Rec 9/24/18

(5)

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. James Brian Robertson**	
Department/Office **Biology / SCI 2053**	Phone # (Office) **898-2066**
MTSU Box # **60**	Phone # (Cell)
E-mail **james.robertson@mtsu.edu**	Submittal Date 9/24/18

2. Project Categories (Select One)			
Select the category that best describes the project.			
	Energy Conservation/Efficiency		Sustainable Design
X	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
Cellulase production and optimization for conversion of cellulose into ethanol biofuel

3b. Project Cost Estimate
$3,980
Supplies are itemized in US dollars below:

Plastics	500
Enzymes	700
Talon Beads	300
Chemicals	500

Media	500
Primers	150
Sequencing	150
Glassware	150
Mol.Bio. Kits	525
Glucose strips	50
Chromatography	200
Tubing	75
Antibodies	180
	$3,980

3c. Source of Estimate

Based on MTSU prices from venders (e.g. Fisher Scientific and VWR) for work of similar nature and scope.

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

Dr. Robertson was funded in 2014 for a proposal titled "Development of a novel microbial consortium approach to produce ethanol biofuel from tree and grass" which made substantial findings regarding the limitations of using the cellulose harvested from natural sources as a feedstock for microbial fermentation. This proposed work differs from the previous work in that this work focuses on optimizing combinations of enzymes (not microbes) to convert a refined cellulose source (not natural woody materials) into ethanol. This work makes use of the findings from the previous project to overcome/bypass limitations in the production stream.

Dr. Robertson was funded in 2012 for a proposal titled "Development of a Biosensor to Detect Hydrogen Production in Photosynthetic Microbes", and although that project similarly incorporated Dr. Robertson's expertise and interests in microbiology and biofuels, it shares no similarity with this proposed work.

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

In 2007, the EISA (Energy Independence and Security Act of 2007) set a nationwide target of 21 billion gallons of biofuel by 2022. Ethanol (ethyl alcohol) is a liquid fuel similar to gasoline; however, it is not a fossil fuel. Ethanol is a renewable biofuel that is produced by yeast when they ferment sugars. Currently the source of those fermentable sugars is starchy grains like corn, and therefore using grain for ethanol (as well as the required farm land, irrigation, fertilizers, and motorized farm equipment to harvest the grain) imposes a societal and monetary cost where we choose to "burn" a portion of our grain as fuel rather than eat it.

Cellulosic ethanol production is an alternative way to generate ethanol from woody and leafy plant material (that we don't eat) that is high in cellulose rather than starch. The majority of the dry weight of wood, leaves, and grass is a type of carbohydrate called cellulose which can be converted into sugars and thereby converted in to ethanol. However, cellulosic ethanol production is still in its infancy across America because it has been difficult (and expensive) to convert cellulose into fermentable sugars with the current technology. Molecular biology holds the secret for cracking this industrial obstacle through the production and optimization of a class of enzymes called cellulases (enzymes that convert cellulose into fermentable sugars).

Cellulases exist in dozens of different forms and are utilized largely by bacteria and fungi that eat cellulose (wood, grass, and leaves) for food. Within these organisms, a variety of cellulases are naturally utilized to degrade the various types of cellulose. One class of cellulases (called exocellulases) dissolve larger linear cellulose networks into smaller bundles. A second class of cellulases (called cellobiases) degrade these smaller bundles into sugars. And a third class of cellulases (called endocellulases) help to prevent enzymatic traffic jams. Though these three classes of cellulases work in concerted harmony in nature, we (the scientific community) do not yet understand the mechanics of their synergy. This lack of this knowledge hampers our efforts to efficiently employ these enzymes in an industrial setting. If cellulase cooperation were better understood, it could make cellulosic ethanol production more feasible, thereby saving money and reducing America's dependence on foreign fossil fuels.

The purpose of this proposal is to uncover the synergism of the three different classes of cellulase enzymes. We will determine the most effective mixture of enzymes to determine which permutation most efficiently converts cellulose into fermentable sugar (glucose). To accomplish this objective, the Robertson laboratory will...

1. Engineer strains of E. coli to uniquely produce each singular cellulase (of the 10 cellulases produced by the fungus Trichoderma reesei that fall into the 3 classes of cellulases).
2. Compare the purified cellulases within each of the 3 classes to identify the most efficient one from each class. (There are usually 2-3 cellulases within each class.)
3. Once the optimum cellulase has been identified from each class, we will mix one from each class in different proportions to determine which ratio of enzymes is most efficient at converting cellulose into glucose when they work as a concerted team.
4. Conduct a feasibly study for scaling up our findings to an industrial scale.

