Environment and Natural Resources Trust Fund
2016 Request for Proposals (RFP)

Project Title: **ENRTF ID: 076-B**

Production of Value-Added Materials from Wastewater

Category: B. Water Resources

Total Project Budget: $ 946,431

Proposed Project Time Period for the Funding Requested: 3 years, July 2016 to June 2019

Summary:

We will develop an algae-based approach to water treatment, and employ synthetic biology tools to produce value-added products.

Name: Kechun Zhang

Sponsoring Organization: U of MN

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Location

Region: Statewide

County Name: Statewide

City / Township: Minneapolis

Alternate Text for Visual:

Valude-added products from wastewater

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

PROJECT TITLE: ***Production of value-added materials from wastewater***

I. PROJECT STATEMENT

The food and agricultural industries in Minnesota have generated large amount of waste streams. Integrated efforts to clean up wastewater and to manufacture value-added products will lead to significant environmental and economic benefits. We propose to develop an algae-based approach to water treatment, and employ synthetic biology tools to produce useful materials from algal biomass feedstock.

Algae is an emerging solution for wastewater remediation. This technology can remove abundant nitrogen, phosphorus, and organic matter from wastewater, and capture CO_2 to produce lipid oil. Successful examples include the "Algae to Fuels" technology developed by the University of Minnesota (led by co-PI Roger Ruan) with the Metropolitan Council Environment Services. However, the current process remains to be economically viable: the price of biodiesel product is low, and the cost of lipid extraction and esterification is high.

To address this challenge, we will leverage recent advances in synthetic biology to produce high-value products from algal biomass feedstocks. First we will identify algae species that can generate starch-rich biomass. Then we will engineer microorganism to convert starch into isobutyrate, 1,4-butanediol, and mechanically tunable biodegradable polymers. These products can be raw materials for commercial products such as Plexiglass, Spandex, chewing gum and plastic bags with an annual sale of more than $50 billion. The patent applications for the technology have been filed by the University of Minnesota (led by PI Kechun Zhang). By integrating fermentation technology into wastewater treatment, we can develop platform processes to generate an expanded repertoire of sustainable products. The project is expected to have significant environmental impact by improving water quality and reducing carbon footprint. The success can also add value to the agricultural and food industries in Minnesota.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: *Identify optimal microalgae species* **Budget:** $193,445

We will screen such microalgal strains that can tolerate high concentrations of centrate in Minnesota water system. Then strains most effective at N, P, and organic matter removal, as well as starch accumulation will be characterized. Culture strategies to enhance algae growth and biomass production will be developed.

Outcome	Completion Date
1. Identify algae species thriving in local municipal waste water	*9/30/2016*
2. Investigate nutrient removal rate and starch content of algae	*1/31/2017*
3. Develop culture conditions for algae growth	*06/30/2017*

Activity 2: *Develop fermentation technology to convert algae into valuable products* **Budget:** $195,688

We will investigate the fermentation process for converting algal biomass to a range of value-added products. The process economics could be optimized by improving the rate and yield of target products. Starch from algae will be subjected to saccharification by either amylase or acid hydrolysis. Engineered E. coli strains will be used to ferment sugar into isobutyrate, 1,4-butanediol and biodegradable polymers. Fermentation conditions such as sugar feeding rate, growth media, temperature, pH and dissolved oxygen level will be determined experimentally.

Outcome	Completion Date
1. Fermentation of algae to isobutyrate	*12/31/2017*
2. Fermentation of algae to 1,4-butanediol	*06/30/2018*
3. Fermentation of algae to biodegradable polymers	*12/31/2018*

Activity 3: *Integrated system for wastewater treatment and fermentation processing* **Budget:** $403,610

We will develop, design, and construct an integrated facility for process testing and improvement. This facility will consist of our proprietary multi-layer continuous flow enclosed photobioreactors. To culture algae, concentrated wastewater and CO_2 from flue gas will be supplied. During growth, algae remove N, P, trace element (e.g., metal ions) and COD from waste water. The harvested algae will be processed to ferment fuels and chemical as described in Activity 2.

