VOC Emisions and Categorization in Axenic Growth Conditions by *Phaeodactylum tricornutum.*

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Abstract

Volatile organic compounds (VOCs) play an integral role in climate change and as a carbon source for organisms living in a marine environment. These compounds have many sources, but the impacts of biogenically produced VOCs is not fully known. *Phaeodactylum tricornutum* was studied under axenic ideal growth conditions to determine the amounts, ranges, and types of VOCs released. Through PTR-TOF/MS, 21 statistically significant compounds were found out of 348 detected m/z+1 values. These compounds included esters, ketones, hydrocarbons, and VOCs known to be used as secondary metabolites by cohabitating bacteria. Understanding The interaction between marine organisms, the environment, and the atmosphere is vital to understanding the carbon cycling events that take place there and how those events fit into the larger question of global climate change.

Introduction

What are VOCs?

Volatile organic compounds (VOCs) are a class of chemical compound that is light weight, often uncharged and sufficiently non-polar to diffuse across cell membranes and have synergistic and antagonistic impacts on biotic and abiotic environments. VOCs include several families of chemicals including ketones, aldehydes, terpenes, acids, and alcohols. VOCs are so named for their low boiling points, usually below 250 degrees Celsius. Extremely volatile compounds can boil between 0-100 degrees C (Montero-Montoya et al., n.d.). Due to their low molecular weights and lipophilic moieties these compounds can pass through cell membranes easily (Schulz-Bohm et al., 2017), causing toxicity in some cases and serve as signaling molecules or growth resources in other cases. Some VOCs are found in abundance in natural environments such as oceans, soils, sediments, forests, and agricultural areas, especially where photosynthesis occurs, as well as in urban and industrial areas. VOCs are produced in an array of ways, including synthesis through cell processes and normal pathways of organismal metabolism (Moore et al., 2020; Schulz-Bohm et al., 2017). Harmful effects of VOCs are both biotic and abiotic. For example, benzene and vinyl chloride are classified as Group 1 carcinogens by the International Agency for Research on Cancer (Montero-Montoya et al., n.d.),

and chloromethanes released into the atmosphere cause ozone depletion, in the stratosphere as well as creation in the troposphere (Tsai, 2017).

What roles do VOCs play in the environment?

Man-made VOCs are primarily produced for use as household cleaning chemicals, paints and paint thinners, and degreasers. These household sources constitute a large percentage of exposure to harmful VOCs to the public. Other sources of VOCs are burning of petroleum and natural gas. These activities release benzene and methane into the atmosphere, for example, which then cause harm to the environment.

VOCs facilitate communication between organisms representing different domains of life. For example, plant roots release certain volatiles to attract not only beneficial fungi but also bacteria which facilitate antipathogenic properties through competition. Bacteria in the rhizosphere can acquire carbon from the carbon-based VOCs given off by plant roots (Wenke et al., 2010). These bacteria in turn release compounds that fend off bacteria that are parasitic to the plant root. A fascinating use of plant generated VOCs is as an indirect defense against arthropod predators. The act of feeding on the root systems of some plants such as tulip bulbs by *Aceria tulipae* (a type of rust mite) caused the release of compounds which attracted female *Neoseiulus cucumeris*, a predator of the rust mites (Wenke et al., 2010). In addition to antibiotic properties, VOCs produced by microorganisms serve as growth stimulants for plants, fungi, and other bacteria. VOCs produced in the rhizosphere of soil promoted the growth of neighboring bacteria, specifically in motility (Schulz-Bohm et al., 2017), which served as antifungal agents to combat specific fungal plant pathogens. Further effects of bacteria-bacteria interactions promote increased resistance to antibiotics produced by other neighboring species. Volatiles such as ammonia, trimethylamine and others modulate biofilm generation and can increase motility, allowing bacteria to navigate to areas with less competition for food sources. Bacteria-plant interactions also involve beneficial chemical transfer. A varied colony of plant-growth promoting rhizobacteria (PGPR) were observed using volatiles to aid in plant antimicrobial resistance, root growth, seed germination, and root hair density, although it was noted further experimentation should be carried out in conditions mirrored closer to natural environmental habitats (Farag et al., 2013).

