DOE NEPA Tracking Number

U. S. DEPARTMENT OF ENERGY, OFFICE OF SCIENCE
INTEGRATED SUPPORT CENTER—CHICAGO OFFICE

NATIONAL ENVIRONMENTAL POLICY ACT (NEPA)
ENVIRONMENTAL EVALUATION NOTIFICATION FORM

To be completed by "Applicant," i.e., organization with responsibilities for a "Federal action" involving application to DOE for a permit, license, exemption or allocation, or other similar actions. For assistance with this Form, refer to "Instructions for Preparing ISC-CH F-560, Environmental Evaluation Notification Form."

Solicitation/Award No. (if applicable): DE-SC0022091

Organization Name: The Ohio State University (Columbus, Ohio)

Proposed Action Title: Metabolic modeling and genetic engineering of enhanced anaerobic microbial ethylene synthesis

Total DOE Funding/Total Funding: $1,049,986.00

I. Project Description: ***(Use explanation pages if additional space is required)***

A. Proposed Project/Action (if applicable, delineate Federally funded/Non-Federally funded portions)

All of the following proposed actions are new and Federally funded. Need: At present, ethylene is derived almost exclusively from fossil fuels and a small percentage from bioethanol by energy intensive processes, resulting in substantial carbon emissions. Thus there is a clear need for the development of robust and efficient pathways for the microbial conversion of renewable lignocellulose and CO2 feedstocks into impactful levels of ethylene. Purpose: The proposed actions for this work is the genetic modification and bench-scale demonstration of enhanced ethylene synthesis by industrially viable bacteria (Rhodospirillum rubrum and Clostridium cellulolyticum) from CO2 and lignocellulose. The long-term goal beyond the scope of this present project is the scale-up and industrial implementation of these engineered bacteria in ethylene synthesis. For the bacterial synthesis of ethylene, the anaerobic ethylene cycle converts methionine derivatives to ethylene and subsequently regenerates (see North JA, et al., 2020, Science). However, in the native organism, the anaerobic ethylene cycle is both genetically regulated and flux limited. To overcome these limitation on ethylene yields, the following actions will be taken:(Continued on explanation page 1)

B. Would the project proceed without Federal funding? Yes ☐ No ☑

If "yes," use explanation page.

II. Description of Affected Environment: ***(Use explanation pages if additional space is required)***

The Actions 1 and 3 from IA above with potential environment effect are performed indoors. The location in which bacteria will convert CO2 and lignocellulose into ethylene is the Ohio State University, Biological Science Building, 484 W. 12th Avenue, Columbus, OH. The total occupancy of the building is approximately 300 persons spread across 70,000 sq ft gross area. Only those in immediate proximity of the actions are the 6 authorized lab members occupying 400 square feet of space over which the actions are performed. These actions are bench scale (5 ml - 2 L culture) and all strains are euthanized post experimental analysis, collected in regulated bio-hazard containers, and disposed of by Ohio State University Environmental Health and Safety division in accordance with state and federal regulations. These bacteria can only fix CO2 in the absence of oxygen, and thus must be supplied with a defined gas mixture of nitrogen and CO2 prepared commercially. They cannot directly fix CO2 from the atmosphere due to the presence of oxygen.

III. Preliminary Questions:

		Yes	No
A.	Is the DOE-funded work routinely administrative or *entirely* advisory or a "paper study?"	☐	☑

If "Yes", ensure that the description in Section I reflects this and go directly to Section V.

B. Is there any potential whatsoever for: (***Provide an explanation for each "Yes" response)***

1.	Work to be performed outdoors?	☐	☑
2.	Major modification of a building interior?	☐	☑
3.	Threat of violation of applicable statutory, regulatory, or permit requirements for environment, safety, and health?	☐	☑
4.	Siting, construction or major expansion of waste treatment, storage, or disposal facilities?	☐	☑
5.	Disturbance to hazardous substances, pollutants, or contaminants preexisting in the environment?	☐	☑
6.	The presence of any environmentally-sensitive resources?	☐	☑
7.	Any potential whatsoever for high consequence impacts to human health or the environment?	☐	☑
8.	The work being connected to another existing/proposed activity that could potentially create a significant impact?	☑	☐
9.	Nearby past, present, and/or reasonably foreseeable future actions such that collectively significant impacts could result?	☐	☑
10.	Scientific or public controversy, uncertainty over potential impacts, or conflicts regarding resource usage?	☐	☑

If "No" to ALL Section III.B. questions, go directly to Section V.

