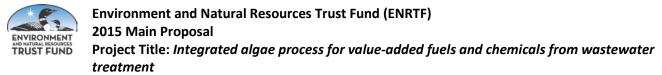
Environment and Natural Resources Trust Fund 2015 Request for Proposals (RFP)

Project Title: ENRTF ID: 050-B	
Integrated Algae Process for Value-Added Fuels and Chemicals	
Category: B. Water Resources	
Total Project Budget: \$ 919,662	
Proposed Project Time Period for the Funding Requested: <u>3 years, July 2015 - June 2018</u>	
Summary:	
This project is to develop an integrated process that will treat wastewater, generate starch-rich algal biomass and ferment value-added fuels and chemicals. The project will improve water quality, reducing carbon	
Name: Kechun Zhang	
Sponsoring Organization: U of MN	
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Minneapolis MN 55455	
Telephone Number: (612) 626-0635	
Email kzhang@umn.edu	_
Web Address https://www.cems.umn.edu/about/people/faculty.id21395.html	_
Location	
Region: Statewide	
County Name: Statewide	
City / Township: Minneapolis	
Alternate Text for Visual:	
project scheme	
Funding Priorities Multiple Benefits Outcomes Knowledge Base	

____ Capacity Readiness _____ Leverage

_____ TOTAL



PROJECT TITLE: Integrated algae process for value-added fuels and chemicals from wastewater treatment

I. PROJECT STATEMENT

Algae-based wastewater treatment is considered to be one of the ideal solutions for wastewater remediation. 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. While current technologies can remove abundant nitrogen, phosphorus, and organic matter from wastewater, and capture CO₂ to produce lipid oil, the

overall economics of this process still requires significant improvement. The reason is that the price of biodiesel is less than \$1/kg, and the cost of lipid extraction and esterification is too high. To address this challenge, we propose to leverage recent advances in synthetic biology to produce value-added fuels or chemicals (>\$2/kg) from algal biomass feedstocks. This proposal builds on our success in engineering E. coli and yeast to biosynthesize advanced biofuel isobutanol, isobutyrate and 1,4-butanediol (raw materials for commercial products such as *Plexiglass* or *Spandex* with an annual sale of more than \$10 billion). The patent applications for the technology have been filed by the University of Minnesota (Led by PI Kechun Zhang). By integrating this biosynthetic strategy into wastewater treatment process, we can avoid the use of glucose feedstock and thus the competition for food and water use in additional to lower the cost of wastewater remediation. The project is expected to have significant environmental impact by improving water quality and reducing carbon footprint. The success could generate potential income for the state with the commercial production of valuable renewable energy and chemicals.

II. PROJECT ACTIVITIES AND OUTCOMES

Activity 1: Identify optimal microalgae species

Algae with high starch content will be good feedstock for downstream fermentation. We will screen such microalgal strains that can tolerate high concentrations of centrate in Minnesota water system. Then strains most effective at nitrogen, phosphorous, and organic matter removal, as well as starch accumulation will be characterized. Since the lipid and starch biosynthesis share the same pathway in most of microalgae species, selection of the mutants that inactivate enzymes that closely related to lipid biosynthesis through UV or chemical treatment will be conducted and evaluated in this study. 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	9/30/2015
2. Investigate nutrient removal rate and starch content of algae	1/31/2016
3. Develop culture conditions for algae growth	06/30/2016

Activity 2: Develop fermentation technology to convert algae into valuable products

Budget: \$194,707 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 isobutanol, isobutyrate or 1,4-butanediol. Fermentation conditions such as growth media, will sugar feeding rate, temperature, pH and dissolved oxygen level be determined experimentally.

	Completion Date
Outcome	
1. Fermentation of algae to isobutanol	12/31/2016
2. Fermentation of algae to isobutyrate	06/30/2017
3. Fermentation of algae to 1,4-butanediol	12/31/2017

Budget: \$192,707

Environment and Natural Resources Trust Fund (ENRTF) 2015 Main Proposal Project Title: Integrated algae process for value-added fuels and chemicals from wastewater treatment

Activity 3: Integrated system for wastewater treatment and fermentation processing Budget: \$389,708 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	06/30/2017
2. Production and harvest system development	12/31/2017
3. Scale-up fermentation development	06/30/2018

Activity 4: Techno-economic analysis and life-cycle assessment (LCA)

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. The final results of the process will be compared with the conventional processes for isobutanol, isobutyrate or 1,4-butanediol production.

