccharides in SSA. In microcosm 3, the enrichment of both xylose and arabinose in super-micrometer SSA lagged after the addition of HB by at least 2 days, which is likely due to slower processing by HB.⁵³ The findings of this study help to explain the high variability of saccharide EF observed in prior

studies^{14,24} as a result of phytoplankton and HB populations. Consequences of the modifications of saccharides by HB include changes to the properties of SSA that impact the Earth's climate (e.g., hygroscopicity and CCN and IN activities).^{15,17,18,31,85}

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsearthspacechem.0c00167>.

Range and average concentration of the total saccharides quantified in seawater during microcosms 2 and 3 (Table S1), concentrations of saccharides in seawater for galactose, fucose, rhamnose, fructose, mannose, and ribose during microcosm 3 (Figure S1), concentrations of sodium and saccharides in SSA collected during three periods over the course of microcosm 3 (Table S2), EFs of saccharides in SSA calculated for three periods over the course of microcosm 3 (Table S3), temporal variation during microcosm 2 in the seawater of the control tank and HB tank for chl *a* and HB abundance (Figure S2), concentrations of saccharides in seawater for galactose, fucose, rhamnose, fructose, mannose, and ribose during microcosm 2 (Figure S3), concentrations of sodium and saccharides in SSA collected during three periods over the course of microcosm 2 (Table S4), and EFs of saccharides in SSA calculated for three periods over the course of mesocosm 2 (Table S5) (PDF)

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Notes

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