2019 Project Abstract
For the Period Ending June 30, 2023

PROJECT TITLE: Transformation of Plastic Waste into a Valued Resource
PROJECT MANAGER: Brett Barney
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04j as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

APPROPRIATION AMOUNT: $225,000.00
AMOUNT SPENT: $216,713.00
AMOUNT REMAINING: $8,287.00

Sound bite of Project Outcomes and Results
Our project identified prominent strains within microbial communities obtained from Minnesota waters that are able to degrade problem plastics such as polyethylene. In many cases, individual microbial strains were isolated and sequenced to provide a blueprint of strain features that enable this ability to degrade plastics.

Overall Project Outcome and Results
The goals of our project were to construct reactors to enrich microbial communities from Minnesota for the biodegradation of problematic plastics, and then utilize these reactors to isolate a variety of communities that can biodegrade these plastics. Once enriched, we analyzed the communities for microbial composition and sequenced various microbial strains that showed promise toward the biodegradation of specific plastics. We made significant progress on the goals of this project and isolated seven different microbial communities that demonstrated enhanced biodegradation of problem plastics such as polyethylene. Four of these communities were analyzed for community structure, revealing that each of the communities were composed of only a small number of primary microbes. We made nice progress toward isolating a number of microbial strains from various communities and then sequenced these strains, laying out a path forward for more detailed analyses to determine the mechanisms that are being used by these different microbes to biodegrade plastics. These results are now being prepared for presentation through various scientific journals, and the sequences of the isolated strains are being shared through national databases so they can be accessed by other researchers across the globe. These results are a first step toward developing a clear understanding of how certain microbes can adhere to and degrade plastic materials so they can be used as a source of food and energy, while being converted into biomass and carbon dioxide that can reenter global carbon cycles. Having a clear understanding of these mechanisms will allow us to predict likely amounts of time required for these plastics to fully degrade within the environment and should further assist in developing inoculums that could be used to treat contaminated waters across Minnesota in the future.

Project Results Use and Dissemination
Resources developed as part of this project have already been shared through the National Center for Biotechnology Information (NCBI), a national repository for genomic information, which is a tool for the broader scientific community. Results from this project are being formulated into a series of manuscripts that will be published in peer-reviewed journals, which will detail some of the successful approaches we used to isolate microbial communities able to degrade problem plastics.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2019 ENRTF Work Plan Final Report.

Today's Date: 6/4/2024

Final Report

Date of Work Plan Approval: 6/5/2019

Project Completion Date: 6/30/2023

PROJECT TITLE: Transformation of Plastic Waste into a Valued Resource

Project Manager: Brett Barney

Organization: University of Minnesota

College/Department/Division: College of Food, Agricultural and Natural Resource Sciences/Bioproducts and Biosystems Engineering

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Location: Statewide

Total Project Budget: $225,000.00

Amount Spent: $216,713.00

Balance: $8,287.00

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Appropriation Language: $225,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to develop technologies that use microbes to convert plastic waste into useful chemical compounds and fuels, lowering the likelihood that these materials end up in the environment. This appropriation is subject to Minnesota Statutes, section 116P.10.

M.L. 2022 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2023]

I. PROJECT STATEMENT:

CONCEPT – We will develop technologies that utilize indigenous microbes to convert waste plastics into useful chemical compounds and fuels. By converting this waste stream into valuable commodity chemicals and a potential source of energy, we will increase the demand for this material, which will lower the likelihood that these materials to end up in our natural waters following disposal. This effort will also lay the groundwork for developing future methods to remediate plastics from contaminated soils and waters by identifying natural species from Minnesota that have the ability to degrade these undesirable contaminants.

BACKGROUND – Microplastics are small plastic beads that have been added to exfoliating soaps or skincare products, and also result from the general photochemical degradation process of plastics in our environment that results from exposure to sunlight. These are often unseen based on a visual inspection, but quickly become apparent when viewed under a microscope and based on collection techniques with precision screens. These microplastics have permeated into the food chain and act to concentrate environmental pollutants. Recent reports citing high levels of microplastics in freshwater lakes such as the Great Lakes have confirmed concerns that the accumulation of microplastics in the environment is not an issue facing only water bodies such as the Pacific Ocean, where this topic has been highlighted as a key element of the 'Great Pacific Garbage Patch'. Indeed, ***microplastics have infiltrated many standing bodies of water throughout the world and across the state of Minnesota***. Plastic waste within the environment contributes to the illness and deaths of countless fish, reptiles, marine mammals and bird species, and also diminishes the pristine nature of our public waters which are a valuable aspect of recreation in Minnesota. This unanticipated and detrimental result of our wide-scale adoption of plastics over the past century is an issue that will face generations to come.