Fungi produce VOCs that impact their environment in a variety of ways. Saprophytic fungi from the *Trichoderma* and *Phoma* genera produce VOCs such as isobutyl alcohol, isopentyl alcohol, 2-methyl-propanol that promote plant growth through increased chlorophyll

and biomass production (Farh & Jeon, 2020). Aside from promoting plant growth directly, fungal VOCs include antifungal and antimicrobial agents as well as compounds that aid plants' tolerance to biotic or abiotic stressors. Pathogenic fungi produce a smaller array of unique VOCs that act primarily as info-chemicals which attract or repel certain species of bacteria (Farh & Jeon, 2020). A species of root rot fungus was found to produce terpenic VOCs which increased production of a chemotaxis protein in bacteria, enhancing its movement and enabling access to growth substrates. Certain fungal VOCs also enhance photosynthetic capabilities of the fungal host plant through post-transcriptional modifications to proteins used in photosynthesis, along with regulation of enzymes that facilitate the process (Ameztoy et al., 2019).

In certain species of bacteria, VOCs are produced as antibacterial antagonists (Schulz-Bohm et al., 2017). These VOCs have been harnessed to combat common plant pathogens as biocontrol agents. *Streptomyces albidoflavus* produced VOCs which were absorbed by *Bacillus subtilis*, causing a bacteriostatic effect in the latter (Schulz-Bohm et al., 2017). In another study, it was found that certain *Bacillus* spp. produced benzaldehyde and other compounds that inhibited transcription factors associated with motility in bacteria responsible for wilt disease (Schulz-Bohm et al., 2017)

VOCs appear to be promising compounds that could be detectable signatures of bacterial and viral infections in humans as well, through unique VOC generation which offers identifying information about the specific infection. Most studies that attempt to identify these organisms through this method have been so far unable to reliably produce unambiguous results, mostly due to some compounds being common between multiple organisms (Bos et al., 2013).

VOCs were also used as growth resources by bacteria living in a community with species of phytoplankton. *Pelagibacter* consumed the VOCs given off by the diatom *Thalassiosira pseudonana* (Moore et al., 2020). To confirm that the VOCs were metabolized by *Pelagibacter,* isoprene, acetone, hexanal, toluene, and acetonitrile were incubated with cultures of starved cells. The VOCs stimulated production of ATP. Bacterial VOC consumption induced stress in *T. pseudonana* because the pool of the VOCs produced by the diatom was apparently needed for diatom growth and was depleted by the bacteria.

Biotically generated VOCs contribute to global greenhouse gases, effecting the atmosphere through emissions of acetone, DMS, acetonitrile, methanol, and chloromethylated

VOCs (Davie-Martin et al., 2020; Tsai, 2017). These interactions with the atmosphere can have both positive climate feedbacks and destructive consequences. In the troposphere, VOC species interact with OH^- and NO_x to cause photochemical ozone formation. Ozone formation in the lower atmosphere is harmful to human health and the health of vegetation. Chloromethanes are also liable to react with OH^- to form carbonyl species, which are then hydrolyzed in the atmosphere into acid rain (Tsai, 2017). The amount of chlorinated VOCs that make it to the stratosphere is limited however due to OH^- attacks in the troposphere (Tsai, 2017).

Understanding how compounds are cycled between the marine and atmospheric environments allows for a greater understanding of global carbon budgets and contributions by biotic VOCs to greenhouse gases. *T. pseudonana* produces acetaldehyde, isoprene, and acetone, which are all common greenhouse gases, in light-driven reactions. These compounds are cycled not only into the atmosphere but can be consumed by bacteria in the abundant *Pelagibacter* family as growth resources (Halsey et al., 2017).