IV. Potential Environmental Effects: (***Provide an explanation for each "Yes" response)***

A. Environmentally Sensitive Resources: Could the proposed action potentially result in changes and/or disturbances to any of the following resources?

		Yes	No
1.	Threatened/Endangered Species and/or Critical Habitats	☐	☑
2.	Other Protected Species (e.g., Burros, Migratory Birds, Pollinators)	☐	☑
3.	Sensitive Environments (e.g., Tundra/Coral Reefs/Rain Forests)	☐	☑
4.	Cultural or Historic Resources	☐	☑
5.	Important Farmland	☐	☑
6.	Non-Attainment Areas for Ambient Air Quality Standards	☐	☑
7.	Class I Air Quality Control Region	☐	☑
8.	Special Sources of Groundwater (e.g. Sole Source Aquifer)	☐	☑
9.	Navigable Air Space	☐	☑
10.	Coastal Zones	☐	☑
11.	Areas with Special National Designation (e.g. National Forests, Parks, Trails)	☐	☑
12.	Floodplains and/or Wetlands	☐	☑

B. Regulated Substances/Activities: Would the proposed action involve any of the following regulated Items or activities?

13.	Natural Resource Damage Assessments	☐	☑
14.	Invasive Species or Exotic Organisms	☐	☑
15.	Noxious Weeds	☐	☑
16.	Clearing or Excavation greater than one acre or Removal of Trees Governed by Local Requirement	☐	☑
17.	Dredge or Fill (under Clean Water Act, Section 404, greater than one acre)	☐	☑

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B. Regulated Substances/Activities: Would the proposed action involve any of the following regulated Items or activities? (*continued*)

		Yes	No
18.	Noise (in excess of regulations)	☐	☑
19.	Asbestos Removal	☐	☑
20.	Polychlorinated biphenyls (PCBs)	☐	☑
21.	Import, Manufacture, or Processing of Toxic Substances	☐	☑
22.	Chemical Storage/Use	☐	☑
23.	Pesticide Use	☐	☑
24.	Hazardous, Toxic, or Criteria Pollutant Air Emissions	☐	☑
25.	Liquid Effluents	☐	☑
26.	Spill Prevention/Surface Water Protection	☐	☑
27.	Underground Injection	☐	☑
28.	Hazardous Waste	☑	☐
29.	Underground Storage Tanks	☐	☑
30.	Radioactive or Radioactive Mixed Waste	☑	☐
31.	Radiation Exposure	☑	☐
32.	Nanoscale Materials	☐	☑
33.	Genetically Engineered Microorganisms/Plants or Synthetic Biology	☑	☐
34.	Ozone Depleting Substances	☐	☑
35.	Greenhouse Gas Generation/Sustainability	☐	☑
36.	Off-Road Vehicles	☐	☑
37.	Biosafety Level 3-4 Laboratory	☐	☑
38.	Research on Human Subjects or other Vertebrate Animals	☐	☑
39.	Facility footprint exceeds 5,000 Square Feet	☐	☑

C. Other Relevant Information: Would the proposed action involve the following?

		Yes	No
40.	Disproportionate Nearby Presence of Minority and/or Low Income Populations	☐	☑
41.	Existing, Modified, or New Federal/State Permits	☐	☑
42.	Involvement of Another Federal Agency (e.g. license/permit, funding, approval)	☐	☑
43.	Action in a State with NEPA-type law	☐	☑
44.	Expansion of Public Utilities/Services	☐	☑
45.	Depletion of a Non-Renewable Resources	☐	☑
46.	Subject to an Existing Institutional Work Planning and Control Process	☐	☑
47.	Other Pertinent Information Which Could Impact Human Health or the Environment	☐	☑