Conventional plastics are widely believed to be non-biodegradable. Various reports of microbes that are capable of degrading common plastics such as those found in beverage bottles (PETE), Styrofoam (polystyrene) and those used to store everything from milk to household chemicals (polyethylene; HDPE or LDPE) are now challenging this belief. These studies are important because they have identified specific bacteria and fungi that can degrade many current common plastics, shattering the misconception that all petroleum-derived commodity plastics are non-biodegradable. Our project will build upon the foundations of these reports and preliminary studies in our own laboratories, and further incorporate the emerging realization that diverse microbial communities are better adapted than single organisms to degrading complex chemicals such as those that are found in conventional plastics.

GOAL – The goal of this project is to develop alternatives for disposing of problem-plastics by converting plastic waste materials into a valuable resource using conditions similar to what is commonly found in the lower gut of many plastic-degrading insects. Through this approach, we will create new markets for many of the problematic plastics found in our recycling and waste streams. By adding value and incentive to repurpose the waste, we will decrease levels of plastics reaching the environment, including our lakes and rivers.

II. OVERALL PROJECT STATUS UPDATES:

First Update March 1, 2020

This full project was initiated in July of 2019 building upon some preliminary studies funded through a Seed Grant from MnDRIVE. We are making the expected progress as outlined in the initial work plan, and do not anticipate any changes to the work scope. We have recruited several additional students to work on this project, including high school participants from Great River Montessori School in St. Paul. We have also hired several undergraduate student who are assisting in the laboratory, and will be recruiting one additional graduate student to start on this project next fall. We have some nice initial results that support the project goals and should enable many of the more complex studies we plan in later years. Materials have been purchased and a broad number of reactors are currently running with complex cultures in the laboratory, while we have started to construct some of the more complex reactors we have planned in later years.

Second Update September 1, 2020

We continue to make the expected progress as outlined in the initial work plan, and do not anticipate any changes to the work scope. Due to the current pandemic that broke out in the United States shortly after the last update, we did have to dial back some of our outreach efforts with high school students out of an abundance of caution and as required by State and University directives. Several students assisted with collection of new samples and strains in the months prior to the pandemic, adding to our culture collection, which has been maintained throughout this pandemic. We have collected a significant number of samples and continue to enrich for microbial communities capable of degrading various commodity plastics. While the pandemic has complicated some of our efforts, it has not dramatically slowed our progress. We are still pursuing active laboratory studies associated with this project.

Third Update March 1, 2021

We are ramping up additional efforts related to this project as restrictions are beginning to be lifted from the ongoing pandemic. We will be working again to expand our outreach efforts with high school students and have hired a number of undergraduate students to work on this project over the summer. We continue to maintain cultures, and have been expanding laboratory studies to characterize plastic degrading microbes in the laboratory. We will return to some of our efforts related to collection of plastics from sites across the state to seed cultures of additional plastic degraders. Many of our efforts are shifting toward a community analysis of plastic biodegraders, but we are also working to determine the mechanisms that some specific bacterial and fungal strains are using to biodegrade specific polymers.

Fourth Update September 1, 2021

We have enjoyed a productive summer with strong progress on this project. We have several different microbial communities that are degrading various polymers and plastics, and have been focusing on using alternative chemicals that mimic the same properties as plastics, but are easier to quantify, so we can make faster progress toward identifying the enzymes and mechanisms that are key to the biodegradation of these various plastics. We had several undergraduate students who worked on these projects during the summer months. We are also able to work with high school students and are renewing some of our efforts toward outreach with these students. We provided several students laboratory tours, and will be hosting several high school students to assist with projects in the near future.