Outcome	Completion Date
1. optimize process parameters for algae culturing system	*06/30/2018*
2. Production and harvest system development	*12/31/2018*
3. Scale-up fermentation development	*06/30/2019*

Activity 4: *Techno-economic analysis and life-cycle assessment (LCA)* **Budget:** $153,688

Based on the data provided from activity 1-3, a techno-economic analysis will be conducted to show the initial investment and estimated operational costs, and to provide the economic validation of the waste-to-algae process for value-added fuels and chemicals. We will also conduct the LCA which will include assessment of environmental impacts of materials, chemicals, and energy inputs.

Outcome	Completion Date
1. Develop a techno-economic model to assess the production cost	*06/30/2018*
2. Develop a LCA model to evaluate the environmental impact of the whole process	*12/31/2019*
3. Finalize both models using the optimized parameters obtained from Activity 3	*06/30/2019*

III. PROJECT STRATEGY

A. Project Partners

The project will be carried out by a team of researchers and engineers from UMN.

Dr. Kechun Zhang, Assistant Professor, Department of Chemical Engineering and Materials Science, UMN, will be the PI & project director. He will be responsible for overall project planning and budget management. His expertise is in synthetic biology, genetic engineering, algae culturing and fermentation processing.

Dr. Roger Ruan, Professor, Director, Center for Biorefining, Department of Bioproducts and Biosystems Engineering (BBE), UMN, will be the co-PI. He will be responsible for designing and evaluating photobioreactors, culturing and harvesting algae.

Dr. Min Addy, Research Associate, P.E., Department of Bioproducts and Biosystems Engineering (BBE), UMN, will be the co-PI. She will be responsible for the techno-economic analysis and LCA of the whole process.

B. Project Impact and Long-Term Strategy

The long-term research goal is to develop an economically viable process to treat wastewater. We envision such a goal will be accomplished by converting organic waste into value-added fuels/chemicals rather than low value biodiesels. Our proposed work could also contribute to scientific and technological advances for the emerging bioeconomy. More specifically, the proposed work will discover algae strains that have the potential to rapidly remove nutrients from Minnesota municipal wastewater as well as accumulate high starch content. Then valuable products could be biosynthesized from this nonfood feedstock. The successful implementation of this integrated strategy would have a broader impact beyond Minnesota.

C. Timeline Requirements

It takes about one year to identify suitable algae strains that accumulate high starch content as well as treat waste water effectively. Then one and half year will be spent on developing fermentation conditions for converting algae biomass. In parallel, scale up system for waste water treatment, algae culturing and fermentation production will be constructed. The project is expected to finish within the 3 year timeframe.

