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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 **Dr. Mary Farone**	
Department/Office **Biology**	Phone # (Office) **904-8341**
MTSU Box # **MTSU Box 0060**	Phone # (Cell) **(615) 653-6537**
E-mail **mary.farone@mtsu.edu**	Submittal Date **September 20, 2012**

2. Project Categories (Select One)			
Select the category that best describes the project.			
x	Energy Conservation/Efficiency	x	Sustainable Design
	Alternative Fuels		Other
	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 **Bioremediation of Barbiturates for Eco-Friendly Composting v2.0**
3b. Project Cost Estimate Bioremediation Project Total Request $14320.00

Phenomenex C18 LC columns (2)	$1300.00
Strata X Solid Phase Extraction 100 ct 1 mL tubes	$ 600.00
Fisher Scientific HPLC Grade Acetonitrile (5 L)	$ 500.00
Fisher Scientific Certified ACS Methanol (2 L)	$ 200.00
Sodium acetate trihydrate	$ 100.00
Fisher HPLC grade acetic acid	$ 150.00
Millipore syringe microfilters 100 ct	$ 250.00
HiPrep 26/60 Sephacryl S100 column	$ 770.00
Mono P 5/200 GL column	$2020.00
Polybuffer 74	$ 300.00
Mimetic Green 1 Ligand Affinity column	$ 320.00
Bacterial sequencing primers	$ 150.00
Bacterial sequencing reagents	$ 2000.00
Disposable micropipette tips	$ 300.00
Protein analysis reagents	$1500.00
Gene Cloning Kit	$1000.00
Protein purification reagents	$ 900.00
Culturing reagents and supplies	$1000.00
Sodium pentobarbital	$ 110.00
Deuterated pentobarbital as standard	$ 100.00
HPLC Vials	$ 350.00
Centrifugal Concentrators	$ 400.00

3c. Source of Estimate

USDA; MTSU Department of Agribusiness and AgriScience, Dr. David Whitaker; Fisher Scientific Co.; GE Healthcare; research publications

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

We have accomplished the first goal of the initially funded work in which we have successfully detected barbital to the parts/million level in different soil types. However, after numerous attempts, we have had difficulty isolating a new bacterium that will degrade barbital. We therefore propose to use gene engineering to take a barbital-degrading enzyme gene, called barbiturase, from a naturally occurring bacterium, called *Rhodococcus,* and genetically engineer this gene into another bacterium, *E. coli,* that has been designed to produce this enzyme in large amounts. Compared to the low amounts of enzyme produced by *Rhodococcus,* the engineered *E.* coli will produce barbiturase in suitable amounts for bioremediation of large-animal disposal sites. It would also be more favorable to add an enzyme to a compost pile instead of introducing a bacterium into the environment. We have utilized the majority of the generous funding from the previous Clean Energy award as a part of this work but have also given many students invaluable experiences in the classroom and laboratory as well as in presenting their research. Their preliminary results suggest that the current direction of the project will be successful.

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

We propose to take an existing barbital-degrading enzyme gene, barbiturase, from a naturally occurring bacterium called *Rhodococcus* that produces barbiturase in low amounts and engineer this enzyme gene from *Rhodococcus* into another bacterium, a safe strain of *E. coli* that cannot grow in the environment outside of the laboratory. This laboratory strain of *E. coli* is designed to produce the enzyme in large amounts suitable for bioremediation of large-animal disposal sites.

A secondary goal of this project will be to determine the mechanism by which this enzyme operates. Once the gene has been inserted into *E. coli*, studies will be performed to understand how this enzyme functions related to its protein structure. This may allow further modification of the enzyme to increase its efficiency.

4b. Scope: Benefit Statement

The ownership of horses, ponies, and other equines often includes making inevitable decisions about end-of-life events and disposal of deceased animals. Proper disposal of these animals is required of all owners including the Horse Science Center at MTSU whether the cause of death was natural or humane euthanasia. Under the Tennessee Department of Environment and Conservation rules and regulations, carcasses should be disposed of within 48 hours of death. In Tennessee, owners have had several disposal options including rendering, burial, composting, incineration, or landfills. However, many of these options are no longer available. Because of new federal or local regulations, many rendering companies and landfills will no longer accept large animal carcasses, including horses. If the landfill does accept large animal carcasses, it charges a very large fee. Therefore as a practical matter most large carcasses must be disposed of by burial/composting or incineration. Incineration is a *very energy-intensive* process requiring large amounts of fossil fuels such as propane and the renting of large pieces of equipment. Burial/composting is a more practical, energy-efficient process. However since most horses are euthanized with large amounts of pentobarbital the possibility exists that the pentobarbital will leach into the soil and eventually make its way into water sources resulting in a potential environmental hazard to humans and wildlife.