Fifth Update March 1, 2022

Our plastic degrading community cultures are now growing sufficiently fast enough that we are collecting samples to determine the modes of degradation employed by the various communities. We are going back through the fifty strains of bacteria and fungi that have isolated and identified or sequenced as part of this work to do more detailed analysis of the mechanisms of plastic degradation using these new methods. New techniques developed in the past year are better informing experimental design, and we have begun to submit sequencing samples to begin the final aim of determining biological mechanisms that are employed by these microbes to facilitate degradation.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

We have received microbial community results from four different communities that were developed to degrade polyethylene plastic precursors. From this data, we now have several very strong target microbes that appear to be capable of degrading polyethylene that are found in the environment, and we are in the process of quantifying the rates of degradation. These results look very promising, and as a result, we will sequence these bacteria to determine if these would be amenable to potential scale-up. This project has made very nice progress in the past few months after some delays over the summer while we awaited community sequencing results.

Seventh Update as of March 1, 2023:

We have isolated important microbes from several of our microbial communities and tested the ability of these to degrade polyethylene plastic precursors. We submitted the strains for genomic sequencing, and have just recently received the results. We are in the process of assembling these genomes and comparing these to similar model strains that have been sequenced. The results seem to indicate a specific class of microbes have a high potential for biodegrading polyethylene at a reasonable rate for more extensive futures studies. We are finalizing figures and writing a draft manuscript related to these results, that we expect will be of significant interest to many other researchers in the field.

Amendment Request April 26, 2023

Changes were made to the budget based on increased costs associated with sequencing services and restrictions placed on travel during COVID. Money originally budgeted for travel and some of the money from laboratory supplies was diverted to cover these increased costs. Price differences were unexpected and a result of dramatic increases due to the pandemic.

Amendment Approved by LCCMR 5/8/23
Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

We have completed the laboratory studies associated with this project. Genomes of specific microbes that were found to biodegrade polyethylene in reasonable time frames have been assembled and submitted to appropriate databases. A full scientific report on the findings from using microbial communities to degrade polyethylene is currently being prepared to share this work with the broader scientific community. We are working on a final draft of a manuscript related to this work, and are using the results to pursue additional research efforts to identify the mechanisms used by this microbes to degrade polyethylene in the future.

Overall Project Outcome and Results

The goals of our project were to construct reactors to enrich microbial communities from Minnesota for the biodegradation of problematic plastics, and then utilize these reactors to isolate a variety of communities that are able to biodegrade these plastics. Once enriched, we sought to analyze the communities for microbial composition and then sequence various microbial strains that showed promise toward the biodegradation of specific plastics. We made significant progress on the goals of this project, and isolated seven different microbial communities that demonstrated enhanced biodegradation of problem plastics such as polyethylene. Four of these communities were analyzed for community structure, revealing that each of the communities were composed of only a small number of primary microbes. We made nice progress toward isolating a number of microbial strains from various communities and then sequenced these strains, laying out a path forward for more detailed analyses to determine the mechanisms that are being used by these different microbes to biodegrade plastics. These results are now being prepared for presentation through various scientific journals, and the sequences of the isolated strains are being shared through national databases so that they can be accessed by other researchers across the globe. The first paper was published in Environmental Microbiology in June 2024. These results are a first step toward developing a clear understanding of how certain microbes are

able to adhere to and degrade plastic materials so that they can be used as a source of food and energy, while being converted into biomass and carbon dioxide that can reenter global carbon cycles. Having a clear understanding of these mechanisms will allow us to predict likely amounts of time that will be required for these plastics to be fully degraded within the environment and should further assist in developing inoculums that could be used to treat contaminated waters across Minnesota in the future.

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1 Title: Collection and Analysis of Plastic-Degrading Microbial Communities
Description: We will enrich several microbial communities collected from Minnesota with the greatest ability to biodegrade targeted plastics. This effort will build upon current studies already underway that have resulted in several microbial communities that biodegrade targeted problem-plastics. This effort may include outreach with secondary school teachers across the state to increase the breadth of sites sampled and also educate students and their communities about the environmental impacts of poor plastic waste management and the impacts on our waters and the environment. This effort will expand our sample size and geographical diversity, while also educating future generations of Minnesotans.

