Rec 2/16/18

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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**	
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E-mail **Anthony.Farone@mtsu.edu**	Submittal Date **February 16, 2018**

2. Project Categories (Select One)

Select the category that best describes the project.

x	Energy Conservation/Efficiency	x	Sustainable Design
x	Alternative Fuels	x	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 Ginseng Plant Waste for Biofuel Using a Pond Microbe

3b. Project Cost Estimate $9000

Total Request	$9000.00
HPLC purification columns (2)	$1500.00
Strata X Solid Phase Extraction 100 ct 1 mL tubes	$250.00
Millipore syringe microfilters	$500.00
Purification Guard columns	$1000.00
Disposable micropipette tips	$1000.00
Plant growth reagents and supplies	$500.00
Lipid analysis reagents	$1000.00
Pipettes	$1000.00
Tissue Culture Supplies	$2000.00
Centrifugal Concentrators	$250.00

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 ginseng plant waste following extraction of compounds to be screened for medicinal use.

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

Following growth in the laboratory, Ginseng tissues will be extracted for analysis of medicinal compounds. Tissue will be collected and incubated in induction medium that has been optimized for maximum growth. Variation in growth rate, bioactive compound production and ginseng plant tissue production will be monitored. Extracted compounds will be analyzed in laboratory for immune stimulating activity. Plant byproduct solid waste will be fed into a biofuel study that will analyze the ability of a microbe found in pond water to grow on the waste and produce biodiesel fuel. Students will conduct plant tissue culture work, plant compound purification, culture of protozoans and biofuel analysis. We propose to have undergraduate students from our laboratory classes learn these marketable techniques in tissue culture 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 by the economically-threatened ginseng while discovering new medicinal compounds from these plants.

4b. Scope: Benefit Statement

Phase 1. Bioactivity of Ginseng polysaccharides: Ginseng root has a long history of medicinal use in the Far East and has become a multi-million dollar industry having beneficial effects on the human immune system. In addition to the well characterized ginsenoside compounds, ginseng plants also produce unique polysaccharides, but these compounds have been less-thoroughly investigated. Because the tissue culture-grown ginseng can be stimulated experimentally, our hypothesis is there will be more medicinal compounds produced than in the wild ginseng. If more bioactivity is demonstrated in the cultured ginseng, we can scale-up the cultures to produce large quantities of plant tissue more energy efficiently, conserving the economically-threatened wild ginseng.

Phase 2: Use of Plant waste for biofuel production by protozoa:
Tetrahymena is a genus of ciliated protozoa – a micobe found commonly in freshwater systems such as ponds, lakes, and rivers. They grow on nutrient media 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 lipids.
- We have already shown that they ***grow well on nutrients from waste products*** (brewery waste, fresh produce waste, and even on Kudzu).
- ***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 ginseng growth/extraction for as the nutrient source for *Tetrahymena* growth. Plant waste material would be ground in a blender, 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. The lipids produced will be confirmed as those suitable for conversion to biodiesel using mass spectrometry.

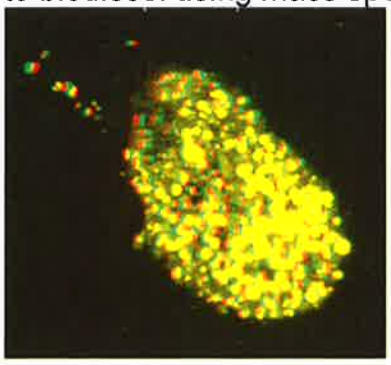

Figure 1. Microscope image of Tetrahymena grown in diluted brewery waste. The cell is full of fluorescent yellow lipid globules (stained with Nile red). Image taken by Sharon Berk.

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 in determining the bioactivity of ginseng extracts.

Dr. Mary Farone, MTSU Biology, will oversee undergraduates and graduate students in the growth of protozoans using plant waste and production of biofuels.

Dr. Elliot Altman, MTSU Biology and Director of TCBMR, will oversee students involved in ginseng plant tissue culture production and extraction.

4e. Student participation and/or student benefit

Marissa Perez– Undergraduate student – MTSU Department of Biology
Chelsey Molina– Undergraduate student– MTSU Biology Department Andrew Swehla– Undergraduate student – MTSU Department of Biology
Marshall Baughman- Graduate student – MTSU Department of Biology
Raj Ghosh- Graduate student - Molecular Biosciences PhD program

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 the tissue culture growth, purification, and analysis of the plant chemical compounds, protozoan growth, purification, analysis of biofuel compounds.

Drs. Altman, M. Farone, A. Farone will include this research as part of their Microbiology, Molecular Genetics, and Immunology courses impacting approximately 200-250 MTSU undergraduate and graduate students/year.

This project will continue to impact large numbers of both MTSU and local high school students. The community at large will benefit through the development of a high school biology laboratory experiment to illustrate the process of biomedical/bioengineering innovations at Central Magnet High School. The first iteration of this experience is currently in progress in Central Magnet High School and this laboratory exercise has been developed by MTSU Biology and Chemistry graduate students. This exercise allows high school students to conduct actual research projects and this exercise will be further developed in the coming years as part of the sustainability of this project.

4f. Future Operating and/or Maintenance Requirements

None. Scale up of this system will be funded by other external mechanisms. The USDA has funded our laboratory for the use of protozoa to produce biofuel from brewer's waste and these technologies will be applied to the ginseng plant waste project in the hopes of receiving additional federal grants to support student research.

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 ($)

Due to overharvesting and 7 years to reach maturity, Ginseng is difficult to find in the wild, hence the "economically-threatened designation," and therefore it is difficult to determine the energy expended by wild ginseng collectors. Depending on the quality, wild ginseng root ranges from $200-$400/pound Preliminary results from our laboratory suggest cultured ginseng can be produced much more rapidly and therefore producing more bioactive compounds/time.

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 ginseng 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 waste products.