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LLNL-TR-809677

Elucidating algal-bacterial community interactions by tracking volatile biomarkers - final report

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May 6, 2020

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This work performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under Contract DE-AC52-07NA27344.

FINAL REPORT
Elucidating algal-bacterial community interactions by tracking volatile biomarkers
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Abstract

This feasibility study project aimed to lay further groundwork for studying and understanding how microorganisms interact with each other at the molecular level using model algal-bacterial co-cultures. A better understanding of such interactions is critical to optimizing and operating biotic platforms for a number of applications, including bioenergy, algal bioproducts and agriculture, and carbon capture. Here we explored the feasibility of characterizing and following these interactions by identifying and tracking volatile metabolites and biomarkers in the complex gaseous headspace of such cultures. We set up an experimental platform using a co-culture of *Phaeodactylum tricornutum* and *Marinobacter* subspecies 3-2 as a model system, used solid-phase microextraction fibers for volatile collection at various time points throughout culture growth, and employed gas chromatography mass spectrometry-based instrumentation available at LLNL for compound identification and quantification. We found that it is feasible to determine volatile profiles from the growth of *Phaeodactylum tricornutum* and *Marinobacter* subspecies 3-2 using our methodology and monitor profile changes over time. We also observed that volatiles distinguishing between cultures of the individual species and the co-cultures could be detected. We expect that results of this project will feed into the ongoing LLNL's DOE BER funded Biofuels Scientific Focus Area (SFA) on algal-bacterial interactions and pave the way for future expanded volatile studies supported by DOE. The approach and technologies developed here would also be applicable and transferable to other microbial communities under study, such as cyanobacteria, rhizosphere communities and biofilms.

Background and Research Objectives

A better understanding of how microorganisms interact with each other at the molecular level is critical to optimizing and operating biotic platforms for a number of applications, including bioenergy, algal bioproducts and agriculture, and carbon capture. Photosynthetic microalgae are responsible for at least 40% of global CO2 fixation [1] and are also a promising bioenergy resource [2]. Algae have evolved in close relationships with other microbes, and interactions with other microbes influence their physiology and growth in natural systems [3], and likely in engineered systems as well. Currently, our ability to predict algal physiology and activity in situ is limited, due to a lack of understanding about these dynamic interactions with other microbes. From a few studies, we know that these interactions involve exchange of both signaling molecules and nutrients [4, 5], mediated by the microscale chemical environment surrounding algal cells [6], known as the *phycosphere*. Here we explored the feasibility of characterizing and following these interactions by identifying and tracking volatile metabolites and biomarkers in the complex gaseous headspace of a model co-culture of the algal taxa *P. tricornutum* and the bacterium Marinobacter subspecies 3-2 – a potential model for a biofuels-producing algal culture in a symbiotic relationship with bacteria.

This feasibility study aimed to address three key challenges and questions: 1) Can we detect key volatile biomarkers of interest and their time profile in the complex headspace? 2)

Can we use isotope labeling to identify producers of these biomarkers and link with exchange of metabolites between algae and bacteria? and 3) Can we find volatile signatures of interactions between algae and bacteria? We proposed to address these challenges by perform systematic headspace studies employing a unique combination of advanced mass spectrometry-based instrumentation available at LLNL for compound identification and quantification. Overall, this project met 2 of these 3 challenges. We found that it is feasible to determine volatile profiles from the growth of *P. tricornutum* and *Marinobacter* subspecies 3-2 using our methodology and monitor profile changes over time. We also observed that volatiles distinguishing between cultures of the individual species and the co-cultures could be detected. We also considered the use of 13-C isotope labeling to strengthen the identification of relevant compounds but decided not to pursue this path during the limited time of this feasibility project because the mass resolution of the available instrumentation was not sufficient to draw strong conclusions from such labeling. Instead, we focused our efforts on the other two challenges.