ACTIVITY 1 ENRTF BUDGET: $ 110,000.00

Outcome	Completion Date
1. Construct laboratory reactors to enrich microbial communities for the biodegradation of problematic plastics such as polyethylene (HDPE and LDPE), polystyrene (Styrofoam) and PETE (Water bottles).	Dec 15, 2019
2. Prepare sites to house simple microcosms to enrich natural organisms capable of using different plastics as a growth substrate (including insects, soil and water samples).	May 1, 2020
3. Determine the composition of enriched microbial communities to identify the diversity and abundance of plastic degrading organisms across Minnesota.	July 30, 2021

First Update March 1, 2020: Update waived by LCCMR 4/1/2020

Second Update September 1, 2020

Laboratory cultures to enrich microbial communities for the biodegradation of problematic plastics such as polyethylene (HDPE and LDPE), polystyrene (Styrofoam) and PETE (Water bottles) continue to be enriched in relation to our efforts toward isolating bacterial and fungal strains degrading these problematic plastics (ACTIVITY 1, Outcome 1). Microcosms remain deployed in several locations (ACTIVITY 1, Outcome 2), though this activity was slowed somewhat by the pandemic and we hope to expand on this work this next spring.

Third Update March 1, 2021

We are working specifically on our efforts toward determining the composition of enriched microbial communities to identify plastic degrading microbes that were collected from across Minnesota (ACTIVITY 1, Outcome 3). Nearly twenty strains of bacteria were sequenced in order to determine if there are any specific novel proteins that are taking part in this process. We are also planning to focus some additional effort on fungal strains. These results are being mined to better understand mechanisms used to break down plastics once they are in the environment. Our efforts toward creating additional microcosms to enrich natural organisms are ongoing, but will be expanded and revisited again, now that work travel restrictions have been lifted (ACTIVITY 1, Outcome 2). Efforts toward enriching microbial communities remain ongoing (ACTIVITY 1, Outcome 1).

Fourth Update September 1, 2021

We have accumulated more than thirty different bacterial and fungal strains with the potential to degrade plastics. Many of these strains were sequenced to provide a genetic blueprint that can be used in further studies of identifying the genes responsible for degrading recalcitrant polymers. Robust methods to characterize the biodegradation of plastics are still lacking in this field, and we have been directing quite a bit of attention at developing improved procedures to characterize and quantify the biodegradation of representative polymers so that we can assign biodegradation potential values to each of these strains. We are also reassembling pseudo-communities of these combined strains, and also have been cultivating a number of natural communities that can rapidly degrade polymers for further characterization. Most of our attention for the next few months will be directed at characterizing the communities of microbes that appear to be working better than independently isolated strains, to determine the contributions of various strains in these complex communities.

Fifth Update March 1, 2022

We have four robust communities that are growing fast enough to pursue the chemical characterization approaches aimed to accurately determine biodegradation potential. Based on recent results, we have developed several new approaches to enrich plastic degrading communities from various bodies of water across the state of Minnesota. We are hoping to obtain an extension on this grant that will allow us to further engage additional high school students and undergraduates to dramatically expand our efforts to collect robust plastic degraders from across the state. In the event that we are able to continue this work for another year, we believe we can dramatically increase the rates of degradation for the plastics. In the event that we are unable to continue this work beyond the June 30 original deadline, we will instead focus our attention toward the final characterization of the samples already in hand. We are thus awaiting confirmation of whether LCCMR will be granting extensions as a result of the COVID pandemic.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

Our four robust communities have now been characterized to determine the complexity of the communities. Interestingly, the communities are less complex than we had anticipated, with four or five dominant species in each community. This encouraged us to go ahead and try to isolate some of the primary microbes that were found in each community, and we have made nice progress here as well. One very nice caveat to all of these results was that each of the communities did share one specific microbial genus among them, indicating that there may be a specific organism that is essential in these processes. With the extension of another year on our funding, this is allowing us to make a good deal of progress, and may enable us to do a more thorough analysis of specific microbes in the coming months. Most of our progress has been made in relation to Outcome 3.

Seventh Update as of March 1, 2023:

We have now isolated and sequenced about seven strains of bacteria that were prominent in our community cultures. We have also constructed minimal communities based on the several prominent strains that we found in these communities, and demonstrated that these minimalistic communities were able to degrade polyethylene mimics at rates similar to the more complex communities. We also have confirmed that on specific genus of microbes that is present in each of the communities is able to achieve significant biodegradation of these plastic mimics as a single isolate, indicating that this strain is the primary degrader in these studies, and is likely the strain that has the required cellular machinery to biodegrade this class of plastics. We are working to publish these results as we work to complete the last remaining tasks of this grant.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

All microbes that were isolated and showed a promising potential for biodegradation of polyethylene have now been sequenced and these sequences deposited to appropriate databases. The analysis of microbial communities has also been completed, and the results have been assembled into a draft manuscript that is being submitted for peer review. All microcosms have been collected and used to generate a series of communities to support these studies. The methods developed as part of this work will be used in future work to better understand the mechanisms that are being used by these microbes for biodegradation of polyethylene.