V. Applicant certification that to the best of their knowledge all information provided on this form is accurate:

Does this disclosure contain: classified, sensitive business, or other exempt information that DOE would not be obligated to disclose pursuant to the Freedom of Information Act. Yes ☐ No ☑

A. Organization Official (Name and Title): Justin Andrew North — Research Scientist

Signature: DocuSigned by: 4690E3F4258F49F... Date: 08/18/2021

e-mail: north.62@osu.edu Phone: 614-292-4313

B. Optional Secondary Approval (Name and Title): Tracy Coleman Burdett — Sr. Sponsored Program Officer

Signature: DocuSigned by: FE8F3E5A9232474... Date: 08/18/2021

e-mail: burdett.16@osu.edu Phone: 614-247-8348

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Remainder to be completed by DOE

VI. DOE Concurrence/Recommendation/Determination:

A. DOE Project Director/Program Manager or Contract/Grant Management Specialist:

	Yes	No
Has the Applicant completed this Form correctly?	☑	☐
Does an existing generic categorical exclusion apply?	☐	☑

If yes, indicate: _____

Name and Title: Daniella Duverne, Contract Specialist

Signature: Daniella Duverne Daniella Duverne Digitally signed by Daniella Duverne Date: 2021.08.19 11:19:07 -05'00' Date: 08/19/2021

B. DOE NEPA Team Review (if requested):

	Yes	No
Is the class of action identified in the DOE NEPA Regulations (Appendices A-D to Subpart D (10 CFR § 1021))?	☒	☐

If yes, specify the class(es) of action: B3.6

Name and Title: _____

Signature: _____ Date: _____

C. DOE Counsel (if requested):

Name and Title: _____

Signature: _____ Date: _____

D. DOE NEPA Compliance Officer:

The preceding pages are a record of documentation required under DOE Final NEPA Regulation, 10 CFR § 1021.410.

☒ Action may be categorically excluded from further NEPA review. I have determined that the proposed action meets the requirements for Categorical Exclusion referenced above.

☐ Action requires approval by Head of the Field Organization. Recommend preparation of an Environmental Assessment.

☐ Action requires approval by Head of the Field Organization or a Secretarial Officer. Recommend preparation of an Environmental Impact Statement.

Comments/limitations if any:

NEPA Compliance Officer:

Name: Peter Siebach Digitally signed by Peter Siebach Date: 2021.08.19 12:40:05 -05'00'

Signature: _____ Date: _____

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Optional Additional Narrative: (add additional detail to description to Sections I and II or explanations to responses in Sections 3 and 4.

Section IA Continued:
Action 1 performed by Ohio State and Colorado State Universities. This action will overcome the known flux and regulation constrains of the anaerobic ethylene cycle. Known genetic elements that suppress synthesis of anaerobic ethylene cycle genes will be genetically engineered to be in the active state or replaced with other compatible active elements. Furthermore, non-native anaerobic etheyle cycle genes with enhanced and alternate functionality that overcome pathway flux limitations will be introduced. This in turn will convert more CO2 and lignocellulose into product.
Action 2 performed by the Department of Energy Pacific Northwest National Laboratory. This action will construct and employ systems-level predictive metabolic models of photosynthetic and lignocellulosic bacteria. Predictive model simulations will provide deep insights into regulation strategies and best combinations of top-performing ethylene cycle genes required to optimize ethylene production while minimizing trade-off costs to the cells.
Action 3 performed by Ohio State University. This action will engineer photosynthetic and cellulolytic bacteria for high-yield ethylene production from CO2 and lignocellulose. The best genes from Action 1 and best strategies from Action 2 will be concurrently engineered into host photosynthetic and lignocellulose bacteria in a combinatorial and modular manner. Strains are placed in sealed anaerobic growth vessels and supplied with nitrogen gas and either CO2 or lignocellulose. As such, these systems are isolated from the surrounding air atmosphere in which the personnel work. Ultimately this will result in engineered bacterial systems that produce robust ethylene yields from CO2 and lignocellulose. At minimum this this technology has the capacity for reducing CO2 emissions by offsetting the fossil fuel-based ethylene industry. In the future, if direct carbon capture from air technology is employed to deliver CO2 from the atmosphere to the bacteria, it can potentially result in a net reduction of global CO2 levels.

