Environment and Natural Resources Trust Fund
2011-2012 Request for Proposals (RFP)

LCCMR ID: 171-F3+4

Project Title: Biofuel Production and Nutrients Removal From Manure Wastewater

Category: F3+4. Renewable Energy

Total Project Budget: $ $154,767

Proposed Project Time Period for the Funding Requested: 3 yrs, July 2011 - June 2014

Other Non-State Funds: $ 0

Summary:

A fungal cultivation process is proposed to follow the anaerobic digestion of manure wastewater in order to remove all the nutrients as well as to accumulate biomass/lipid for bioenergy production.

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Location

Region: Statewide

Ecological Section: 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: Biofuel Production and Nutrients Removal from Manure Wastewater --- Integration of Anaerobic Digestion with Fungal Cultivation

I. PROJECT STATEMENT

As one of the largest agricultural producer in the nation, Minnesota needs proper disposal or treatment to prevent the animal manure polluting the environment. Meanwhile, manure also is a potentially large source of organic carbon that is currently under utilized. Anaerobic digestion (AD) has been widely commercialized to treat manure wastewater while harvesting biogas as an energy source. However, AD only has limited capability to remove all the pollutants from the wastes. After the digestion, still around 40%-60% of COD remains, mainly as fine fibers. Total N and P remain constant although AD converts organic N and P to ammonia and phosphate. A cell cultivation step is recommended followed the AD treatment so that cells can grow on the nutrients in the AD effluent. It can remove the remaining COD, ammonia and phosphate, while the cell biomass can be harvested for the bioenergy/lipid production. There are several options to choose the microbial species in the cultivation step. Obviously microalgae species have been receiving tremendous attentions recently due to its high content of oil in certain stressed conditions and its capability to assimilate ammonia and phosphate during the cell growth. The majority of microalgae in manure wastewater still grow on heterotrophic conditions because rich organic nutrients and high turbidity inhibit the microalgae to grow on sun light and CO_2, and 5 to 10 times dilution of the AD effluent is needed since microalgae cannot tolerate the high concentration of ammonia and phsphate.

Here we are proposing to culture filamentous fungi species, for example *Mucor circinelloides*, after manure is treated with the AD. We believe that Integration of AD with fungal cultivation, instead of microalgae, have a better performance in many aspects. 1) Fungi generally grow much faster than microalgae, and they can tolerate extremely unfavorable conditions as wastewater may have. 2). *Mucor circinelloides* can not only accumulate up to 40%-60% of lipid, but also obsessively high concentration of phosphorus because the phosphorus inside this cell is not only in the form of organophosphorus (the form microalgae utilize phosphorus), but also in the form of polyphosphate, similar as the biological phosphorus removal bacteria (PAO). 3). Many filamentous fungi can form self aggregated pellets, a much easier form to separate via simple filtration method; while microalgae cells need flocculent chemicals to help with the harvest process. 4). Fungal pellets have strong bioadsorption capacity, widely applied in the wastewater to remove the fine particles, color, and heavy metals. 5). *Mucor circinelloides* has much higher capability to utilize fibers due to several hydrolysis enzymes it produces. This is specifically important as the major COD remaining of AD effluent is fine manure fiber. In comparison, microalgae species generally have very limited capability to directly utilize polymers.

This project will develop a process to integrate AD and fungal cultivation (Fig 1), for the GOAL of biofuel production, COD, N and P removal, and easy harvest for fungal biomass. The specific OUTCOMES of the project will be a community based simple design to produce extra local renewable energy products, a typical demonstration of our next generation agricultural wastewater treatment, where *"wastes" are "resources".*

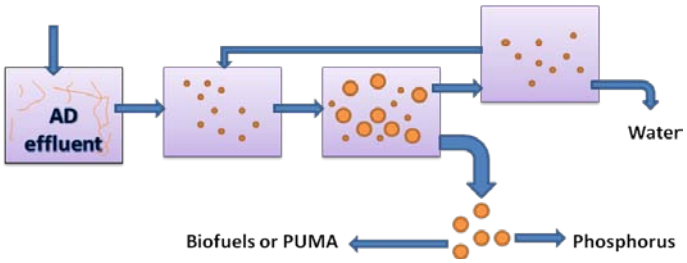

Fig. 1 The proposed integration of AD with fungal cultivation

II. DESCRIPTION OF PROJECT ACTIVITIES

Activity 1: *Pelletized fungal fermentation* **Budget:** $ 56,260

Filamentous fungi tend to aggregate together and grow via pelletized cultivation, characterized for its high surface area, excellent mass transfer, enhanced biomass content. The pellets formation also significantly facilitates harvest and separation of the biomass.

Task 1: Pellet formation. We will study the influence of fermentation factors. The outcome will be optimized culture conditions that stimulate the pellet formation at right size.

Task 2: Pellet adsorption. We will study the bioadsorption of pellets to small particles. The outcome will be a maximized turbidity decrease by introducing pelletized fungal cultivation.

Outcome	Completion Date
1. Optimized cultivation conditions to form fungal pellets	*Jan 1st 2012*
2. Turbidity removal by fungal bioadsorption	*July 1st 2012*

Activity 2: *Nutrients utilization and biomass accumulation* **Budget:** $ 48,223

The primary research efforts will focus on the cultivation of pellets on the AD effluent and culture the pellets for nutrient removal.

Task 3: Fiber hydrolysis and utilization by *Mucor*. As a plant pathogen, Mucor has the capability to degrade the lignin and utilize cellulose and hemicelluloses. The outcome will be degradation, at least part of, and utilization of fine fibers.