ACTIVITY 2 Title: Construction of Model Insect Gut Digesters to Transform Plastic Waste
Description: We will construct a laboratory-scale continuous system that will utilize waste plastics as a feedstock supply to produce useful commodity chemicals, methane and hydrogen gas. The goal of this activity will be to provide a proof of concept for the reactor design and approach, which could then be deployed across the state in the future as an alternative solution to landfilling waste plastics. Our efforts will target problem-plastics that do not have sufficient markets for recycling, and which are often found as contaminants in our lakes and rivers. Through the development of these reactors and the enrichment of strains able to biodegrade these problem-plastics, we will also isolate natural strains that could be used in future efforts to treat contaminated areas. Additional reactor designs will be tested as well to determine optimal methods to treat microplastics.

ACTIVITY 2 ENRTF BUDGET: $ 115,000.00

Outcome	Completion Date
1. Construct a laboratory-scale insect gut digesters to convert target plastic materials into methane and hydrogen for energy production.	Oct 15, 2020
2. Construct aerobic reactors to determine the potential to apply indigenous microbes as a means of bioremediation to plastics in the environment.	Feb 15, 2021
3. Analyze genes and genomes of different species from isolated communities to identify genes involved in plastic waste degradation.	June 1, 2022

First Update March 1, 2020

Laboratory reactors to enrich microbial communities for the biodegradation of problematic plastics such as polyethylene (HDPE and LDPE), polystyrene (Styrofoam) and PETE (Water bottles) have been constructed and used to enrich a number of cultures obtained from locations near the twin cities, and bacterial and fungal strains that are degrading these problematic plastics have been isolated (ACTIVITY 1, Outcome 1). To date, we have isolated about thirty strains that are able to degrade different polymers (plastics and plastic related polymers), and are working toward identifying these species and sequencing the genomes so that we can pursue studies to identify genes that might be associated with plastic waste degradation (ACTIVITY 2, Outcome 3). Microcosms have been designed and constructed, and several have been deployed (ACTIVITY 1, Outcome 2), with additional microcosm deployment planned for early this summer. Digester construction will begin in earnest this summer.

Second Update September 1, 2020

As a result of this work, we submitted eighteen bacterial strains that appear to be actively biodegrading certain plastics and polymers for genome sequencing projects (ACTIVITY 2, Outcome 3), and have already submitted the genomes of six strains to National Bioinformatics databases to share these with the broader scientific community. Various bioreactors continue to be maintained in relation to this project (ACTIVITY 2 Outcomes 1 and 2), and additional analytical tools to quantify polymer biodegradation are being developed. We have completed the construction of several of our aerobic bioreactors for efforts to develop larger bioreactors aimed at studying scale-up with the microbial communities we have already obtained.

Third Update March 1, 2021

We are currently working to develop a series of experiments that will help us better pinpoint the genes involved in plastic waste degradation (ACTIVITY 2, Outcome 3). This includes more detailed studies to identify and confirm specific proteins that are participating in this process. Our first wave of genomes were assembled and released to National Bioinformatics databases to share these with the broader scientific community. Various bioreactors continue to be maintained in relation to this project (ACTIVITY 2 Outcomes 1 and 2), and additional analytical tools to quantify polymer biodegradation are now functioning in the laboratory, which are allowing us to track degradation of representative polymers, which will be a powerful tool in confirming enzyme activities in the future. Some of our efforts are now shifting toward building communities of indigenous microbes for seeding contaminated sites in the future.

Fourth Update September 1, 2021

We have developed several protocols to improve the likelihood that we can isolate specific genes involved in the biodegradation of plastics. This effort, combined with our new protocols to track the biodegradation of representative polymers over shorter time frames will provide improved feedback on the specific genes that are responsible for biodegrading conventional plastics (ACTIVITY 2, Outcome 3). Our model bioreactors are all up and running, which will allow us to test the potential to convert problem plastics and polymers into simple fuels like hydrogen and methane in anaerobic digesters. These reactors often take a long time to establish active cultures, so having the reactors up and running is an important step to determining if the anaerobic aspect (ACTIVITY 2, Outcome 1) of this research work will be successful. In the meantime, we are seeing a good deal of success on the other aims of this research project.

