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MTSU Clean Energy Initiative Project Funding Request

There are five (5) sections of the request to complete before submitting. See http://www.mtsu.edu/sga/cleanenergy.shtml for funding guidelines. Save completed form and email to cee@mtsu.edu or mail to MTSU Box 57.

1. General Information	
Name of Person Submitting Request **Anthony Farone, PhD**	
Department/Office **Biology**	Phone # (Office) **898-5343**
MTSU Box # **MTSU Box 0060**	Phone # (Cell) **615-653-6537**
E-mail **Anthony.Farone@mtsu.edu**	Submittal Date **October 1, 2021**

2. Project Categories (Select One)			
Select the category that best describes the project.			
x	Energy Conservation/Efficiency		Sustainable Design
x	Alternative Fuels		Other
x	Renewable Energy		

3. Project Information

a. Please provide a brief descriptive title for the project.
b. The project cost estimate is the expected cost of the project to be considered by the committee for approval, which may differ from the total project cost in the case of matching funding opportunities. **Any funding request is a 'not-to-exceed' amount. Any proposed expenditure above the requested amount will require a resubmission.**
c. List the source of project cost estimates.
d. Provide a brief explanation in response to question regarding previous funding.

3a. Project Title

Sustainable Design: Recycling of Fermentation Waste for Biofuel Using a Pond Microbe

3b. Project Cost Estimate

Total Request	$8500.00
Purification columns	$1500.00
Millipore syringe microfilters	$500.00
Fluorescent microscopy supplies	$1000.00
Disposable micropipette tips	$1000.00
Culture reagents	$500.00
Lipid analysis reagents	$1000.00
Pipettes	$1000.00
Culture Flasks	$2000.00

3c. Source of Estimate

Fisher Scientific (FisherSci.com)

3d. If previous funding from this source was awarded, explain how this request differs?

Previous funding from this source was used for different research topics, e.g. to determine the degradation of toxic barbiturates in soil by microbes. This work resulted in a student-authored* research publication: Bagsby C*, Saha A*, Goodin G*, Siddiqi S*, Farone M, Farone A, and Kline P. 2017.Stability of pentobarbital in soil. *J Environ Sci Health*, 53:207-13. *Indicates student authors.

The current request will research biofuel production by microbes grown on fermentation waste.

4. Project Description

(Completed in as much detail as possible.)

a. The scope of the work to be accomplished is a detailed description of project activities.
b. The benefit statement describes the advantages of the project as relates to the selected project category.
c. The location of the project includes the name of the building, department, and/or specific location of where the project will be conducted on campus.
d. List any departments you anticipate to be involved. Were any departments consulted in preparation of this request? Who? A listing may be attached to this form when submitted.
e. Provide specific information on anticipated student involvement or benefit.
f. Provide information for anticipated future operating and/or maintenance requirements occurring as a result of the proposed project.
g. Provide any additional comments or information that may be pertinent to approval of the project funding request.

4a. Scope: Work to be accomplished

Fermentation byproduct solid waste will be fed into a biofuel study that will analyze the ability of a microbe found in pond water, *Tetrahymena*, to grow on the waste and produce biodiesel fuel. Students will conduct tissue culture of protozoans and biofuel analysis. We propose to have undergraduate students from our laboratory classes learn these marketable techniques in culturing and purification processes. Student authors will present their work at MTSU Scholar's Day and publish their results. Their work will help us understand how to better utilize the biomass produced for alternative energy.

4b. Scope: Benefit Statement

Use of Plant waste for biofuel production by protozoa:

Tetrahymena is a genus of ciliated protozoa – a microbe found commonly in freshwater systems such as ponds, lakes, and rivers. They grow on nutrient medium in the laboratory and can divide in as little as 2-3 hours. Culturing of *Tetrahymena* is relatively easy and cost-effective. During the past few years our laboratory has been exploring *Tetrahymena* as a biodiesel feedstock -- a microbe that can produce the specific lipids (oils) that are the precursors for biofuel production. Algae are an example of another biofuel feedstock microbe.

Several advantages of *Tetrahymena* as biodiesel sources are as follows:

- *Tetrahymena* has a remarkable ability to accumulate lipids (oils). See Fig. 1.
- We have found that they contain the same lipids as algae and have high concentrations of the C-18 ***fatty acids necessary for biofuel production.***
- *Tetrahymena* ***grow much faster than algae*** and are ready to harvest for lipids in only 2-3 days (vs. several weeks to months for algae).

- They would have a cost-effective, low energy advantage over algae because they ***do not have cell walls to mechanically or chemically break*** to harvest the lipid fuel.
- We have already shown that they can ***grow on nutrients from waste products*** (brewery waste, fresh produce waste, and even on Kudzu).
- ***Finally, light is not needed for culturing*** (another energy-saving advantage).

Technical approach for the proposed study:
For the proposed studies, we plan to use the waste from fermentation as the nutrient source for *Tetrahymena* growth. Waste material supplied by local microbreweries will be mixed with spring water, and filtered to create a sterile solution. Our laboratory strains, already known to produce high amounts of biofuel precursor lipids, would be tested for their lipid content using fluorescence of cells stained with the fluorescent lipid-binding dye, Nile Red (Fig. 1). The lipids produced will be confirmed as those suitable for conversion to biodiesel using mass spectrometry.