In the stratosphere, VOCs contribute to increased warming through greenhouse effects and ozone depletion due to photochemical reactions (Tsai, 2017). The release of VOCs from marine environments contributes to emissions on a level that is not thoroughly understood, but is drawing more attention recently (Davie-Martin et al., 2020; Halsey et al., 2017)

Why do cells make VOCs?

VOC production occurs because of a range of processes, and these pathways and their ultimate use are not entirely understood. Many VOCs seem to be produced as byproducts of metabolism (Kai et al., 2009; Moore et al., 2020), serving as a waste product which some organisms have evolved the capability to use. Other uses can generally be categorized by inter and intra kingdom communication signals, anti-biotics, and growth promoters (Kai et al., 2009).

Owing to the characteristics of VOCs, they may be produced and emitted from cells unintentionally which allows certain species to make use of them as growth substrates Some syntropic relationships form between archaea and bacteria to reduce CO^2 to methane. The partnerships between anaerobic methanotrophic archaea and sulfate reducing bacteria seems to be facilitated through the generation of VOCs, including methane thiol, allowing the bacteria to use the compounds produced by the archaea as an externally made electron supplied energy sinks (Skennerton et al., 2017)

Stated previously, many relations formed between bacteria and fungi, fungi and plants, and plants and bacteria utilize VOCs to engage in intra-kingdom communication, defend themselves from pathogens, or enhance the growth of their respective structures (Farag et al., 2013; Farh & Jeon, 2020; Schulz-Bohm et al., 2017; Wenke et al., 2010).

What algae are known to make VOCs?

Algae are single celled photosynthetic protists and bacteria that are known to produce a wide range of VOCs, although a thorough assessment of VOC production in this diverse group of organisms has not yet been done. It appears that green algae make the fewest volatile compounds, while red algae produce the most and brown algae, including the diatoms, produce a moderate amount. *Laurencia*, a type of red algae, is one of the more prolific producers of halogenated VOCs and is typically found in tropical, subtropical, and temperate coastal water zones (Cabrita et al., 2010). *Plocamium cornutum* is another species of red alga that produces unique halogenated compounds, including a novel species of chloroquine which is used in combating malaria causing parasites. *Callophycus serratus* also produces VOCs that seem to be effective antibacterials against resistant strains of *Staphylococcus aureus* and *Enterococcus faecium* (Cabrita et al., 2010). Brown algae seem to produce mainly terpenes, with an Australian species *Sargassum fallax* producing a fallachromenoic acid which showed promising antitumor properties in a strain of Leukemia cells.

Phytoplankton, which are also called algae, are currently receiving more attention regarding VOC production after evidence emerged about their roles in communal relationships with other microorganisms. Generally, phytoplankton is a broad term encompassing cyanobacteria, green and red algae, diatoms, haptophytes, dinoflagellates, as well as many other protists that are capable of photosynthesis. Cyanobacteria are of primary research interest due to their production of toxins during harmful algal blooms (Cabrita et al., 2010). Cyanobacteria are of particular research interest due to their ubiquity and involvement in the marine-atmosphere gas exchange. Photosynthetic organisms that fix carbon through light-driven processes play an important yet hitherto understudied role in VOC dynamics, ozone generation and depletion, and sea-air trace gas exchange.

What specific VOCs are made?

The array of volatiles produced by microalgae is incredibly diverse. VOCs fall into some major categories such as terpenoids, furans, sulfo compounds, alkanes, alkenes, alcohols,

aldehydes, ketones, and esters (Zuo, 2019). In co culture with *T. pseudonana, Pelagibacter* consumed acetone, hexanol and other chemicals to maintain ATP production. Isoprene, α-Ionone, 2-Methylisoborenol are examples of terpenoids, with 2-Methylisoborenol being one compound responsible for musty odors of lake water. *Thalassiosira weissflogii, T. pseudonana, Pleurochrysis carterae,* and *Rhodomonas salina* produced high levels of isoprene when kept at intense light levels, along with some monoterpenes (Zuo, 2019). After synthesizing these VOCs, cells commonly release them quickly due to a lack of storage ability. Halogenated hydrocarbons are also common in marine microalgae. CH_3I, $CHBrCl_2$, and CH_2I_2 are examples of compounds produced during times of intense sunlight. The increase in photosynthesis causes the productions of reactive oxygen species to increase as well, promoting the formation and emission of halogenated hydrocarbons.