Scientific Approach and Accomplishments

Co-culture model and control samples: In order to evaluate the feasibility of collecting and detecting volatile organic compounds (VOCs) emitted from the headspace of co-cultures of algae and bacteria we set up a co-culture model system of algae *Phaeodactylum tricornutum* (*P. tricornutum*) and bacteria *Marinobacter* subspecies 3-2. Those species are currently also utilized by the DOE-funded BioSFA project at LLNL and were readily available. VOC samples were acquired from several different culture types, including co-cultures of algae-bacteria, and cultures of P. tricornutum-only and Marinobacter spp. 3-2 only as controls. VOC samples were also obtained from exudates (filtrates) of cultures from each organism, combinations of exudates and organisms, and growth media alone (as control).

VOC sampling: We used commercially available, field-portable solid-phase microextraction (SPME) fibers (from Supelco, Bellefonte, PA) for the collection of VOCs from each culture or control samples. As shown in Figure 1, SPME fibers were inserted into culture flasks and typically exposed to the headspace for 2-3 hours. VOCs were sampled every 24 hours over the course of 8-10 days of growth (experiment dependent), concurrent with fluorescence measurements of chlorophyll to monitor the algal densities.

VOC analysis and biomarker identification: The VOC analysis and biomarker identification a procedure similar to the one described in a recent paper by some of the researchers involved here [7]. VOC analyses were performed on an Agilent 5975T GC-MSD system. Data acquisition was performed using ChemStation (version E.02.02). A commercial GC-MS reference standard (S-22329; AccuStandard, New Haven, CT) was used to evaluate day-to-day performance of the GC-MS system and to calculate retention indices. Annotation of these compounds was performed by retention index matching and searching mass spectra against the NIST14 mass spectral database. After data acquisition, data processing procedures and criteria were applied to identify taxa-specific biomarkers, similar to the work described in [7]. In essence, the initial VOC analysis identified a large number of compounds per sample (typically, a few hundred) and the data processing and filtering criteria removed compounds that were also present in the media control or were not present reproducibly. This left a much smaller number of compounds that were identified as putative biomarkers.

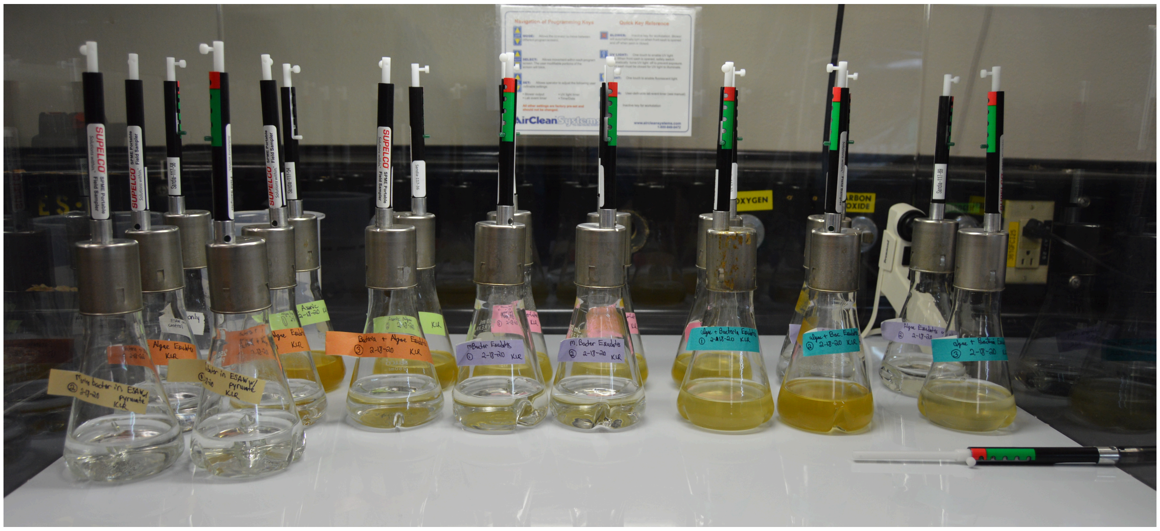

Figure 1. Image of experimental setup during passive sampling of algal and bacterial culture emissions (Day 7 post culture inoculation) using SPME fibers (n=1 fiber per 250 mL vessel; average exposure time ~2.5 hours); replicate cultures indicated by similar-colored labels.