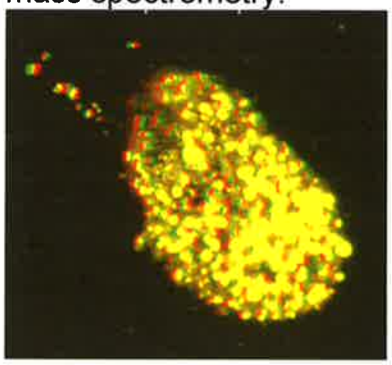

Figure 1. Microscope image of Tetrahymena grown in diluted brewery waste. The cell is full of fluorescent yellow lipid globules (stained with Nile red). Image taken by research assistant Sharon Berk (MTSU).

4. Project Description (continued)

4c. Location of Project (Building, etc.)

Science Building Room 2040, 2070 and 2080.

4d. Participants and Roles

Dr. Anthony Farone, MTSU Biology, will oversee the project and mentor undergraduate and graduate students. He will oversee undergraduates and graduate students in culturing of protozoans and mass spectrometer analysis.

Dr. Mary Farone, MTSU Biology, is a member of the Fermentation Science Program and will oversee student work with plant waste and production of biofuels.

4e. Student participation and/or student benefit

Sydney Ferguson– Undergraduate student – MTSU Department of Biology
Shivam Patel– Undergraduate student– MTSU Biology Department
Tessa Allen—Undergraduate student—MTSU Biology Department
Marshall Baughman- Graduate student – MTSU Department of Biology
Dan Bryant- Graduate student - Molecular Biosciences PhD program

The Farone laboratory typically mentors 5-6 students/year so additional students will be recruited as well as those currently working in the research laboratory.

Students will gain knowledge, skills, and experience in scientific research. Included in the techniques the students will master in this work are: the operation of sophisticated equipment in culturing/growth, purification, and analysis of chemical/biofuel compounds.

Drs. M. Farone, and A. Farone will include this research as part of their Biology courses impacting approximately 200 MTSU undergraduate and graduate students/year.

The community at large will benefit through the development of partnerships with local breweries. Dr. Farone has sent MTSU students to positions at Hap and Harry's, Fat Bottom, Turtle Anarchy Breweries.

4f. Future Operating and/or Maintenance Requirements

None. Scale up of this system will be funded by other external mechanisms such as the USDA.

4g. Additional Comments or Information Pertinent to the Proposed Project

This is a modification of a previous submission that was not funded. Dr. A. Farone has subsequently met with Dr. Kelley and we have attempted to clarify some of the technical details in the current submission, as well as enhance the student impact of this project. We are also excited about our recent successes with biofuel production from *Tetrahymena* strains.

While the proposed funding will provide support for more student research experiences, the project can be scaled back if not fully funded.

5. Project Performance Information

Provide information if applicable.

a. Provide information on estimated annual energy savings stated in units such as kW, kWh, Btu, gallons, etc.
b. Provide information on estimated annual energy cost savings in monetary terms.
c. Provide information on any annual operating or other cost savings in monetary terms. Be specific.
d. Provide information about any matching or supplementary funding opportunities that are available. Identify all sources and explain.

5a. Estimated Annual Energy Savings (Estimated in kW, kWh, Btu, etc.)

Based on the conversion from the government website (https://www.afdc.energy.gov/fuels/fuel_comparison_chart.pdf), 1 gallon of gasoline = 33.7 kWh of electricity, the estimated fossil fuel energy expended to harvest wild ginseng from rural areas is much greater than the electrical energy required to produce laboratory ginseng.

Currently, a gallon of algal biofuel is approximately $7/gallon (http://www.biofuelsdigest.com/bdigest/2014/10/13/where-are-we-with-algae-biofuels/). Much of this cost is reflected by the light energy needed to grow algae—a process which could take up to 3 months to harvest biomass suitable for lipid extraction. Authors Beal et al. estimate that lighting for algal growth requires 860.60 kJ, or 0.24 kWh for every liter (0.26 gal) of bio-output from algae (Beal et al. 2012. *Energies.* 5:1943-1981; doi:10.3390/en5061943; http://www.mdpi.com/1996-1073/5/6/1943). Algae also have thick cell walls requiring additional mechanical energy to break open the cells. *Tetrahymena* represent a significant energy savings because they grow much faster than algae and do not require the additional energy input.

5b. Annual Energy COST Savings ($)

5c. Annual Operating or Other Cost Savings. Specify. ($)

Because the goal of this project is research-based, the annual operating cost savings will be determined depending upon how efficiently the plant waste byproducts are found to be converted to usable biofuels by the protozoans.

Electricity rates in Tennessee for industry are 6.66 cents/kWh (https://www.electricitylocal.com/states/tennessee/murfreesboro/). Based on kWh per gallon of *crude* algae bio-output, eliminating **just** the light needed for algae growth would save approximately 6 cents for every gallon of biofuel (0.24 kWh/0.26 gal). This does not include the cost savings of mechanical energy for breakdown of algal cells nor the increased time and energy (fans, pumps, and filtration) required for growth.

5d. Matching or Supplementary Funding (Identify and Explain)

The Biology Department has supported student research through undergraduate research course (BIOL 4280) and graduate student stipends for this project and through laboratory space, basic supplies (e.g. Petri dishes) and common reagents (e.g. sodium chloride).

Current funding from the USDA has also already allowed us to identify strains of *Tetrahymena* that successfully produce the oils needed for biofuel production when grown on other plant waste.