Under what conditions do phytoplankton make VOCs?

Microalgae species produce more VOCs during times of intense sunlight, particularly midday (Zuo, 2019). During this time, isoprenes and monoterpenes are produced at increased rates, as well as halogenated hydrocarbons. Temperature plays a role in controlling the release rates of volatile compounds as well, with higher temperatures causing the release of C6 green leaf volatiles. These compounds are released because of the higher temperatures creating more reactive oxygen species. Ocean nutrients can regulate the emission of VOCs. For example, phosphorus is considered a limiting nutrient due to the propensity for it to precipitate in an insoluble salt in marine conditions, making it unavailable (Zuo, 2019). VOCs appear to be released to inhibit the growth of competing algae for this resource (ref). Stress responses play an important role in the emission of volatile compounds. For example, *Chlamydomonas reinhadtii* releases a diverse spread of VOCs when exposed to stressors like acetic acid and elevated levels of salt.

Different species of VOCs are also produced at different stages of bacterial growth. Aldehydes were typically produced in larger percentages in the declining phase of population growth, but *Dicrateria inornate* produced more aldehydes during exponential phase (Zhou et al., 2017). The main aldehydes produced were nonanal, decanal, and 2,4-pentadienal. Alkanes showed the opposite relationship to stage of growth and production, being mainly generated in the exponential phase. Alcohols did not have a standard pattern of generation as the other compounds did, with the stage associated with the highest levels being dependant on the tested species of algae. *Nitzschia clostrium, Platymonas helgolandi, D. inornate,* and *Chaeroceros*

calcitrans released alcohols in larger quantities in the stationary phase, whereas *Nannochloropsis* spp. releases the alcohol species in the declining phase.

To understand the VOC emission and categorization of phytoplankton, *Phaeodactylum tricornutum* was studied under ideal growth conditions. This study used PTR-TOF/MS was used to detect very small amounts of these VOCs and GLOVOCS was referenced to categorize the m/z+1 values into possible identities. The results show that *P. tricornutum* produces a range of VOCs in an axenic environment, mostly in the stationary phase. These data are important for understanding both the compounds released into the surrounding environment and clues to how the atmosphere or other organisms might be impacted by these emissions.

Materials and Methods

Culture Conditions

P. tricornutum was grown in f/2 +Si artificial seawater medium. Cultures were inoculated at about 10^4 cells per ml in a volume of 100ml. f2+Si medium has the following chemical composition:.0.02g/L $SrCl_2*6H_2O$, .03g/L H_3BO_3, 0.10g KBr, 0.70g/L KCl, 1.45g/L $CaCL_2*2H_2O$, 4.00g/L Na_2SO_4, 10.8g/L $MgCl_2*6H_2O$, 23.50g/L NaCL, 20.0g/L $NaHCO_3$. f/2 vitamins were added at 340 µL Na_2SeO_3, 2mL of trace metals, and 880 µM $NaNO_3$. This solution was autoclaved for 45 minutes. After cooling overnight, nutrients were aseptically added to the solution consisting of 234 µL of $FeCl_3*6H_2O+Na_2EDTA$, 0.10 µM $Na2SiO_3*9H_2O$, 36 µM NaH_2PO_4. $FeCl_3*6H_2O+Na_2EDTA$ was made with 1.26g $FeCl_3*6H_2O$, 1.74g NaEDTA, and filling that solution to 47 mL.

P. tricornutum was grown axenically in this solution at 19 degrees C over a continuous 24 hour light cycle at a light intensity of 50-70 µmol photons m^{-2} s^{-1}. The culture VOCs were measured during exponential phase of growth when the cell density reached 10^5 cells per mL and in stationary phase two days after the cell density had reached 10^6 cells per mL. Cell densities were measured using a particle counter (Coulter).