Outcome	Completion Date
1. Develop a techno-economic model to assess the production cost	06/30/2017
2. Develop a LCA model to evaluate the environmental impact of the whole process	12/31/2017
3. Finalize both models using the optimized parameters obtained from Activity 3	06/30/2018

III. PROJECT STRATEGY

A. Project Partners

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

<u>Dr. Kechun Zhang</u>, 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.

<u>Dr. Roger Ruan</u>, 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.

<u>Dr. Min Min</u>, 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.

Budget: \$142,539

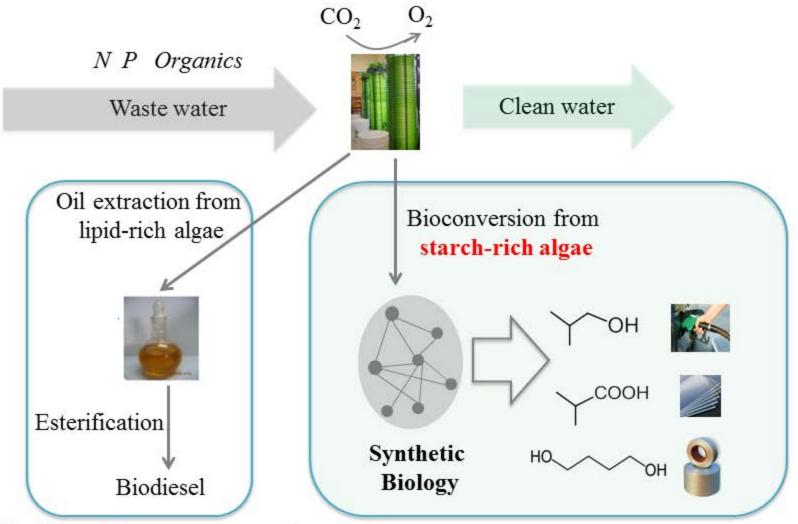
2015 Detailed Project Budget

Project Title: Integrated waste-to-algae process for value-added fuels and chemicals

TV. TOTAL EINRTF REQUEST BUDGET 3 years	1		
BUDGET ITEM (See "Guidance on Allowable Expenses", p. 13)		AMOUNT	-
Personnel: Kechun Zhang, PI, 0.08 FTE, (66.4% salary/33.6% fringe) - \$43,315;	\$		694,162
Roger Ruan, co-PI, 0.08 FTE, (66.4% salary/33.6% fringe) - \$56,762;			
Min Min, co-PI, 0.5 FTE, (66.4% salary/33.6% fringe) - \$109,520;	Post-		
doc Associate, 0.5 FTE (79.25 % salary/ 20.75% fringe) - \$146,501;	3		
Grad Research Assistant, 1.5 FTE,(77% salary/23% fringe) \$338,064			
Contracts:		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):		N/A	
Travel: Travel in Minnesota to collect Algae and build fermentation facilities.	\$		7,500
Additional Budget Items:		N/A	
TOTAL ENVIRONMENT AND NATURAL RESOURCES TRUST FUND \$ REC	UEST = \$		919,662

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	<u>Status</u>
Other Non-State \$ To Be Applied To Project During Project Period:	N/A	
Other State \$ To Be Applied To Project During Project Period: /	N/#	A
In-kind Services To Be Applied To Project During Project Period: Unrecovered F&A	N/ <i>4</i>	A
Funding History:	N/A	4
Remaining \$ From Current ENRTF Appropriation: S	N/#	4



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 fuels and chemicals

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 with Prof. James C. Liao as a postdoctoral fellow at UCLA as from 2007 to 2010. Prof. Liao is the recipient of 2010 Presidential Green Chemistry Challenge Award, and his works are the basis of 1 NASDAQ-traded company (Gevo Inc., production site in Luverne Minnesota) and a new startup company (EASEL Biotechnologies). Dr. Zhang's research output has contributed to such successes. Since Dr. Zhang started his independent career, he has generated 6 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 and American Heart Association National Scientist Development Grant Award.

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 millions 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.