Fifth Update March 1, 2022

We continue to maintain several anaerobic reactors to collect a range of bacteria in a simulated insect gut system. Our results to date indicate a higher degree of biodegradation with aerobic cultures. This is promising, as it indicates more promise in many of the environments where these plastics are accumulating, such as in our lakes and rivers, which are typically aerobic in most of the regions where plastics like polyethylene and polystyrene would accumulates (at the surfaces of the water). Once we identify some of the enzymes utilized from aerobic cultures, it may be possible to determine if these cultures can be used interchangeably in both anaerobic and aerobic environments, and thus used to seed additional reactors for the biodegradation of plastics.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

Our results from our work thus far seems to indicate that specific microbes are able to break down very long chain wax materials that are similar to polyethylene. Based on these results, we should be able to project the time that some of these microbes might take to break down microplastics in the environment. This is a promising result, as it does indicate that there are some microbes found naturally in Minnesota with a natural ability to break down long polymers that are similar to those found in polyethylene and polypropylene. Work is currently focused on Task 3 of Activity 2.

Seventh Update as of March 1, 2023:

As mentioned under Activity 1, much of our work is now focused on Task 3 of Activity 2, and we recently obtained the sequencing results for several different isolated microbes of a specific genus of bacteria that show promise for degrading polyethylene. Once we have completed the genome assemblies, we will begin to compare these genomes with other related strains to determine if there are conserved genes that these organisms harbor. We are also testing advanced genetic approaches that could reveal the identity of the metabolic pathways that are used to degrade polyethylene by this class of microbes. These efforts are more exploratory while we work on the computational aspects of assembling the genomes, though we would like to pursue these experiments in the future if we are able to acquire additional funding.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

The analysis of genes that might be important in the biodegradation of polyethylene and similar polymers is now the primary focus of our work as we move away from the laboratory studies completed as part of this project and into the data analysis portion of this work. We anticipate continuing work in relation to this final goal, and pursuing additional laboratory studies related to this aspect in the future once we obtain additional funding.

We constructed the laboratory-scale insect gut digesters and operated these for nearly a year but did not ever identify significant amounts of methane or hydrogen production. It is possible that this is due to very low yields and very slow rates of degradation in these reactors. We also constructed the aerobic reactors to test methods for bioremediation of plastics in the environment, and as a result of these reactors and other approaches developed to isolate communities of microbes, we were able to isolate several communities that were characterized and are the primary topic of a manuscript that is in revision at the journal Environmental Microbiology. We have addressed all of the concerns of three separate reviewers and are awaiting a final editorial decision on the manuscript. Finally, we fully sequenced approximately 25 microbial isolates and published the genomes of the microbes to national databases. These genomes are currently being analyzed to identify specific genes that are involved in plastic waste degradation.

IV. DISSEMINATION:

Description: Results obtained from this project will be disseminated in several manners. The first is through peer-reviewed publication, which allows the results to be scrutinized by other experts in the field, and then shared with the larger research community. Since we also anticipate including various teachers and school children across the state, we aim to develop a webpage that narrates the project, goals and approaches, and provides an update of results that are obtained. This will allow those members of the public who are interested in this complex problem to see how we approach this problem and evolve our efforts over time based on the results that are obtained. Finally, it is expected that we will make several presentation as part of our outreach with other scientists and also with the community. The webpage will be developed as a component of the PI's current website, listed above.

The Minnesota Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENRTF Acknowledgement Guidelines.

First Update March 1, 2020

We have begun writing a draft manuscript related to some of the approaches used in this study to isolate various strains, which we plan to use to accompany the publication of several of the completed genomes that have been sequenced as part of the initial phase of this project. We are also working with students from Great River Montessori School in St. Paul, and are planning to expand this effort to include additional high school students from across the state as part of our laboratory outreach efforts. We have also begun a website related to this project that is posted as part of the laboratory's general website, which will highlight some of the progress being made on this project.

Second Update September 1, 2020

We have submitted three genomes to the National Center for Biotechnology Information through the National Institutes of Health, with plans to submit another three and another twelve currently in assembly stages. Our efforts to do some of the proposed outreach were hampered by the current pandemic, and we are evaluating other approaches to continue this outreach work while limiting contact to maintain social distancing protocols.