VOC Measurement

In triplicate, 100 mL of culture was added to a dynamic stripping chamber and measured by PTR-TOF/MS. VOC signals were analyzed over 5 second cycles for 60 cycles. The first 30 seconds were discarded as during that time the lines and headspace of the chamber were being purged and the gasses measured were not associated with VOC production. The stripping chamber was kept at 19 degrees C and air was bubbled through the chamber at 50 mL min^{-1} to strip the VOCs from the growth medium. Axenic control media was used as the media blank (negative control) as described in Halsey et al (2017). VOCs were measured by H_3O^+ hydronium ionization after being stripped from the culture or abiotic medium. VOCs were measured ranging from 16-340 a.m.u. and represented mass per charge +1, due to the hydrogen ion transferred from protonated water to the VOCs.

PTR-TOF/MS data was processed using PTRwid (Holzinger, 2015). PTRwid automates calibration of signals and time-of-flight by using internal calibrants. Data sets for the *P. tricornutum* and negative control (media blank) were gathered by PTRwid.

Data analysis.

M/z+1 values received from PTRwid were processed initially through Microsoft Excel (Microsoft corp. Ver 16051.14). All cycles for both exponential and stationary phases, from 30 seconds to 5 minutes, were averaged and standard deviation \ calculations were carried out. Signals from VOCs in the media blank were subtracted from signals from corresponding VOCs in the exponential and stationary samples to eliminate background compounds that are associated with lab atmosphere, media composition and media preparation. Following this step, the total number of m/z+1 values detected in the samples was 178 compounds. Further analysis was carried out in R studio (ver 2021.09.0+351). m/z+1 values that were found to be significantly different in concentration compared to the media blank (p-value <.05) were corrected for possible false positives using the bonferroni method to account for multiple pairwise comparisons. The reverse log base 2 of these data were used for graphical representation in excel due to the large range in ppbv observed for the m/z+1 values. The total number of m/z+1 values that were determined to be significantly different in concentration in the different growth phases was 21, and these were alcohols, amines, aldehydes, ketones, and esters. The highest amounts of VOCs were found in the stationary phase with 20 of 21 VOCs produced in this stage. One compound (75.021) was exclusively produced in exponential phase.

Results

VOC production

We hypothesized that *P. tricornutum* would produce different VOCs in exponential and stationary phases, and different VOCs would be produced in different quantities. Of the 178 m/z +1 values initially identified, 27 VOCs were found in concentrations significantly higher than the media blank. These 27 compounds were further compared against known contaminants introduced during the experimental these were removed from the list of m/z+1 values, reducing the total count to 21. M/z+1 values ranged from 35.041 to 330.925. 20 of the 21 compounds showed significant differences in the concentrations present in exponential and stationary phases, although it was not controlled for the increase in cell density in the stationary phase. m/z+1 value 75.021 was the m/z+1 value present at the highest concentration in exponential phase and m/z+1 value 143.938 was present at the highest concentration in stationary phase. The GLOVOCS database (citation) was used to help identify VOCs by their m/z+1 ratios (Table1). m/z+1 value 72.06 has many possible identities. Although PTR-TOF is highly accurate in measuring m/z values, it is difficult to distinguish between possible stereoisomers. Four of the 21 m/z+1 values were present in both exponential and stationary phases of growth, and m/z+1 values 90.95 and 107.957 were present in higher concentrations in the exponential phase compared to stationary phase and m/z+1 values 105.938 and 106.96 were present in higher concentrations in stationary phase compared to exponential phase.

