Environment and Natural Resources Trust Fund

2027 Request for Proposal

General Information

Proposal ID: 2027-327

Proposal Title: Biological Deodorization of Biogas

Project Manager Information

Name: Bo Wang

Organization: U of MN - College of Food, Agricultural and Natural Resource Sciences

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Project Basic Information

Project Summary: A bioprocess will be developed to use soil bacterium Cupriavidus necator to remove major smelly and harmful compounds in the off-gas from anaerobic digesters. Potential valuable byproducts will be generated.

ENRTF Funds Requested: $297,000

Proposed Project Completion: June 30, 2030

LCCMR Funding Category: Small Projects (G)

Secondary Category: Resiliency (A)

Project Location

What is the best scale for describing where your work will take place?

Region(s): Metro

What is the best scale to describe the area impacted by your work?

Statewide

When will the work impact occur?

During the Project and In the Future

Narrative

Describe the opportunity or problem your proposal seeks to address. Include any relevant background information.

Anaerobic digesters (AD) are becoming increasingly popular facilities due to the demand for more environmentally friendly ways to dispose of municipal and farm solid waste while generating profit out of it. There are 57 AD at Minnesota wastewater treatment plants (WWTPs) and many operations at dairy farms, with the number of AD increasing more rapidly in recent years. The off-gas streams from anaerobic digesters contain decent concentrations of methane, making it ideal for burning as fuel. However, one problem associated with AD is the problematic off-gas impurities including hydrogen sulfide (H2S), ammonia and various volatile organic compounds (VOCs). These impurities from the raw off-gas are usually highly stinky, toxic and corrosive. If not well treated, they will cause serious environmental damage and health issues. Current strategies for deodorization and detoxifying off-gas from anaerobic digesters necessitate a multi-stage approach encompassing desulfurization, removal of ammonia, adsorption of VOCs, etc. The conventional approach is usually relatively fast, but it produces wastes, requires consumable materials or frequent material regeneration, leading to relatively high energy costs. There has been high demand for more affordable and consolidated processes to detoxify the off-gas from the growing number of AD in Minnesota.

What is your proposed solution to the problem or opportunity discussed above? Introduce us to the work you are seeking funding to do. You will be asked to expand on this proposed solution in Activities & Milestones.

We propose developing a biotechnology to use soil bacterium Cupriavidus necator as the workhorse to deodorize and detoxify the off-gas from AD. The C. necator H16 strain used in this study was initially isolated from soil, and it is well known for its ability to accumulate high levels of bioplastics polyhydroxyalkanoates (PHA). It is able to detoxify various types of smelly gases from the AD off-gas, including aromatic compounds (such as toluene), ammonia, short-chain fatty acids (such as acetate, propionate, and butyrate), and H2S. While C. necator's native capacity in oxidizing H2S is inferior to some other sulfur-oxidizing bacteria, its versatile biodegradation capability and high-performance in converting waste into PHA makes it more appealing than other bacteria in the context of circular economy. Additionally, the H2S removal capacity of C. necator can be improved through an engineering approach. Here, we will (1) use adaptive laboratory evolution and metabolic engineering approaches to enhance the H2S removal capacity of C. necator, (2) systematically characterize the kinetics in toxic compound removal and the bioplastics PHA formation by C. necator cells grown with mimicked and pilot AD off-gas, and (3) Develop a more efficient bioprocess for harvesting C. necator cells and thiosulfate.

What are the specific project outcomes as they relate to the public purpose of protection, conservation, preservation, and enhancement of the state's natural resources?

In Minnesota, the agricultural, dairy, and food processing industries generate significant organic waste, leading to an increasing number of anaerobic digesters (AD). Developing robust bioprocess for the treatment of off-gas from anaerobic digesters (AD) directly serves the public interest in Minnesota through reducing air pollutant emission from these waste management facilities. In addition to the removal of the smelly and toxic AD off-gas impurities, our research provides a promising sustainable bioplastics production opportunity. This project aligns with the state's goals for environmental stewardship and the circular economy.