The goals of this study are to reduce the amount of sodium pentobarbital that could leach into the surrounding soil from a buried horse carcass by using a barbiturate-degrading enzyme to reduce the amounts of barbiturate in the soil to environmentally safe levels. This would allow the on-farm composting and burial of large animal carcasses environmentally friendly and both cost and energy-efficient for horse owners.

4. Project Description (continued)

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

Davis Science Building Room 238

Davis Science Building Room 201 (Cold room for protein purification)

Davis Science Building Rooms 105 and 102

MTSU Horse Science Center

4d. Participants and Roles

Dr. Mary Farone MTSU Biology– Project Director and Cloning of *Rhodococcus* gene

Dr. Paul Kline MTSU Chemistry – Development of soil screening methods for presences of barbiturates and isolation and characterization of barbiturate degrading enzymes

4e. Student participation and/or student benefit

Matthew Rodgers – MS student – MTSU Department of Biology

Chasity Suttle – MS student – MTSU Department of Chemistry

Brandon Stewart – Undergraduate – Biology Department

Matthew, under the supervision of Dr. Mary Farone, would carry out the experiments necessary to produce the barbiturase involved in the bioremediation of barbital. He would also be involved in the work necessary to characterize the enzyme under different environmental conditions.

Brandon, a Presidential Scholar in the Honors College will work with Dr. Farone and Matthew to learn the the molecular biology techniques to conduct the gene cloning methods for this project.

Chasity, under the supervision of Dr. Kline, will continue to develop the techniques necessary to measure the amount of barbital present in soil samples following enzyme treatment. She will also be involved in the purification and characterization of the enzyme(s) responsible for the breakdown of barbital from Matthew's work. Chasity is scheduled to present the results of her work from the previous grant at 2013 Annual

Meeting of the American Academy of Forensic Sciences in Washington DC and the Southeast Regional Meeting of the American Chemical Society and Matthew and Chasity have presented their work at MTSU Scholar's Day and Matthew will present his work at the Tennessee Academy of Sciences Meeting.

The students will gain knowledge skills, and experience in scientific research. Included in the techniques the students will master in this work are the operation and data analysis of mass spectral data, enzyme-linked immunosorbent assay (ELISA), protein purification, and DNA sequencing and gene cloning/engineering.

The MTSU campus and the community at large will benefit by the reduced energy expenses incurred in the disposal of large animals, by the reduced contamination of soils and surrounding watersheds, and the development of a sustainable method for the disposal of large animal carcasses.

Further, a part of this project has been the continued development of a high school biology laboratory experiment to illustrate the process of bioremediation of toxins in the environment. The second iteration of this bioremediation laboratory is currently in progress in Rutherford and Metro Nashville High Schools 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 improved and further developed in the coming years as part of the sustainability of this project. This aspect of the project will continue to impact large numbers of both MTSU and high school students.

4f. Future Operating and/or Maintenance Requirements

N/A

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

N/A

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.)

Incineration of one horse carcass requires 2 million BTU's of fossil fuel, mainly propane. In addition each carcass requires the use of heavy equipment for transportation to the cremation site.

Burial of a horse carcass requires 30 min of diesel tractor time which consumes approximately 0.25 gallon of diesel fuel. The translates to approximately 60,000 BTUs. (http://www.provehicles.co.uk/john-deere-310g-backhoe-loader).

Therefore burial of each horse carcass would conservatively save 1,940,000 BTU's over incineration. Each year MTSU euthanizes 5 horses, while in the United States approximately 90,000 horses are euthanized. (http://www.avma.org/advocacy/federal/legislative/110th/issue_briefs/ahspa.asp) Therefore MTSU would save approximately 9.7 million BTUs, while the United States would save 174.6 billion BTUs.

5b. Annual Energy COST Savings ($)

Burial of the 5 horses MTSU euthanizes each year would cost approximately $10.00, the cost of 2-3 gallons of diesel fuel. The cost of incineration of a single horse is estimated to be between $600-$1000 depending on the current price of propane. (http://www.extension.org/pages/20164/horse-disposal-options) Therefore the cost to MTSU of disposing of 5 horses per year would be between $3000-$5000. This results in an annual savings to MTSU of between $2990-$4990.

The annual cost to the United States of composting 90,000 horses per year versus incineration would be $1,400,000 versus $54,000,000 at the low end and $90,000,000 at the upper end.

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

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

The Biology and Chemistry Departments typically provide approximately $300-$500/ student in support of their research.