m/z+1	P-value	Stationary Phase Signal (ppbv)	Exponential Phase Signal(ppbv)	Possible Compound
34.028	0.015332456	2.347761542	0	*
35.041	0.000299407	1.698637591	0	*
45.993	0.000879443	0.93053177	1.180000232	Acetaldehyde
72.926	0.000471553	3.054931194	0	*
75.021	0.028564514	0	4.485867505	Methyl vinyl sulfide Thietane Methyl-thiirane
75.944	0.002693543	2.039340432	0	1,3-Propanediamine
77.01	0.002273065	2.587372924	0	Thioacetic acid
88.952	0.01047375	2.342852145	0	*
90.95	0.047417935	1.901159404	3.930547763	4-Amino-1-butanol
93.965	0.002501304	2.294032209	0	*
94.043	0.048395405	2.393955452	0	Phenoxy radical
95.961	0.032614869	2.361713032	0	*
101.934	0.030277243	2.341822852	0	Cis-3-hexenol Hexanal 2-Hexanone Cyclohexanol 3-Hexanone 2,2-Dimethyltetrahydrofuran 3,3-Dimethyl-2-butanone Oxepane 2-Hexenol Isobutylmethylketone
102.953	0.03139138	2.291510468	0	*
105.938	0.006953334	2.371491703	1.661083593	1,3-Dimethoxy-propane 1,5-Pentanediol
106.96	0.000593743	1.985693901	1.564874558	Dicarbonic acid
107.957	0.025823841	0.900760909	3.365426181	2-Chloro-N-methylacetamide*
125.98	0.01330931	0.390048683	0	*
143.938	0.003023862	5.449395494	0	Trichloroacetonitrile
162.95	0.023024415	3.786360202	0	*
234.948	0.015713965	5.067430222	0	*

Table 1. I would think 45.993 is acetaldehyde M/z+1 values identified as being present in exponential and stationary phases of growth in *P. tricornutum.* m/z+1 values are listed with identities of probable VOCs. Multiple VOCs are listed

for compounds where there are various stereoisomers exist. Asterisks denote areas where documentation on the VOCs could not be found. All p-values were determined after correction by the Bonferroni test.

In exponential phase fewer VOCs were present compared to the stationary phase (figure 1), with 6 out of 21 detected compounds identified. Those m/z+1 values present were observed to be at the higher end of the mass range. VOCs produced in the exponential phase were generally present in higher concentration than those produced in stationary phase, except for 105.938 and 106.96.

The stationary phase contained the bulk of detected VOCs at 19 of 21 VOCs. The largest diversity of compounds lay between the ranges of 75.943 and 106.96. Higher mass range compounds, 143.938, 162.95, and 234.948 were present in the highest concentrations. 19 of the 21 VOCs were captured in the stationary phase, with the same one VOC (125.98) showing weak statistical significance as in the exponential phase.