Activities and Milestones

Activity 1: Use adaptive laboratory evolution and metabolic engineering approaches to enhance the H2S removal capacity of C. necator

Activity Budget: $96,107

Activity Description:
This task aims to improve the C. necator's capacity in removing the smelly and toxic compound, H2S. While the wild-type C. necator H16 studied in this research possesses excellent capability in degrading other toxic compounds in the AD off-gas, its ability to handle high concentrations of H2S still needs to be improved. The C. necator H16 strain can oxidize 0.1 mM H2S in aqueous solutions (corresponding to 1000 ppm in the headspace), but the reaction rate was slow and its H2S removal capability might be further inhibited when exposed to AD off-gas where the H2S concentrations can be up to 3000 ppm. Overexpression of key genes in the H2S oxidization pathway was proven effective to improve C. necator's H2S conversion rates. Here, we will use two strategies to improve C. necator's capacity in oxidizing H2S. One is overexpressing a more efficient H2S oxidization pathway consisting of sulfide:quinone oxidoreductase (SQR) and persulfide dioxygenase (PDO). The second one is to combine random mutagenesis with exposing C. necator to elevated concentrations of H2S for adaptive laboratory evolution. The mutant C. necator cells with higher H2S oxidizing capacity will outcompete other cells. The outcome will be C. necator strains exhibiting improved H2S

Activity Milestones:

Description	Approximate Completion Date
Overexpressing a more efficient H2S oxidization pathway consisting of sulfide:quinone oxidoreductase (SQR) and persulfide	June 30, 2028
Random mutagenesis and adaptive laboratory evolution for improving C. necator's H2S removal capability	December 31, 2028

Activity 2: Systematic characterization of toxic compound removal and the bioplastics formation kinetics for C. necator cells grown with mimicked and pilot

Activity Budget: $98,881

Activity Description:
This task aims to systematically characterize the ability of the engineered C. necator strains acquired in Activity 1 to effectively remove smelly and toxic compounds from the AD off-gas. As C. necator can utilize volatile fatty acids (VFAs; rich in AD liquid digestate) as the sole carbon and energy sources, the cultures of C. necator strains will be resuspended in culture media supplemented with AD liquid digest as the carbon and energy source, and then placed in a glass reactor. We will prepare an artificial gas mixture to mimic the AD off-gas, consisting of H2S, ammonia and toluene. The system will then be purged with pure CO2 to remove dissolved O2. The artificial AD off-gas mixture and a small amount of O2 will be injected into the reactor and sealed. The overall injected gas consists of 2% of O2, 0.3% of H2S (3000 ppmv), 0.01% of ammonia (100 ppm), 0.005% of toluene (50 ppm) relative to the total headspace volume (100%). The concentrations of these compounds, the VFAs, the cell density and the intracellular PHA will be monitored over a time course. Off-gas from a pilot AD within our department will be subsequently tested for deodorization using

Activity Milestones:

Description	Approximate Completion Date
Systematic characterization of toxic compound removal and the bioplastics formation kinetics for C. necator cells	June 30, 2029
Characterization of biotransformation kinetics for C. necator cells grown with pilot AD off-gas	June 30, 2029

Activity 3: Develop an efficient bioprocess for harvesting C. necator cells and thiosulfate

Activity Budget: $102,012

Activity Description:

This task aims to develop a process for efficient separating PHA-containing C. necator cells from the thiosulfate-containing supernatant. As C. necator cells can utilize VFAs, toluene, ammonia to grow and make biodegradable plastics PHAs while oxidizing H2S, the final products at the end of this bioprocess will be intracellular polyhydroxyalkanoates (PHA) and the thiosulfate in the culture supernatant. PHA are a type of biodegradable plastics that hold great potential in a variety of applications such as packaging and agricultural mulch films. Thiosulfate is commonly used for removing residual chlorine or chloramine from water for aquaculture systems, and it is also a more environmentally friendly reagent for leaching and recovering gold. Since C. necator cells are small (1-2 micron, rod-shaped), separating the cells from the culture supernatant is usually energy costly and membrane-fouling during filtration is a common problem. Here, various flocculation pretreatment methods (e.g., chitosan and alginate) will be investigated to examine their efficacy in triggering cells to aggregate before cells are loaded onto membrane filtration. The findings of this task will lay a solid foundation for developing a more economical bioprocess for harvesting both valuable PHA and thiosulfate, which will make this AD off-gas treatment process more economically.

Activity Milestones:

Description	Approximate Completion Date
Investigate the capacity of different flocculants in aggregating C. necator cells	June 30, 2030
Preliminary economic analysis for the AD off-gas treatment and byproduct harvest process	June 30, 2030

Project Partners and Collaborators

Name	Organization	Role	Receiving Funds
David Schmidt	University of Minnesota	Assisting the collection of pilot AD off-gas	No

Dissemination

Describe your plans for dissemination, presentation, documentation, or sharing of data, results, samples, physical collections, and other products and how they will follow ENRTF Acknowledgement Requirements and Guidelines.
Results from this project will be disseminated to stakeholders, resource managers, and the public to support improved management of odorous emissions and protection of Minnesota's environment and communities. Key stakeholders, including livestock producers, wastewater utilities, and environmental agencies, will be engaged throughout the project. Our goal is to translate scientific findings into practical recommendations that livestock producers, wastewater treatment facilities, and other operators can use to improve odor control and reduce environmental impacts. Project updates and findings will be shared with the Minnesota Pollution Control Agency and the Minnesota Department of Natural Resources through technical reports, webinars, and project meetings.

We will also communicate results to Minnesotans through plain-language summaries, infographics, and outreach presentations. For example, the results will be shown to the public during our department's annual Open House and Recruitment events. We plan to showcase our progress and findings at our college's booth at Minnesota State Fair.

Research outcomes will also be communicated with the scientific community through presentations at regional, national and international conferences and publication at peer-reviewed journals. We will take advantage of the UMN's University Digital Conservancy (UDC) to make our articles open and free to the public. To ensure long-term accessibility, project datasets, analytical methods, and reports will be archived in publicly accessible UMN data platforms - Data Repository for the University of Minnesota (DRUM). We will always acknowledge support from the Environment and Natural Resources Trust Fund.

Long-Term Implementation and Funding

Describe how the results will be implemented and how any ongoing effort will be funded. If not already addressed as part of the project, how will findings, results, and products developed be implemented after project completion? If additional work is needed, how will this work be funded?
The proposed research will be carried out by Bo Wang and his students at University of Minnesota. After successful demonstration of the technology at lab scale, Dr. Bo Wang will seek collaboration with bioprocess engineers and Minnesota WWTPs and dairy farms that operate anaerobic digesters (AD) to acquire a larger grant for scaling up this bioprocess for deodorizing and detoxifying the AD off-gas. The potential additional funding for scaling up and onsite demonstration will be sought through the Minnesota PCA, Minnesota Department of Health, as well as federal funding agencies such as NSF, EPA and USDA.

Project Manager and Organization Qualifications

Project Manager Name: Bo Wang

Job Title: Assistant Professor

Provide description of the project manager's qualifications to manage the proposed project.
Bo Wang is an assistant professor at the Department of Bioproducts and Biosystems Engineering at the University of Minnesota (UMN). Bo is also a member of the BioTechnology Institute which aims to advance multidisciplinary research and innovation at the forefront of biotechnology. Bo earned his bachelor's and master's degrees in chemical engineering

