Environment and Natural Resources Trust Fund
2012-2013 Request for Proposals (RFP)

Project Title: **ENRTF ID:** **020-B**

Phosphorus Removal by a Special Phosphorus Accumulating Fungus

Topic Area: B. Forestry/Agriculture/Minerals

Total Project Budget: $ 250,000

Proposed Project Time Period for the Funding Requested: 3 yrs, July 2013 - June 2016

Other Non-State Funds: $ 0

Summary:

We found a special filamentous fungus with capabilities to storage high content of phosphorus. We want to recover phosphorus from digested manure with fungal culture and apply it as fertilizer.

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Sponsoring Organization: U of MN

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Location

Region: Statewide

County Name: Statewide

City / Township:

_____ Funding Priorities	_____ Multiple Benefits	_____ Outcomes	_____ Knowledge Base
_____ Extent of Impact	_____ Innovation	_____ Scientific/Tech Basis	_____ Urgency
_____ Capacity Readiness	_____ Leverage	_____ Employment	_____ TOTAL _____%

PROJECT TITLE: Phosphorus Removal by a Special Phosphorus Accumulating Fungus

I. PROJECT STATEMENT

WHY –Many of the crop fields with phosphorus needs are using chemical fertilizers to provide phosphorus. High quality phosphorus rock for fertilizer production is predicted to be depleted in 50 to 100 years under current rate of excavation. The increasing cost of extraction and refinement of poor phosphorus rock and the rising demands for food production boosts the price of phosphorus fertilizer. On the other hand, application of dairy and swine manure to the field as an organic fertilizer is one way to recycle the phosphorus; however, its application is limited to the site close to the dairy farm due to the low nutrient content of manure wastewater and subsequent high transportation cost. Application rates based on crop nitrogen requirements typically result in phosphorus application rates that are more than double crop requirements. The situation becomes worse with the increasing size of dairy and swine farms, especially in the area where livestock raising is highly concentrated (for instance, Winona, MN). The surplus digested manure applied on soil increases P concentration in agricultural runoff, causing environmental problems like eutrophication. Onsite treatment of surplus digested manure and phosphorus recovery reveals its importance both in resources utilization and environmental considerations. The most common phosphorus removal technology is chemical precipitation. However, the precipitated phosphate salt is hardly dissolved and its bio-availability is debatable. Some bacterial phosphorus removal processes have been explored, however, the phosphorus content of the sludge generated by these processes are very low and the direct application of this sludge as organic fertilizer is not feasible.

Our research group is currently funded by the University of Minnesota's Grant-in-Aid program to screen oleaginous fungal strains and to develop new cultivation methods for biodiesel production. We accidentally found out that one of our filamentous *Mucor* strains can obsessively accumulate high amount of phosphorus during its cell growth, as the phosphorus storage. The phosphorus content of this fungus can easily reach to 6-10% of the dry cell biomass. Compared with bacteria, filamentous fungi have the advantage of being easy to harvest due to the mycelium growth of these cells and thus show promises in phosphorus recovery. We recently developed a process and applied a US patent to take advantage of the filamentous feature of the fungal cells to induce their cell pelletization during the cultivation. Key advantages for this type of cell cultivation method are in the harvest of these cells. Once the microbial cells are pelletized, we can use a simple sieve to harvest these cell pellets, much easier than individual cells, which size are in the range of micrometers and energy intensive methods for instance centrifugation are needed to harvest.

GOALS – Set up a biological phosphorus removal process by the phosphorus accumulating fungus and convert the phosphorus recovered from animal manure to be used as a fertilizer. The project will benefit large size dairy farms, all the crop fields with needs of phosphorus fertilizer and will have a commercial interest from fertilizer producers.

OUTCOMES – The fungal cell biomass containing polyphosphate has relatively higher phosphorus purity and density, and therefore it can be transported to phosphorus deficient area at an acceptable cost. If the phosphorus in the manure digestion effluent can be recovered to be exported from the animal farm to be used as fertilizer elsewhere, the excess nutrient problems in the animal farm can be solved, and associated water quality concerns can be addressed

HOW – This special fungus strain will be cultured on the digested manure water and the phosphate will be assimilated and stored in these filamentous fungal cells, then, the cell biomass will be harvested to process as a biological phosphorus fertilizer. We will first conduct lab research to study the feasibility of the process, then, a pilot demonstration at our lab and a field testing will be carried out for its effectiveness as the phosphorus fertilizer.