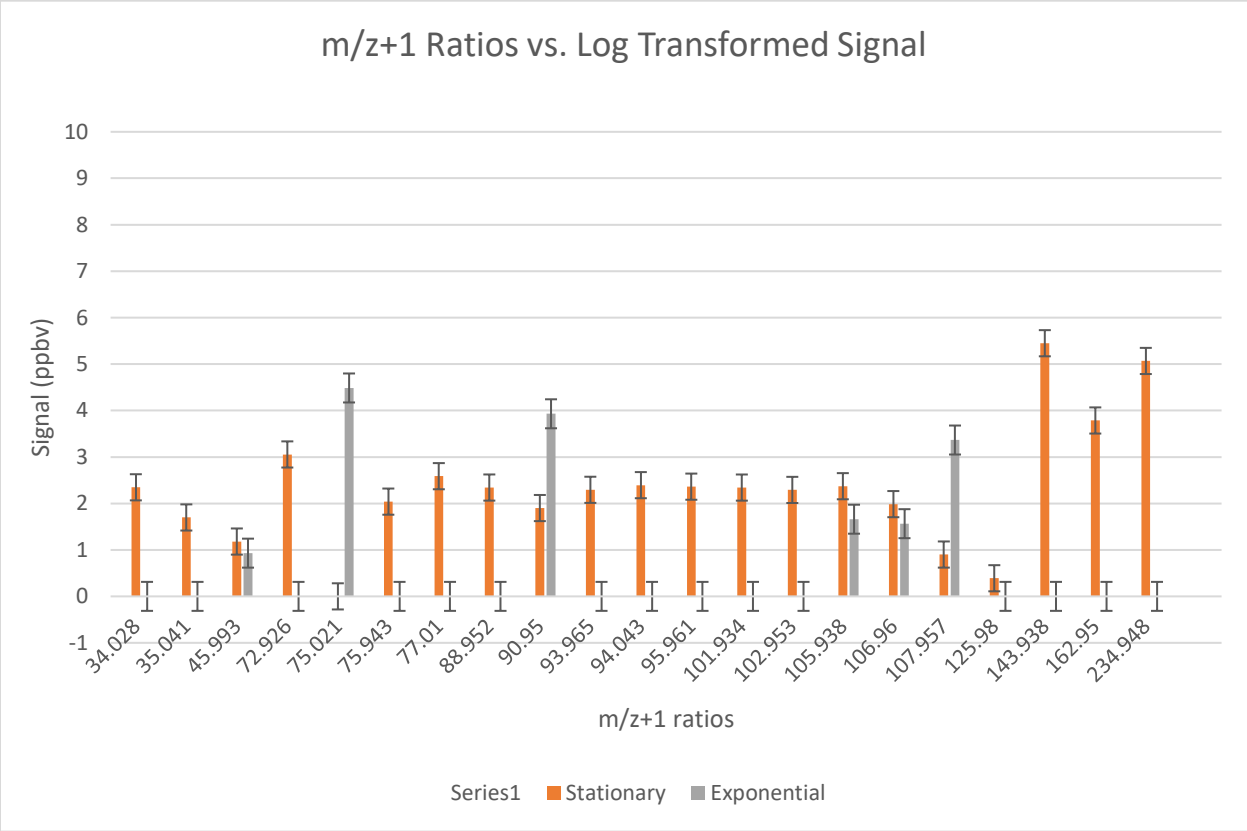

Figure 1. More VOCs were produced in the stationary phase than in exponential phase. The amount of the unique VOCs is greater I the stationary phase when compared to the exponential phase. The signal was log base 2 transformed due to the large volume of data.

Discussion

Volatile organic compounds produced by plankton are an important yet inadequately explored piece of understanding the exchange of carbon between the atmosphere and the ocean level (Halsey et al., 2017). The amounts and identifies of VOCs that contribute to dissolved organic carbon in the ocean is still being explored (Davie-Martin et al., 2020). Here, we have designed an experiment to measure the levels of VOCs produced during the two major phases of cell growth and to provide insight into the specific VOCs measured. These VOCs can be used readily as secondary metabolites for bacterial species sharing the same region as the *P. tricornutum* or can be used to understand impacts on other organisms or the atmosphere.

Another diatom, *T. pseudonana,* has previously been shown to produce VOCs such as acetone, isoprene, and acetaldehyde (Halsey et al., 2017; Moore et al., 2020) under certain light conditions. Cyclohexanol was also observed to be consumed by *Pelagibacter*, a bacterial species commonly found in the vicinity of *T. pseudonana* (Moore et al., 2020). This experiment grew a different diatom, *P. tricornutum*, in monoculture under ideal growth conditions allowing for very efficient use of the metabolites for growth. The efficient use of these metabolites may explain the relatively low numbers of diverse VOCs in the exponential phase because these volatile intermediates did not accumulate inside the cell and leak out into the medium for detection by PTR-MS. m/z+1 values 75.021 (Methyl vinyl sulfide, Thietane, Methyl-thiirane), 90.95 (4-Amino-1-butanol), 105.938 (1,3-dimethoxy-propane, 1,5-pentanediol), 106.96 (dicarbonic acid), and 107.957 (2-chloro-N-methylacetamide) were detected in the exponential phase in elevated levels. Once released by the cell, these VOCs would become available to interact in the environment. These compounds could be metabolized by bacteria or interact with the atmosphere.