2016 Detailed Project Budget

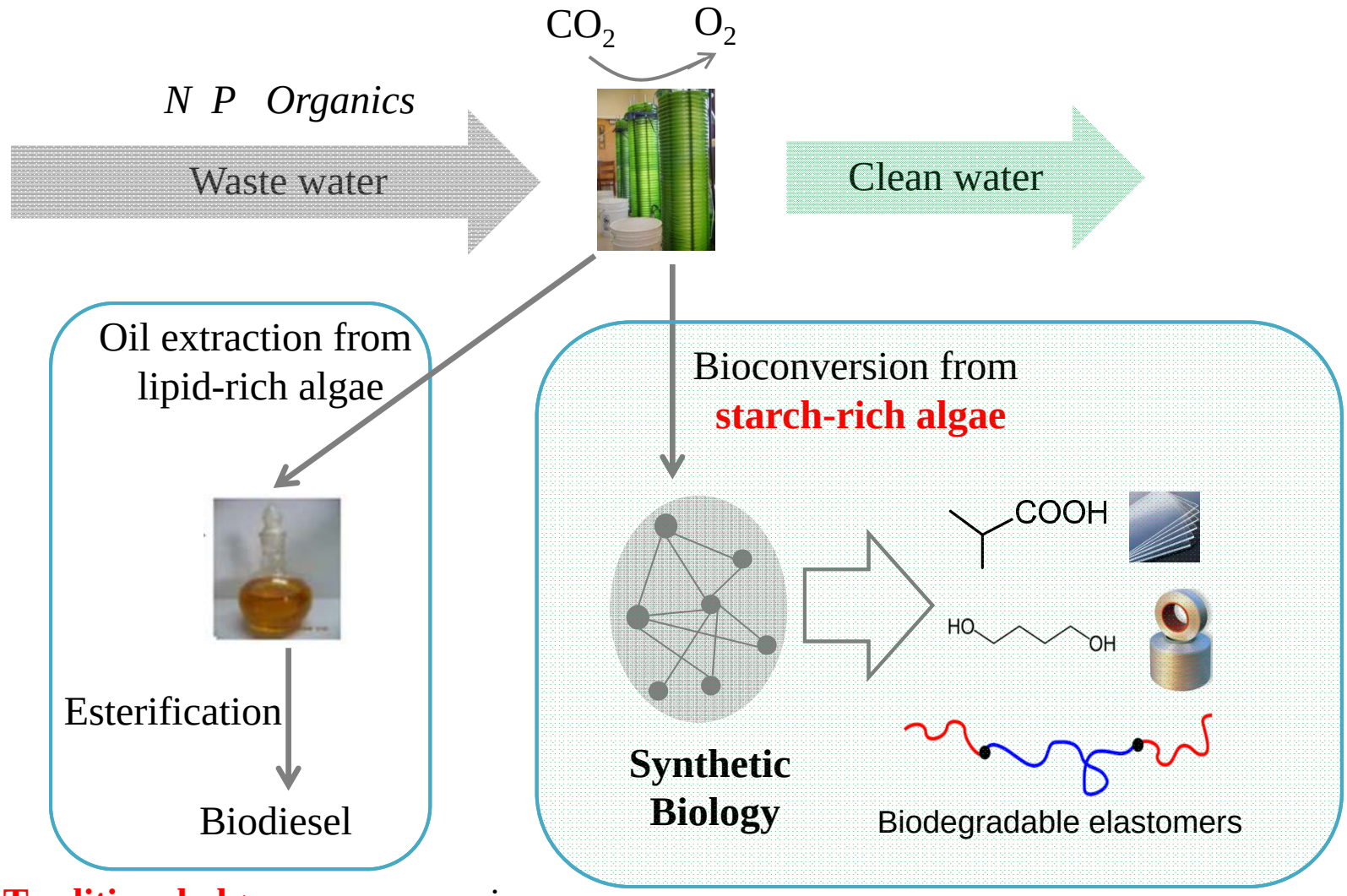
Project Title: *Production of value-added materials from wastewater*

IV. TOTAL ENRTF REQUEST BUDGET *[Insert # of years for project]* **years**

BUDGET ITEM *(See "Guidance on Allowable Expenses", p. 13)*	**AMOUNT**
Personnel: *Personnel: Kechun Zhang, PI, 0.08 FTE, (66.3% salary/33.7% fringe) - $44,808; Roger Ruan, co-PI, 0.08 FTE, (66.3% salary/33.7% fringe) - $58,339;*	$ 720,931
Professional/Technical/Service 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. If an RFP will be issued, state that.*	N/A
Equipment/Tools/Supplies: *pilot scale bio-conversion demo system, Algae cultivation and harvesting demo; - $40,000; services, repairs - $63,000; misc lab supplies, - $115,000*	$ 218,000
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.*	N/A
Travel: *Travel in Minnesota to collect Algae and build fermentation facilities.*	$ 7,500
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 determined One row per*	$ -
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND $ REQUEST =	$ 946,431