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: Conduct lab research on the phosphorus recovery from manure water. **Budget:** $122,537

We will study the flask culture conditions for the fungal cells to accumulate polyphosphate, including the starvation of phosphorus then followed by abundant addition. The final phosphorus content of the cell biomass will be used as the criteria to evaluate the overall potential for this strain to accumulate phosphorus. Cell pelletization method will also be studied to combine this technology for easier harvest of phosphorus containing cells. Finally, combine the manure management and utilize digested manure waste water as the raw material to support the fungal cell growth for polyphosphate accumulation.

Outcome	Completion Date
1. Flask culture conditions of this fungus	Dec 1, 2013
2. Cell cultivation and harvest of this fungus	April 1, 2014
3. Phosphorus accumulation from digested manure	Dec 1, 2014

Activity 2: Pilot demonstration to test the feasibility of the process **Budget: $41,654**

A lab scale demonstration will be set up on a 5L bioreactor to optimize all the cultivation conditions on digested manure. These demonstrations will generate around 10 lbs of dry biomass during the entire testing period and these biomass samples will be taken as fertilizer in the activity 3. A techno-economic analysis will be carried out to estimate the overall cost of the fungal biomass fertilizer and compare to current chemical fertilizer.

Outcome	Completion Date
1. Stable production of fungus biomass containing polyphorsphate	April 1, 2015
2. Generation of 10 lbs of fungus biomass	April 1, 2015
3. Estimation of the phosphorus cost from this process	June 30, 2015

Activity 3: Field testing of using phosphorus accumulating fungus as fertilizer **Budget: $85,809**

Since the project is still in the early exploratory stage, no collaborating partners are involved in current proposal. However, once the process is proved to be feasible, we will collaborate with farms and fertilizer companies on the field testing of phosphorus containing biomass as the fertilizer. Certain area of field will be chosen to apply the fungus biomass obtained in activity 2, and the results will be compared with cases with pure fertilizer applications at the same testing at the adjacent location.

Outcome	Completion Date
1. Comparison of chemical fertilizer and fungal biomass in crop growth	June 30, 2016
2. Phosphorus removal rate, compared to chemical precipitation	June 30, 2016
3. Final reporting	June 30, 2016

III. PROJECT STRATEGY

A. Project Team/Partners

The project team to receive funds from LCCMR includes Dr. Bo Hu and his research team at Department of Bioproducts and Biosystems Engineering, University of Minnesota.

B. Timeline Requirements

The project will be finished within 3 years. We will start objective 1 and 2 for the first two years and work on objective 3 the field test at year 3.

C. Long-Term Strategy and Future Funding Needs

This process once is proved to be feasible, further research fund will be requested from other funding agencies and we will be working with the fertilizer companies to turn the process into industrial reality.

2012-2013 Detailed Project Budget

INSTRUCTIONS AND TEMPLATE (1 PAGE LIMIT)

Attach budget, in MS-EXCEL format, to your "2012-2013 LCCMR Proposal Submit Form".

(1-page limit, single-sided, 10 pt. font minimum. Retain bold text and DELETE all instructions typed in italics. ADD OR DELETE ROWS AS NECESSARY. If a category is not applicable write "N/A" , leave it blank, or delete the row.)

IV. TOTAL ENRTF REQUEST BUDGET: *3* years

BUDGET ITEM *(See list of Eligible and Non-Eligible Costs, p. 11)*	**AMOUNT**
Personnel: *In this column, list who is getting paid to do what and what is the % of full-time employment for each position. List out by position or position type - one row per position/position type. For each, provide details in this column on the inputs: i.e., % dollars toward salary, % dollars toward benefits, time period for position/position type, and number of people in the position/position type.*	$ 226,769
Contracts: *In this column, list out proposed contracts. Be clear about whom the contract is to be made with and what services will be provided. If a specific contractor is not yet determined, specify the type of contractor sought. List out by contract types/categories - one row per type/category.*	$ -
Equipment/Tools/Supplies: *In this column, list out general descriptions of item(s) or item type(s) and their purpose - one row per item/item type.*	$ 7,776
Acquisition (Fee Title or Permanent Easements): *In this column, indicate proposed number of acres and and name of organization or entity who will hold title.*	$ -
Travel: *Be specific. Only in-state travel essential to completing project activities can be included.*	$ 15,455
Additional Budget Items: *In this column, list any additional budget items that do not fit above categories. List by item(s) or item type(s) and explain how number was reached. One row per type/category.*	$ -
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 250,000