Third Update March 1, 2021

We have submitted more than a dozen genomes to the National Center for Biotechnology Information through the National Institutes of Health. Our efforts toward further outreach are ramping up again as State and University restrictions are being lifted. We have selected three microbial strains with nice polymer-degrading characteristics to study in more detail, while continuing to maintain community cultures in the laboratory. We anticipate publishing our first paper related to this project in the coming months and putting some additional effort into sharing our results through additional media and websites.

Fourth Update September 1, 2021

We are working on our first manuscript related to this project that will detail some of the tools and approaches we have developed to establish polymer degrading communities in the laboratory for further study. This will be important to share with the broader community, as this issue of plastics in the environment is a global problem, and methodology is an area that needs further development. We are also continuing our work to identify specific genes for plastic degrading enzymes.

Fifth Update March 1, 2022

We have completed many of the methods for polyethylene degradation analysis now, and are collecting key results to assemble the core elements of our first manuscript. We hope to get this first manuscript submitted early this summer, with additional manuscripts to follow to further disseminate the results from this project. We hope this work will serve as preliminary results to pursue additional funding in the future so that methods to treat plastic wastes in the environment can become a reality in the future.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

Based on promising results obtained over the summer and fall months of 2022 on this project, we postponed publication of our initial manuscript to be able to include these new results. We are working on a manuscript currently to describe the microbial communities that were obtained and specific species of microbes that seem to be predominant in the cultures that are showing promise to degrade polyethylene type materials.

Seventh Update as of March 1, 2023:

We are still in the process of writing the manuscript that will describe much of the work we have done related to this project. We believe that the approach we have used here will be beneficial to others in the field, and should provide a new research direction to pursue to identify the genes that are involved in the biodegradation of polyethylene and related chemicals. We are excited to publish these results, as we believe they show strong

potential for future work to further develop systems to biodegrade microplastics from contaminated waters and soils.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

Resources developed as part of this project have already been shared through the National Center for Biotechnology Information (NCBI), a national repository for genomic information, which is a tool for the broader scientific community. Results from this project are being formulated into a series of manuscripts that will be published in peer-reviewed journals, which will detail some of the successful approaches we used to isolate microbial communities able to degrade problem plastics.

Our first manuscript has been peer reviewed, edited and published in Environmental Microbiology as of June 2024. We will follow this manuscript with another manuscript describing our efforts to sequence the microbes that were isolated as part of our community analysis. As part of this work, we also developed and maintain a website (https://barneybioproductslab.cfans.umn.edu/plastic-degrading-microbes), and have had outreach efforts with approximately 10 high school students from around the state of Minnesota. We have also provided tours to another 50 students, where aspects of this project were presented. One graduate student, Natalia Mancipe, presented this research work as part of her PhD dissertation (published) and public presentation. The PI has also presented this work at three scientific meetings, and students have presented posters at two national meetings, and six University sponsored events.

V. ADDITIONAL BUDGET INFORMATION:

A. Personnel and Capital Expenditures

Explanation of Capital Expenditures Greater Than $5,000: N/A

Explanation of Use of Classified Staff: N/A

Total Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: We have budgeted such that a graduate student will receive ½ time support (standard research assistantship) for 2 years, so 1.0 FTE. The project manager and co-PIs will receive a total of 0.15 FTEs over the entire course of the grant. We anticipate undergraduate support distributed to multiple undergraduate students equivalent to 1.0 FTE over the course of the entire grant.

Enter Total Estimated Personnel Hours for entire duration of project: Approximately 4500 hours	Divide total personnel hours by 2,080 hours in 1 yr = TOTAL FTE: 2.15

Total Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: N/A

Enter Total Estimated Contract Personnel Hours for entire duration of project:	Divide total contract hours by 2,080 hours in 1 yr = TOTAL FTE:

VI. PROJECT PARTNERS: The research team includes Professor Brett Barney from the Department of Bioproducts and Biosystems Engineering and the BioTechnology Institute at the University of Minnesota, who will oversee the project. Professor Barney's lab has been isolating natural communities of microbes capable of biodegrading plastics for several years. Professor Jeff Gralnick from the Department of Plant and Microbial Biology will grow anaerobic communities and assist with metagenomics studies. Professor Bo Hu from the Department of Bioproducts and Biosystems Engineering is an expert in the area of anaerobic digestion, and will help with reactor design. We are also working with several industry partners that produce commodity plastics. These industry

partners will provide materials that are key to enriching our cultures and confirming that strains are biodegrading the targeted plastics.