Section II Continued:
Given that the organisms cannot directly fix CO2 from air due to the presence of oxygen, the potential to alter the atmospheric CO2 in the vicinity of the activities and beyond the building in an uncontrolled fashion is impossible. Their only negative impact would be a localized production of ethylene that could affect other plant-based experiments within the building. This is mitigated by evacuation of the ethylene produced through a standard laboratory fume hood, which disperses the ethylene into the atmosphere outside of the building to imperceptible levels. In case of accidental environmental release, these engineered strains, which grow slower by virtue of their genetic modifications, would be out-competed by native organisms.

Section 3 responses:
8: The work being connected to another existing/proposed activity that could potentially create a significant impact? A future proposed activity not within the scope of activities of this current project, is industrial scale-up of engineered microbe cultures that convert CO2 and lignocellulose to ethylene. This has the potential to reduce CO2 emission by offsetting the current fossil-fuel ethylene industry as well as actually lowering CO2 levels from current 414 ppm toward 300 ppm goals through microbial fixation of direct air captured CO2. This technology, by virtue of being controllable, would not continuously deplete atmospheric CO2 to detrimentally low levels.

Section 4 responses:
28: Hazardous waste. These activities generating genetically engineered microbes in solid and liquid culture. Cultures are euthanized by sodium hypochlorite (bleach) and collected as biohazardous waste to prevent accidental environmental release. This volume is approximately 30 cubic feet per year, which is disposed of by Ohio State University Environmental Health and Safety division in accordance with federal and state biohazard disposal regulations.

30: Radioactive or Radioactive Mixed Waste. Actions 1 and 3 at Ohio State University will follow the consumption and formation of key ethylene precursory compounds by the microbes using C14 or H3 radioactive tracers. Each experiment requires 10 microcuries of radioactive material (C14 or H3) and produces separate solid (cell debris) and liquid (cell extract) radioactive waste not in excess of 1 mCi per year per radionuclide. All radioactive waste is dispose of by Ohio State University Radiation Safety division in accordance with federal and state radioactive waste disposal regulations. The Ohio State University Research group managed by Dr. Justin North (PI) is permitted and inspected by the Ohio State Radiation Safety division for use of these radionuclide at the indicated levels.

31: Radiation Exposure: Permitted laboratory workers (4 individuals) in the Ohio State University research group managed by Dr. Justin North (PI) are certified for use of C14 and H3 radionuclide by the Ohio State University Radiation Safety division. As indicated in #30, each experiment requires 10 microcuries of radioactive material (C14 or H3) and produces separate solid (cell debris) and liquid (cell extract) radioactive waste not in excess of 1 mCi per year per radionuclide. As calculated by the OSU Radiation Safety Committee for the approved protocols, exposure will not exceed 0.5 rem per year for each worker.

33: Genetically Engineered Microorganisms/Plants or Synthetic Biology. The organisms employed in Actions 1 and 3 are Rhodospirillum rubrum and Clostridium cellulolyticum. They are BLS1 level bacteria, possess defined genetic systems established in the scientific literature, and are familiar to the PIs of this project. Native genes of interest will be deleted from the chromosomes from each organism. Subsequently, homologous genes from other organisms, or native genes modified in nucleotide sequence to enhance activity will be inserted into the organism on a plasmid or on the chromosome. Modified organisms are grown in sealed anaerobic growth vessels in defined minimal salts media with nitrogen atmosphere. CO2 and lignocellulose are supplied and ethylene is produced therefrom, collected, and quantified by gas chromatography.