V. OTHER FUNDS *(This entire section must be filled out. Do not delete rows. Indicate "N/A" if row is not applicable.)*

SOURCE OF FUNDS	**AMOUNT**	**Status**
Other Non-State $ To Be Applied To Project During Project Period: *Indicate any additional non-state cash dollars secured or applied for 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.*	N/A	*Indicate: Secured or Pending*
Other State $ To Be Applied To Project During Project Period: *Indicate any additional state cash dollars (e.g., bonding, other grants) secured or applied for 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.*	N/A	*Indicate: Secured or Pending*
In-kind Services To Be Applied To Project During Project Period: *Indicate any additional in-kind service(s) secured or applied for to be spent on the project during the funding period. For each type of service, list type of service(s), estimated value, and indicate whether it is secured or pending. In-kind services listed must be specific to the project.*	N/A	*Indicate: Secured or Pending*
Funding History: *Indicate funding secured but to be expended prior to July 1, 2016, for activities directly relevant to this specific funding request, including past and current ENRTF funds. State specific source(s) of fund and dollar amount.*	N/A	
Remaining $ From Current ENRTF Appropriation: *Specify dollar amount and year of appropriation from any current ENRTF appropriation for any directly related project of the project manager or organization that remains unspent or not yet legally obligated at the time of proposal submission. Be as specific as possible. Indicate the status of the funds.*	N/A	*Indicate: Unspent? Legally Obligated?*



Traditional algae process requires expensive oil extraction procedure and generates low-value biodiesel

Our proposed new process is cost-effective and produces various value-added materials

Project Manager Qualifications and Organization Description

Project Managers:

The principal investigator Prof. Kechun Zhang is an assistant professor at the Department of Chemical Engineering and Materials Science at the University of Minnesota. Dr. Zhang received his PhD degree in 2007 from the California Institute of Technology and worked as a postdoctoral fellow at UCLA as from 2007 to 2010. Since Dr. Zhang started his independent career, he has generated nine patent applications and one of which is licensed by Ascenix Biotechnolgies LLC to commercialize green Plexiglas technology. Dr. Zhang has received various awards, including 3M Nontenured Faculty award, American Heart Association National Scientist Development Grant Award, Inaugural Early Innovator award**,** McKnight Land-Grant Professorship.

Dr. Roger Ruan, Professor and Director, Center for Biorefining and Department of Bioproducts and Biosystems Engineering, co-leader of Bioenegy and Bioproducts Cluster of the Initiative of Renewable Energy and Environment (IREE), University of Minnesota, is the co-project manager of the proposed project. Dr. Ruan's research focuses on renewable energy and the environment as well as food safety and quality. Dr. Ruan has published over 200 papers in refereed journals, books, and book chapters, and over 300 additional meeting papers and other reports, and holds 12 US patents. He has received over 100 projects totaling over $17 million in various funding for research.

Organization Description:

The University of Minnesota provides lab space and necessary facilities for project. Major equipments for metabolic engineering and bioprocess engineering include: HPLC (Agilent 1200), Fermentor (Bioflo 115), incubator/shaker, centrifuges, freezers, electrophoresis device, PCR thermocycler, UV/Vis spectrophotometer, pH meter, analytical balances, Transilluminator, Electroporator.

The Center for Biorefining is affiliated with the University of Minnesota Initiative for Renewable Energy and the Environment to coordinate the University efforts and resources to conduct exploratory fundamental and applied research; provide education on bioenergy, biochemicals and biomaterials; stimulate collaboration among the University researchers, other public sector investigators, and private investigators involved in biobased production technology development; promote technology transfer to industries; and foster economic development in rural areas. The Center's research programs are founded by DOE, USDA, DOT, DOD, LCCMR, IREE, Xcel Energy, and other federal and state agencies, NGOs, and private companies. The Center is equipped with state of the arts analytical instruments, and processing facilities ranging from bench to pilot scale.