<u>Results:</u> Overall, volatile profiles were not only detectable from the growth of *P. tricornutum* and *Marinobacter* subspecies 3-2 from our methodology, distinguishing volatiles were observed in each culture type. The growth of individual replicates is summarized in Figure 2, indicating that the interaction of algae and bacteria in co-cultures does not inhibit the growth patterns.

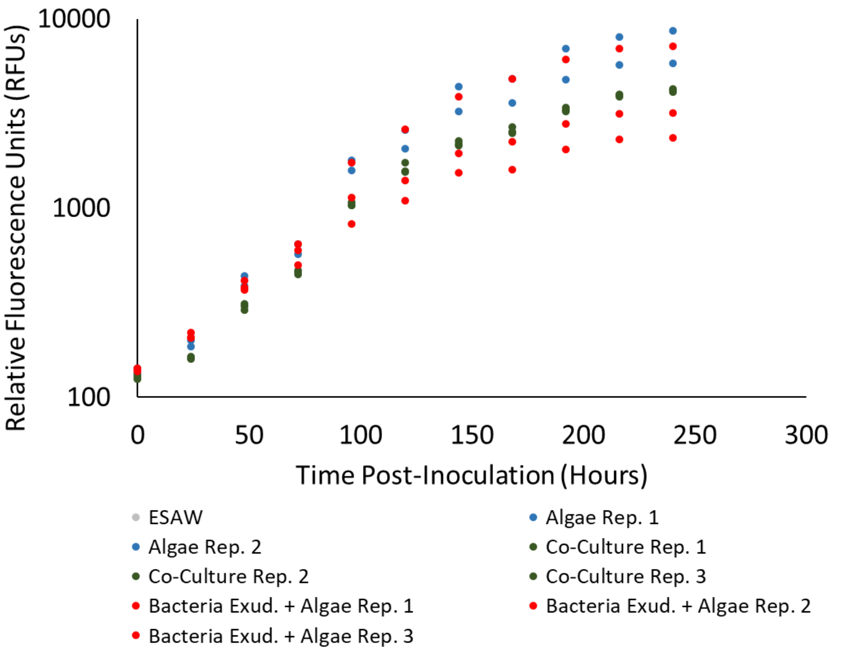

Figure 2. Growth curves of *P. tricornutum* (algae) and *Marinobacter* spp. 3.2 (bacteria) cultures via measurements of relative chlorophyll fluorescence units (RFUs) at various timepoints post-inoculation of cultures. Replicates for each type of culture are plotted separately, so every point represents a single measurement. Simultaneous measurements of RFUs in *Marinobacter*-only cultures was simultaneously acquired but did not exceed 20 RFUs, hence considered to be negligible signal.

Preliminary VOC profiles from select culture types were determined from the obtained data and are summarized in the following. Briefly, when *P. tricornutum* and *Marinobacter* spp. 3-2 were grown together (co-cultured), a total of 33 VOCs were produced that were distinct from emissions from the growth media and sampling devices. An example of compound detection is given in Figure 3 illustrating VOCs found in the co-cultures versus the growth media. Tentative identifications of found compounds against the NIST14 mass spectral database identified a known algal pheromone called ectocarpene (6-[(Z)-1-Butenyl]-1,4-cycloheptadiene) along with compounds whose functions are not presently known, belonging to the compound classes of methyl ketones, carboxylic acids, and cyclic hydrocarbons. Eleven of these compounds were also identified in monocultures of *P. tricornutum* and/or *Marinobacter* spp. 3-2, including ectocarpene, indicating these were produced by the two separate organisms and not as a result of the interaction between the two. Additionally, we tested whether similar volatiles were produced when *P. tricornutum* was grown in liquid media simulating the presence of *Marinobacter* spp. 3-2 by using the exudates of spent bacterial media. Further experiments are necessary to determine if individual compounds or compound classes are consistently indicative of algae-bacterial interactions. Identification of metabolites in these algal-bacterial interactions provides the first steps to understanding their formation, associated co-organism metabolic pathways, and the genetic basis of these pathways.