from Tsinghua University and his Ph.D. degree in biological design from Arizona State University. He was a postdoctoral fellow and research scientist at the National Renewable Energy Laboratory (renamed as National Laboratory of the Rockies), and worked as a research assistant professor at Vanderbilt University before he joined UMN.
Bo has extensive research experience in working with heterotrophic bacteria, cyanobacteria, renewable energy, and bioproducts. His professional background includes chemical engineering, molecular biology, metabolic engineering, and bioprocess engineering, which qualifies him to take the lead in coordinating this project. His research interests include understanding the fundamental metabolism of microorganisms and engineering microbial metabolism for biomanufacturing and bioremediation purposes. Bo has authored >20 articles in peer-reviewed journals or as book chapters and has been awarded 4 US patents on biomanufacturing and biodegradation technologies. Bo had worked on multiple federally funded projects before he joined UMN. At UMN, Bo is a Principal Investigator leading a team centered on studying microbial metabolism and metabolic engineering. He has been a mentor for 3 graduate students and 3 undergraduate students at UMN.

Organization: U of MN - College of Food, Agricultural and Natural Resource Sciences

Organization Description:
In the College of Food, Agricultural and Natural Resources Sciences (CFANS) at the University of Minnesota, we look at the bigger picture. When we envision a better tomorrow, it includes disease-resistant crops, products that protect our health, lakes free from invasive species, and so much more. We use science to find answers to Minnesota and the world's grand challenges and solve tomorrow's problems. Almost 93 percent of students who earn CFANS undergraduate degrees find jobs in their career field or enter graduate school within six months of graduation.

The Department of Bioproducts and Biosystems Engineering, in CFANS, discovers and teaches solutions for the sustainable use of renewable resources and the enhancement of the environment. We discover innovative solutions to address challenges in the sustainable production and consumption of food, feed, fiber, materials, and chemicals by integrating engineering, science, technology, and management into all degree programs.

We have a public impact through community engagement and extension efforts. We develop and deliver high quality, regionally and nationally-recognized research-based programs to meet current and emerging needs of industry and communities. We also have a long-standing tradition of close partnerships with alumni, industry professionals, organizations, government agencies, donors, and community members.

Budget Summary

Category / Name	Subcategory or Type	Description	Purpose	Gen. Ineligible	% Benefits	# FTE	Classified Staff?	$ Amount
Personnel								
Principal Investigator/9 month appointment seeking summer salary		To oversee all research and personnel, design and supervise experiments, perform data analysis, reporting, presenting, and article writing			26.8%	0.24		$43,632
Graduate student full appointment (50% work, 50% education)		Design and perform experiments, analyze experimental results, draft reports, and present findings, and write articles			45.7%	3		$188,326
Undergraduate students		Experiment set and clean up, performing experiments, assisting graduate student, reporting and presenting results			0%	0.6		$22,779
							Sub Total	**$254,737**
Contracts and Services								
UMN Center for Metabolomics and Proteomics	Internal services or fees (uncommon)	To analyze intracellular metabolites from bacterial cell cultures. To elucidate the assimilation kinetics of volatile fatty acids, and degradation kinetics of toxic compounds (such as the aromatic toluene).				0		$9,263
U of M Instrument Services	Internal services or fees (uncommon)	Repairs and maintenance of equipment used, such as rotary shakers, centrifuges, incubators.				0		$1,500
							Sub Total	**$10,763**
Equipment, Tools, and Supplies								
	Tools and Supplies	Laboratory consumables - pipettes, pipettes tips, centrifuge tubes, cuvettes, petri dishes, medium	To conduct experiments and maintain safety of all personnel in lab. Pipettes, pipettes tips, centrifuge					$15,000