Task 4: Ammonia assimilation and biomass accumulation. The research will focus on the ammonia assimilation and its effect on the biomass accumulation. The outcome of the research can be massive fungal biomass accumulation with little or without dilution.

Task 5: Stressed conditions for higher lipid accumulation. The stress factors, for example lower pH, need to apply to the system to increase the lipid content of cells. The outcome is the oil content at the stressed conditions reaching to high level, for example 40-60%

Task 6: Phosphorus removal via polyphosphate production. The primarily outcome is completely remove the P from the waste effluent.

Outcome	Completion Date
3. Optimized cultivation conditions to form fungal pellets	*Sep 1st 2012*
4. Fungal biomass accumulation without massive dilution of effluent	*Jan 1st 2013*
5. High content of oil accumulated in the stressed conditions	*March 1st 2013*
6. Phosphorus completely removed from the effluent	*July 1st 2013*

Activity 3: *Process integration* **Budget:** $ 50,285

The whole process will be integrated to obtain an optimized process for the oil accumulation and nutrient removal.

Task 7: A techno-economic assessment of the feasibility to integrate fungal cultivation with AD will be conducted. The outcome will be a simulation model and lab demonstration of the integrated system for the future commercialization.

Outcome	Completion Date
7. Simulation model for the integrated process	*July 1st 2014*

III. PROJECT STRATEGY

A. Project Team/Partners: The project team includes Dr. Bo Hu as the PI and he is a faculty member of the Biobased and Bioprocessing Engineering Department at University of Minnesota. The funding request will support a graduate student.

B. Timeline Requirements: The research activities will be accomplished with 3 years:

Year 1	Year 2	Year 3
Task 1, 2, 3	Task 4, 5, 6	Task 7

C. Long-Term Strategy and Future Funding Needs: The proposed research activities are the first step of applied research to set up the lab scale integrated wastewater treatment process. Continuous support is needed for the pilot demonstration for future commercialization.

2011-2012 Detailed Project Budget

IV. TOTAL TRUST FUND REQUEST BUDGET *3* years

BUDGET ITEM *(See list of Eligible & Non-Eligible Costs, p. 13)*	**AMOUNT**
Personnel: A Phd student will be getting paid to conduct the experiments of proposed activities. The funds will cover his/her tuition and monthly stipend. Specifically, $72,202 will be paid as the student's three year total salary and wages; and $51,565 will be paid as the total fringe benefits, as requested by University regulation.	$ 123,767
Contracts:	$ -
Equipment/Tools/Supplies:$15,000 for the first year and $5,000 for each of next two years, total $25,000 is requested to cover the material and supply cost for the proposed research. The supply and materials may include the chemicals, lab supplies, analysis for the proposed research activities.	$ 25,000
Acquisition (Fee Title or Permanent Easements):	$ -
Travel: *$2,000 is requested per year for the travel expenses. The travel may include the trip to pick up AD effluent samples, and attending regional outreach*	$ 6,000
Additional Budget Items:	
TOTAL ENVIRONMENT & NATURAL RESOURCES TRUST FUND $ REQUEST	$ 154,767

V. OTHER FUNDS

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ Being Applied to Project During Project Period: *l.*	$ -	
Other State $ Being Applied to Project During Project Period:	$ -	
In-kind Services During Project Period:	$ -	
Remaining $ from Current ENRTF Appropriation (if applicable):	$ -	
Funding History:	$ -	

Project Manager Qualifications and Organization Description

Competence of the applicants to perform the proposed research

In regard to technical expertise, **Dr. Bo Hu** is a new assistant professor with over 8 years of active research experience specifically in the fields within biomass utilization and fermentative conversion. Dr. Hu has been involved in projects to develop the anaerobic digestion system for biohydrogen production and its microbial community study by using 16s rRNA based microbial analysis, projects to study the heterotrophic microalgae fermentation for nutritional oil production, and projects to study the nisin production from cheese whey by using mixed culture system etc. Before joining the faculty at University of Minnesota, Dr. Bo Hu spent two years in Puerto Rico, studying the integration of anaerobic digestion with microalgae cultivation. His paper 'Oil accumulation from waste via heterotrophic/mixotrophic *Chlorella protothecoides'* was recently accepted for publication at Applied Biochemistry and Biotechnology. He received numerous research fundings when he served as the faculty member at UPR, for example, his proposed idea to study the comprehensive bio-utilization of coffee processing waste was funded by USDA Rural Development program.

As a new professor in the Bioproducts and Biosystems Engineering Department, Dr. Bo Hu was offered enough lab space to start his group. He just graduated one master student, recruited a new postdoc researcher and one graduate student in his group at UMN. He is in the process to set up his own lab while he can also access to all the equipments in the department, such as INNOVA 4230 Incubated/Refrigerated Shaker, biosafety cabinet, HPLC, GC-MS, UV-spectrometer, centrifuge, Sartorius 2L Fermenter etc. The PI can also look for help from different research centers for the gene sequence and other services as paid per sample based. Dr. Bo Hu's research has also started to obtain attention and receive support from local industry, for example, Quasar energy group, Schmack Bioenergy (Cleveland, OH) has shown their commitment to support his research in the biofuel field.

Project Management

Dr. Bo Hu will recruit one PhD graduate student 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 graduate student, and he will receive research assistantship to take samples, conduct experiment, and analyze results. Dr. Bo Hu will meet with his graduate student 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, for example, Dr. Roger Ruan, who has received tremendous support from LCCMR program. Being recruited as a Bioprocess Engineer, Dr. Bo Hu's position was part of the University efforts to be a leading center of excellence in the production of biofuels and bioenergy from renewable resources, and to help transform the State of Minnesota to be a leader in the 21st century bio-economy.