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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. Anthony Farone**	
Department/Office **Biology**	Phone # (Office) **898-5343**
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E-mail **anthony.farone@mtsu.edu**	Submittal Date **February 14, 2015**

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 **Isolation of Soil Bacteria that Degrade Toxic Barbiturates from Horses in Landfills**
3b. Project Cost Estimate

Total Request $5400.00	
Phenomenex C18 LC columns (2)	$1000.00
Strata X Solid Phase Extraction 100 ct 1 mL tubes	$ 500.00
Sodium acetate trihydrate	$ 100.00
Fisher HPLC grade acetic acid	$ 100.00
Millipore syringe microfilters 100 ct	$ 250.00
Purification columns	$ 1500.00
Polybuffer 74	$ 200.00
Mimetic Green 1 Ligand Affinity column	$ 250.00
Disposable micropipette tips	$ 300.00
Bacterial growth reagents and supplies	$500.00
Sodium pentobarbital	$ 100.00
Deuterated pentobarbital as standard	$ 100.00
HPLC Vials	$ 250.00
Centrifugal Concentrators	$ 250.00

3c. Source of Estimate

USDA; MTSU Department of Agribusiness and AgriScience; Dr. John Haffner , DVM, and David Whitaker Horse Science Center Director; 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 concentration in different soils. However, we have had difficulty isolating a bacterium that will degrade barbital.

In this proposal we plan to isolate a naturally occurring soil bacterium that has a barbital-degrading enzyme for bioremediation of large-animal disposal sites.

This proposal also includes MTSU student impact with Drs.

Farone and Kline bringing this research into the classroom and impacting more than 200 students each year.

We have utilized the majority of the funding from the previous award and have preliminary results that 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 have students from our laboratory classes bring in soil samples that we will culture for bacteria that grow on barbital-containing medium. Those bacteria that grow on this medium will be isolated and tested for the production of barbital-degrading enzyme, barbiturase. If the enzyme is detected then the barbiturase gene from the soil organism will be produced in by another bacterium, *E. coli,* that is designed to produce the enzyme in large amounts and can be purified away from the bacteria and used for bioremediation of large-animal disposal sites.

4b. Scope: Benefit Statement

The ownership of horses, ponies, donkeys, or mules 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 regulations, many rendering companies will no longer accept large animal carcasses, including horses. Many landfills do not accept large animal carcasses due to county laws or due to the regulations of the landfill operator. 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.

4. Project Description (continued)

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

Science Building Room 2080 and 3080 Science Building

4d. Participants and Roles

Dr. Anthony Farone, MTSU Biology– Project Director, isolation of soil microbes

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

Dr. John Haffner, DVM, Horse Science Center will oversee barbital dispensing for laboratory biochemistry studies.

4e. Student participation and/or student benefit

Kala Grooms– Undergraduate student – MTSU Department of Biology

Marten Melchor– Undergraduate student– Biology Department

Granville Goodin– Undergraduate student – MTSU Department of Chemistry

Kala, under the supervision of Dr. Anthony Farone, would carry out the experiments necessary to engineer the bacteria involved in the bioremediation of barbitals. He would also be involved in the work necessary to characterize the enzyme under different environmental conditions.

Marten, an Army veteran will work with Dr. Farone and Kala to learn the technical skills involved to conduct the molecular biology methods for this project.

Granville, 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. He will also be involved in the purification and characterization of the enzyme(s) responsible for the breakdown of barbital by the organisms identified by Kala.

These 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.

Drs. Farone and Kline will include this research as part of their Microbiology/Immunology and Biochemistry courses impacting approximately 200 MTSU undergraduate and graduate students/year.

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. The first iteration of this bioremediation laboratory 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. 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

Scale up of this system will be funded by other external mechanisms.

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

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. ($)
5d.Matching or Supplementary Funding (Identify and Explain) The Biology and Chemistry Departments have been strong supporters of this project through basic supplies and reagents. **Again, we are appreciative of the funding for this work by the Clean Energy Initiative.**