The methods used to perform this work will involve...

A. Genetically engineering bacteria to produce the cellulase enzymes of fungi - tasks for which the Robertson lab is expert and well published.
B. Expressing and purifying these enzymes from bacteria – a routine operation that Dr. Robertson teaches in his graduate Biotechnology class BIOL 5550, for which students in the course are welcome to assist and participate (if the CEI committee values student involvement in the project).
C. Testing purified enzymes (and their combinations) ability' to convert a purified candidate cellulose (reagent-grade cellulose) into glucose (a fermentable sugar). Reagent-grade cellulose will be used as a substrate for reasons of consistency between experiments. However, this substrate could be expanded to include cellulose from leaves and grass once optimum ratios of enzymes have been determined.
D. Measuring the amount of glucose (fermentable sugar) produced from the enzymatic reactions using a common glucose meter (like a meter used by diabetics to test blood sugar). We have tested such an approach. The meter will perform for this task.

4b. Scope: Benefit Statement

This project has potential benefits on two scales.

Insights gleaned from this research will have broader impacts on lignocellulosic research in general. The determined ratios of cellulases for optimizing the breakdown of cellulose (regardless of source) will benefit the industry as a whole and will result in publishable results in biofuels journals.

More locally, this project could provide MTSU with an alternative energy stream and an alternative use for certain types of refined cellulose like recycled paper (including used paper towels and food-contaminated cardboard that are not currently recycled). Results of our feasibility study could provide an avenue for collaboration with the new Fermentation Science program and the development of a mini-cellulosic ethanol production stream on campus. (Development of such an ethanol-producing stream with The Fermentation Science Program is outside of the focus of THIS proposal, but might be the subject of a future CEI proposal following success of this proposed project.

4. Project Description (continued)

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

This research will be conducted in Dr. Robertson's lab in SCI 2050.

4d. Participants and Roles

Dr. Robertson will supervise the construction of the strains necessary to produce the various cellulases. As a genetic engineer, Dr. Robertson is well versed in the requirements and processes of this work. The work will be primarily performed by one M.S. student in the Biology department, as well as possible undergrads who work in Dr. Robertson's research lab. Where the subject matter of this work is expandable to learning objectives of the Biotechnology course (BIOL 5550), portions of this work may be conducted by students in that course as well. Involving students with this work shows them practical applications of the skills they are learning in the course, as well as excites them about the prospects of alternative energy and biofuels.

4e. Student participation and/or student benefit

This research project will be completely student-driven. Dr. Robertson will lend his expertise and advice to guide the project, but the hands-on construction and development will be done by the students. As mentioned earlier, this project will also provide complementary experiments and laboratory exercises for students in the Biotechnology class BIOL 5550 (average enrolment 12-16 students per year). Completion of this work is also expected to benefit the new Fermentation Sciences program and developing a tie-in for scale-up will be a priority post completion.

4f. Future Operating and/or Maintenance Requirements

This is a one-time investment in research material needed to construct the strains for enzyme production, test their effectiveness when blended, and to carry out a pilot project for feasibility.

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

This proposal is for technology development with a proposed timeframe of two semesters to construct the strains. Large scale production of ethanol is beyond the scope of this proposal. However, should this research be successful, the goal of scaling up production may be worthy of another proposal in the future.

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.) See below
5b. Annual Energy COST Savings ($) This project by itself does not produce energy cost savings, however it is the beginning of a series of steps that can ultimately lead to reducing our nation's dependence on fossil fuels and the need to import them from foreign countries. Successful completion of this work can provide the impetus for MTSU or Tennessee to develop regional ethanol biorefineries so that our locally produced sources of cellulose can provide an avenue for fuel cost savings. Research that benefits development of locally produced renewable fuels reduces transportation costs and provides flexibility to offset the volatility of the fuel market.
5c. Annual Operating or Other Cost Savings. Specify. ($)
5d.Matching or Supplementary Funding (Identify and Explain) All of the equipment needed for this research has been purchased using other funds. Additionally, student labor for this project will be funded through teaching assistantships. Should modest unexpected costs arise, Dr. Robertson can draw upon departmental resources to cover the difference.