Chloromethylated VOCs such as 2-Chloro-N-Methylacetmide can be attacked by hydroxyl groups in the atmosphere, forming acid rain and O3 in the tropospheric zone (Tsai, 2017). Acetone and isoprene have been observed to be produced at higher light intensities than this experiment encompassed (Halsey et al., 2017) however statistically insignificant levels of these VOCs were detected in the present study. Acetone and isoprene are noted greenhouse

gases responsible for impacts in global O3 levels in the lower atmosphere (Dayan et al., 2020). Methyl vinyl sulfide is a source of DMS (biogenic sulfur) in the marine ecosystem. Dimethylsufides (DMS) impact the atmosphere by creating cloud condensation nuclei (Davie-Martin et al., 2020). DMS is a VOC which can be used by other organisms in the surface waters as a metabolite, such as *Pelagibactr*. Methanol in the troposphere causes increased oxidation rates in the lower atmosphere, and a large amount of concentrations are thought to be biogenically produced (Davie-Martin et al., 2020; Dixon et al., 2011).

4-Amino-1-butanol would be a likely candidate for a byproduct produced through lipid metabolism and would be available for bacteria to use for secondary metabolism. Likewise any aminated VOC might be used by other plankton to produce different VOCs (Zuo, 2019). Nitrogen limitation in the environment causes the upregulation of metabolic genes which code for enzymes used in pyruvate metabolism. One result from this upregulation is the increased production of isoprenes, monoterpenes, and sesquiterpenes (Zuo, 2019). This suggests that aminated VOCs in the environment are necessary to regulate the production of other compounds through control of metabolic pathways. The abundant bacterium, *Pelagibacter* is also known to consume DMS both as an energy source and carbon source (Davie-Martin et al., 2020; Moore et al., 2020). Acetaldehyde (45.990) detected in almost equal parts in the stationary phase and exponential phase is used by bacteria as an energy source or creation of biomass (Halsey et al., 2017; Moore et al., 2020). As *P. tricornutum* was grown in monoculture these compounds would be available for bacteria to consume as secondary metabolites which would reduce their prevalence. The lower volumes of VOCs in the exponential phase are likely due to continuous growth and use of metabolites for reproduction.

Of those VOCs produced, the bulk were produced in the stationary phase. 15 of the 21 significant VOCs were exclusively produced in the stationary phase, 5 were produced in both phases, with 105.938 and 106.96 having greater production in the stationary phase. The culture counts for cell growth between each phase were not accounted for, and it is possible this altered the VOC signal levels in each phase. Normalizing the counts between phase may have shown increased signal results for the stationary phase, narrowing the gap between those m/z+1 signals shown in higher strength in the exponential phase (75.021, 90.95, and 107.957) and raising the signal amounts for each of the stationary phase VOCs. In stationary phase, some cells may be dying, meaning that VOCs observed in this stage may be related to decomposition. In some species of phytoplankton the stationary phase causes no change in assimilation of carbon but respiration and use of nitrogen is impacted, which alters the range of VOCs released

(López-Sandoval et al., 2014). Many of the VOCs released in the stationary phase are alcohols, which can be used by organisms as energy and biomass sources. Methanol is one such alcohol, although use in the production of biomass is limited in the absence of some specialized metabolisms allowing cells to use 1-C compounds (Halsey et al., 2012). One of the largest produced VOCs was 143.938, a compound with no entry in GLOVOCs. Research on biogenic VOCs is currently challenged because of the lack of investigations on VOCs and the incompleteness of literature on their identities. Carrying out this experiment while varying conditions of light, metabolism, growth medium, and other factors would enable understanding a broader range of VOC production.

Understanding the impact of carbon cycling between the surface marine biota, the lower atmosphere, and the marine environment will help fill in missing details of climate change and how carbon is sequestered or released. Further investigations into the ability of microorganisms to utilize VOCs dispersed by phytoplankton will lend new insights into understanding how carbon is incorporated into energy strategies and biomass structures.

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