A. Partners outside of project manager's organization receiving ENRTF funding: N/A

B. Partners outside of project manager's organization NOT receiving ENRTF funding: N/A

VII. LONG-TERM- IMPLEMENTATION AND FUNDING: We expect this to be a long-term project. The goals of the project are not the immediate cleanup of any specific site, as it does not make sense to clean a site until we determine ways to eliminate the further addition of these plastics to the environment. Our belief is that the best solution to this problem is to create an incentive for these problem materials to be directed away from the current waste streams. While some of these materials are recyclable, these tend to be difficult recycling streams that are not fully utilized. By developing a technology that converts these materials into a fuel, we are creating new markets and solutions. The research will also contribute to other future directions that could be applied to site specific cleanup strategies.

VIII. REPORTING REQUIREMENTS:

- Project status update reports will be submitted March 1 and September 1 each year of the project
- A final report and associated products will be submitted between June 30 and August 15, 2023

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet
B. Visual Component or Map
C. Parcel List Spreadsheet
D. Acquisition, Easements, and Restoration Requirements
E. Research Addendum

Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2019 Budget Spreadsheet - Final
Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04j
Project Manager: Brett Barney
Project Title: Transformation of Plastic Waste into a Valued Resource
Organization: University of Minnesota/CFANS/BBE
Project Budget: $225,000.00
Project Length and Completion Date: 4 Years, 6/30/2023
Today's Date: 8/15/2023

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Amendment 4/26/23	Amount Spent	Balance
BUDGET ITEM			
Personnel (Wages and Benefits)	$ 167,000	$ 161,988	$ 5,012
Brett Barney, Project Manager (75% salary, 25% benefits), Associate Professor, 9 Month Appointment, Summer Salary; 5% FTE for 2 years, $14,000		$ -	
Jeff Gralnick, co-Project Manager (75% salary, 25% benefits), Professor, 9 Month Appointment, Summer Salary; 2% FTE for 2 years, $9,000		$ -	
Bo Hu, co-Project Manager (75% salary, 25% benefits), Associate Professor, 9 Month Appointment, Summer Salary; 2% FTE for 2 years, $8,000		$ -	
1 Graduate Research Assistant, UMN (Twin Cities), Laboratory Experiment Data Analysis, supervised by Barney and Gralnick (56% salary, 44% fringe), 50% FTE for 2 years, $100,000		$ -	
Undergraduate Research Assistants, UMN (Twin Cities), Laboratory Experiment and Field Study Data Collection, Supervised by Barney/Gralnick/Hu (100% salary) approximately 800 hours per year, 3 years, $36,000		$ -	
Professional/Technical/Service Contracts			
Lab Services - DNA sequencing for metagenomics work, performed at University of Minnesota Sequencing Facilities. Six sequencing runs at $2,500 each.	$ 20,000	$ 19,355	$ 645
Equipment/Tools/Supplies			
Laboratory Supplies: General Laboratory Chemicals, Media, and Reagents ($400 per month) and Kits for Performing Routine Molecular Biology ($400 per kit), Analytical Reagents, DNA Synthesis of Primers ($100 per month), Liquid Nitrogen for Strain Storage ($400 per year). Combined laboratory supplies for the labs for all 3 PIs (Barney, Gralnick, Hu).	$ 38,000	$ 35,370	$ 2,630
Capital Expenditures Over $5,000			
		$ -	$ -
Fee Title Acquisition			
		$ -	$ -
Easement Acquisition			
		$ -	$ -
Professional Services for Acquisition			
		$ -	$ -
Printing			
		$ -	$ -
Travel expenses in Minnesota			
Travel by Brett Barney and students between the Twin Cities campus and various field sites across Minnesota, to be reimbursed by the University Compensation Plan.	$ -	$ -	$ -
Other			
		$ -	$ -
COLUMN TOTAL	$ 225,000	$ 216,713	$ 8,287

OTHER FUNDS CONTRIBUTED TO THE PROJECT	Status (secured or pending)	Budget	Spent	Balance
Non-State:		$ -	$ -	$ -
State:		$ -	$ -	$ -
In kind: Unpaid Indirect Costs		$ 105,000	$ -	$ 105,000

PAST AND CURRENT ENRTF APPROPRIATIONS	Amount legally obligated but not yet spent	Budget	Spent	Balance
Current appropriation:		$ -	$ -	$ -
Past appropriations:		$ -	$ -	$ -