V. OTHER FUNDS

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ Being Applied to Project During Project Period: *Indicate any additional non-state cash dollars to be spent on the project during the funding period. For each individual sum, list out the source of the funds, the amount, and indicate whether the funds are secured or pending approval.*	$ -	*Indicate: Secured or Pending*
Other State $ Being Applied to Project During Project Period: *Indicate any additional state cash dollars (e.g. bonding, other grants) to be spent on the project during the funding period. For each individual sum, list out the source of the funds, the amount, and indicate whether the funds are secured or pending approval.*	$ -	*Indicate: Secured or Pending*
In-kind Services During Project Period: *Indicate any in-kind services to be provided during the funding period. List type of service(s) and estimated value. In-kind services listed must be specific to the project.*	$ -	
Remaining $ from Current ENRTF Appropriation (if applicable): *Specify dollar and year of appropriation from any current ENRTF appropriation for any directly related project of the project manager or organization that remains unspect or not yet legally obligated at the time of proposal submission. Be as specific as possible. Describe the status of funds in the right-most column.*	$ -	*Indicate: Unspent? Not Legally Obligated? Other?*
Funding History: *Indicate funding secured prior to July 1, 2013, for activities directly relevant to this specific funding request. State specific source(s) of funds.*	$ -	

Project Manager Qualifications and Organization Description

Competence of the applicants to perform the proposed research

In regard to technical expertise, **Dr. Bo Hu** is an Assistant Professor at Department of Bioproducts and Biosystems Engineering, University of Minnesota. With over 10 years of active research experience specifically in biomass utilization, fermentative conversion, and molecular biology, he has led projects on microbial oil production from waste materials via mixotrophic microalgae and oleaginous fungal fermentation, and projects to develop the modified anaerobic digestion system for biohydrogen production and its microbial community change study by using 16s rDNA based microbial analysis. Dr. Hu's team at UMN has set up several standard procedures such as 16s rDNA fingerprint screening for microbial species in the wastewater treatment facilities, ITS sequences to identify oleaginous fungal species; and several conversion platforms such as pelletized fungal fermentation, solid and hemi- SolidSF to accumulate oil from lignocellulosic materials.

Dr. Hu's lab is located at BAE 320B, adjacent to Dr. Hu's office. The lab space is around 1000 sqft and it is equipped with the two laminar flow hoods and one clean bench. Our lab has all the necessary equipment and facilities for this project, including a refrigerated shaker, two open air shakers, one incubation shaker, two incubators, one fermentation bioreactor, GC-FID-TCD, PCR thermal cycler, several electrophoresis, centrifuge, and ovens. Our research group can also utilize facilities and equipment at **Biotechnology Resource Center** (BRC), on a pay-per-sample base. BRC is a 4,000 square-foot laboratory/pilot plant facility with state-of-the-art equipment for research and development in fermentation, animal cell culture technology, molecular biology, protein expression, and separation of a wide range of biological molecules. We have a long-term agreement with BIL laboratories at BRC to use their equipment HPLC for $40 per sample set (up to 200 samples) for our analysis of sugars and other chemicals.

Project Management

Dr. Bo Hu will recruit one postdoc researcher to co-work on this project. Dr. Bo Hu will oversee the whole project and be responsible for experiment design, student training, filing the annual report, and project dissemination to the local farmers and commodity groups. The grant from LCCMR will primarily cover the expenses of the postdoc researcher, and he/she will receive research assistantship to take samples, conduct experiment, and analyze results. Dr. Bo Hu will meet with his postdoc researcher regularly to discuss about the research results and modify the research plan etc. The travel request will cover the transportation fees to pick up samples, and to disseminate our research results at local conference meetings.

Organization Description

Dr. Bo Hu joined the faculty at Department of Bioproducts and Biosystems Engineering at University of Minnesota in August 2009. Bioproducts and Biosystems Engineering Department has very dynamic research activities and numerous excellent scientific researchers have received grant supports from LCCMR program.