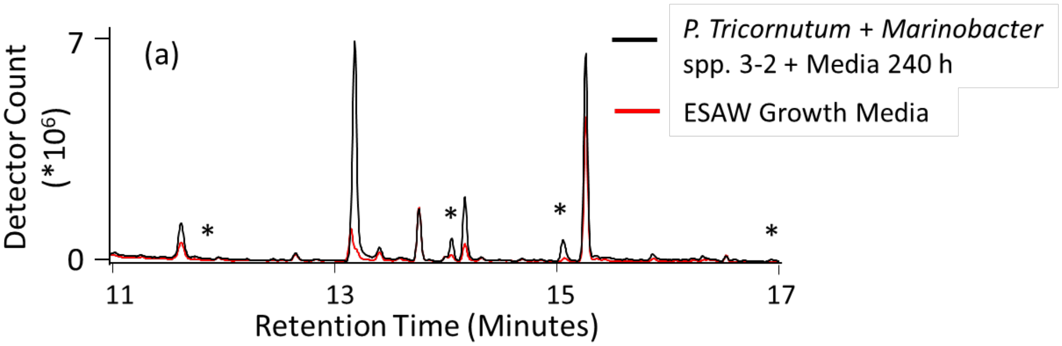

Figure 3. Example total ion chromatograms for observed VOCs sampled from the co-culture of *P. tricornutum* + *Marinobacter* spp. 3-2 at 240 hours post inoculation of cultures, with indicated VOCs being potential markers of algae-bacterial interactions.

Further analysis and compilation of this data is currently in progress as part of the Ph.D. thesis work of LLNL graduate student researcher and Michigan State University student Kristen Reese, who was the main contributor to this work. More details on the results coming out of this study will be published as part of her Ph.D. thesis in summer 2020. We also expect a peer-reviewed publication to result from this work.

Impact on Mission

The results of this feasibility study indicate that measuring VOC emissions from algal cultures or algal-bacterial co-cultures may be a useful non-invasive tool to monitor algal growth and potential synergistic interactions with bacteria. Such a tool would support cutting edge research on microbial interactions that would leverage and provide additional capabilities to ongoing work under the DOE/BER Bioenergy SFA and, thus, support the Lab's bioenergy mission. Such a tool could also be adapted and applied to much larger culture systems used for biofuel production based on algae. The approach and technologies developed here would also be applicable and transferable to other microbial communities under study, such as rhizosphere and biofilms, that are also of great scientific interest and of interest to other sponsors including DoD.

Conclusion

This work has shown that the proposed method and experimental approach is suitable for studying volatile emissions from algal-bacterial co-cultures and their evolution over time during culture growth. Future work may include additional algal and bacterial species and larger number of experimental replicates to solidify these results. Future work in this area should also explore the option of stable isotopic labeling of metabolic products to determine the origins of VOCs amongst *P. tricornutum*, *Marinobacter* subspecies 3-2, and their co-cultures, work originally proposed for this project but ultimately not attempted due to limited resolution capabilities of the chosen MS system. This information can in turn inform researchers on the transfer of metabolites between organisms. Some of these VOC biomarkers are believed to be indicative of algal-bacterial interactions and may be useful in optimizing such cultures for biofuel production.

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Publications and Presentations

Further analysis of this data is currently in progress as part of the Ph.D. thesis work of LLNL graduate student researcher and Michigan State University student Kristen Reese, who was the main contributor to this work. Preliminary results were presented at the 2019 ANACHEM (Association of Analytical Chemistry) Conference in Livonia, MI (LLNL IM # LLNL-PRES-795758) by Kristen Reese who received an ANACHEM best student presentation award for her presentation. More details on the results coming out of this study will be published as part of her Ph.D. thesis in summer 2020. We also expect a peer-reviewed publication to result from this work.

Notes to the Editors

N/A