		bottles, hydrogen sulfide test kits, gloves, lab coats, safety goggles	tubes are needed for processing cell culture samples. Cuvettes are for reading the optical density of cell culture. hydrogen sulfide test kits are for detecting hydrogen sulfide from the head space from cell cultures. Gloves, lab coats and safety goggles are for personal protection in the lab and in the field.					
	Tools and Supplies	Laboratory chemicals and reagents: hydrogen sulfide standards, toluene, volatile fatty acids, polyhydroxyalkanoates (PHAs), sodium thiosulfate, derivative reagents for GC-MS analysis, cell culture medium, flocculants, organic solvents.	These chemicals, reagents and tools are necessary for the PI and students to set up and execute the proposed biogas deodorization experiments.					$15,000
							Sub Total	**$30,000**
Capital Equipment								
							Sub Total	**-**
Acquisitions and Stewardship								
							Sub Total	**-**
Travel In Minnesota								
	Conference Registration Miles/ Meals/ Lodging	One conference trip for the PI and two students each year. Registration: $100 per person on average; about 200 miles each year; transport & meals: $200 each year. $500 each year.	PI and students will present the progress and findings on biological deodorization of biogas at conferences held within Minnesota; we will communicate with peers, government agencies and stakeholders to seek collaborations and support.					$1,500
							Sub Total	**$1,500**
Travel Outside Minnesota								
							Sub Total	**-**

Printing and Publication								
	Publication	Publishing peer-reviewed journal articles.	To disseminate the research findings. The University Digital Conservancy (UDC) provides free and world-wide open access to scholarly works authored by UMN faculty. Therefore, no additional budget for publication.					-
							Sub Total	**-**
Other Expenses								
							Sub Total	**-**
							Grand Total	**$297,000**

Classified Staff or Generally Ineligible Expenses

Category/Name	Subcategory or Type	Description	Justification Ineligible Expense or Classified Staff Request

Non ENRTF Funds

Category	Specific Source	Use	Status	Amount
State				
			State Sub Total	**-**
Non-State				
			Non State Sub Total	**-**
			Funds Total	**-**

Total Project Cost: $297,000

This amount accurately reflects total project cost?

Yes

Attachments

Required Attachments

Visual Component

File: f6e5cc1d-acb.pdf

Alternate Text for Visual Component

Bacterium C. necator is able to use volatile fatty acids (acetate, propionate and butyrate) as its 'food' and can remove and convert toxic gases (e.g., hydrogen sulfide, ammonia) from biogas (anaerobic digesters off-gas) while generating cleaner fuel and valuable byproducts - bioplastics and thiosulfate....

Supplemental Attachments

Capital Project Questionnaire, Budget Supplements, Support Letter, Photos, Media, Other

Title	File
Letter of Approval to Submit	3d021924-d27.pdf

Administrative Use

Does your project include restoration or acquisition of land rights?

No

Do you understand that travel expenses are only approved if they follow the "Commissioner's Plan" promulgated by the Commissioner of Management of Budget or, for University of Minnesota projects, the University of Minnesota plan?

Yes, I understand the UMN Policy on travel applies.

Does your project have potential for royalties, copyrights, patents, sale of products and assets, or revenue generation?

Yes

Do you understand and acknowledge IP and revenue-return and sharing requirements in 116P.10?

Yes

Do you wish to request reinvestment of any revenues into your project instead of returning revenue to the ENRTF?

No

Does your project include original, hypothesis-driven research?

Yes

Does the organization have a fiscal agent for this project?

No

Does your project include the pre-design, design, construction, or renovation of a building, trail, campground, or other fixed capital asset costing $10,000 or more or large-scale stream or wetland restoration?

No

Do you propose using an appropriation from the Environment and Natural Resources Trust Fund to conduct a project that provides children's services (as defined in Minnesota Statutes section 299C.61 Subd.7 as "the provision of care, treatment, education, training, instruction, or recreation to children")?

No

Provide the name(s) and organization(s) of additional individuals assisting in the completion of this proposal:

Wendy Moylan

Do you understand that a named service contract does not constitute a funder-designated subrecipient or approval of a sole-source contract? In other words, a service contract entity is only approved if it has been selected according to the contracting rules identified in state law and policy for organizations that receive ENRTF funds through direct appropriations, or in the DNR's reimbursement manual for non-state organizations. These rules may include competitive bidding and prevailing wage requirements

N/A