



**ADVANCED RESEARCH PROJECTS AGENCY - ENERGY
ENVIRONMENTAL IMPACT QUESTIONNAIRE**

SECTION I. PROJECT SUMMARY	
Control Number:	0881-1516
Project Title:	HIGH EFFICIENCY METHANOL CONDENSATION CYCLE (MC2)
Lead Organization:	Regents of the University of California, Los Angeles
Other Participants (Subrecipients, Contractors, etc.):	
Principal Investigator:	James C. Liao
Telephone Number:	310-825-1656
Email:	liaoj@seas.ucla.edu
Location(s) of each Project Activity (City, State):	Los Angeles, CA
SECTION II. CERTIFICATION BY PROPOSER	
I hereby certify that: 1) I am authorized to submit the information in this questionnaire on behalf of the lead organization named below, and 2) the information provided herein is accurate and complete.	
Signature	<i>F. Panovska</i>
Name	Frosina Panovska
Title, Organization	Sr. Grant Analyst
Date	10/11/2013

SECTION III. PROJECT EVALUATION

1. Is the proposed project limited exclusively to intellectual, academic, or analytical activities?

Intellectual, academic, and analytical activities include but are not limited to:

- Literature searches and information gathering,
- Data analysis,
- Computer modeling,
- Analytical reviews,
- Feasibility studies,
- Document preparation,
- Data dissemination,
- Conceptual design, and
- Paper studies.

Note: You cannot answer “Yes” to this question if the proposed project involves any physical experiments, prototypes, pilot-scale, demonstration projects, field tests, or similar activities.

Yes | No

If you have completed Sections I and II and have checked “Yes,” you do not need to answer the remaining questions in this form. If you checked “No,” you must complete the entire questionnaire.



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- Paper studies.
- Feasibility studies,

Note: You cannot answer “Yes” to this question if the proposed project involves any physical experiments, prototypes, pilot-scale, demonstration projects, field tests, or similar activities.

Yes | No

If you have completed Sections I and II and have checked “Yes,” you do not need to answer the remaining questions in this form. If you checked “No,” you must complete the entire questionnaire.

2a. Please select the scale of the proposed activities. If you select "Other," provide a detailed explanation of the scale of the activity in the explanation box provided for Question 2 below.

- Small scale:** activities categorized as "lab" or "bench" scale.
- Pilot scale:** activities of a small scale and short duration related to proof of concept.
- Other:** activities that, by reason of their nature, scope, or duration, do not fall into one of the above two categories.

2b. Will the proposed project involve (a) physical modifications or additions to existing facilities, (b) construction of new facilities, or (c) any change in the use, mission, or operation of existing facilities? If you select "Yes," please provide a detailed description of the activities that will take place in the explanation box provided for Question 2 below, including any potential impacts that project activities may have on the surrounding environment.

Yes | No

2c. In the box below, describe the proposed project, including a brief description of any larger scope of work that the proposed project is a part. Please explain each activity or task as specifically as possible and describe the type of facility where each activity will occur (e.g., urban, industrial, dedicated laboratory facility, agricultural) including the plan for decommissioning or disposition of equipment or materials at the end of the project.

Q2 Explanation: The proposed project is to demonstrate butanol production using a metabolic methanol condensation cycle. The first year of the project we will genetically engineer three microorganisms (Escherichia coli, Bacillus methanolicus, Pichi pastoris) by introduction of non-native pathways to produce butanol(laboratory scale). Meanwhile, we will outsource CNSI MSSR (California NanoScience Institute - Molecular Screening Shared Resource) in UCLA to perform high throughput screening(HTS) to engineer essential enzymes for this pathway. The second year will focus on the highest performance strain and further genetically engineer its genome by removing the competing pathways. In addition, the optimized enzymes from HTS will be introduced to this engineered strain and test butanol production (laboratory scale). The third year (last year), we will test large scale butanol production by using 5L bio-reactor in the laboratory and optimize its condition to maximize the productivity. Work besides HTS will take place in our academic/scientific laboratories on the UCLA campus.

3. Will the proposed project require, in any part, review or permitting by any Federal, state, regional, local, environmental, or regulatory agency (including any existing, renewals, or changes to existing permits)? If you select "Yes," please identify each review or permit needed in the explanation box below.

Yes | No

Q3 Explanation:

4. In the box below, please identify and describe: (1) any potential health and safety risks to the public or project workers that may occur while performing work for the proposed project, and (2) any efforts that will be taken by project participants to mitigate these risks.

Q4 Explanation: Project will involve the use and handling of industrial solvents and standard DNA recombination technologies. All such handling will occur in-lab, and UCLA has instituted dedicated hazardous material disposal practices. All hazardous materials will not be contacted by unrelated persons and will not be allowed to leave the lab. The project activities that involve these materials will pose no risk to the public. In addition, all employees have been trained in the proper use, storage, handling, and disposal of these materials along with the availability of proper safety equipment. Any potential risks employees face as a result of the hazardous materials are substantially mitigated by the required training and equipment.

5a. Does the proposed project have potential to adversely affect any of the following environmentally sensitive resources? Please check any applicable boxes.

- Historical or cultural resources
- Threatened, endangered, or protected species (whether proposed or listed by State or Federal Governments), including their habitat
- Wetlands regulated under the Clean Water Act (33 U.S.C. § 1344) or floodplains
- Tundra, coral reefs, or rainforests
- Geologic resources or minerals (e.g., Underground extraction/injection)
- Areas having a special designation, (e.g., Federally- and state-designated wilderness areas, national parks, national natural landmarks, wild and scenic rivers, state and federal wildlife refuges, and marine sanctuaries)
- Prime farmland, unique farmland, or other farmland of statewide or local importance.
- Special sources of water (e.g., sole-source Aquifers, coastal zones, floodplains)
- Non-renewable resources

None of the boxes above apply to this project. Please proceed to Section IV.

5b. If you checked any boxes in Question 5a, please provide a detailed explanation below that describes: (1) the resources that could be affected and (2) how project activities may affect those resources.

Q5 Explanation:

SECTION IV. EXTRAORDINARY CIRCUMSTANCES

6. Please identify whether the proposed project will involve any of the following activities. If you select "Yes" for any of the questions below, please provide all requested information the explanation box below.

- a. Generation, use, handling, storage, transport, importation, or disposal of toxic or hazardous

chemicals or materials or pesticides.¹ If you checked “Yes,” please provide a detailed description of: (1) the materials, (2) their role in the project, and (3) storage, transport, and disposal procedures for each material.

Yes | No

- b. **Use, development, handling, storage, transport, or disposal of genetically engineered organisms or recombinant DNA.** If you checked “Yes,” please provide a detailed description of: (1) the genetic modifications, (2) the safety procedures in place for their handling and use over the course of the project, and (3) how they will be disposed of. In your response, please indicate if the project team will follow any federally established protocols and/or applicable requirements (e.g., NIH Guidelines for Research Involving Recombinant DNA Molecules or regulations issued by the Animal and Plant Health Inspection Service (APHIS)).

Yes | No

- c. **Use, handling, storage, transport, or disposal of nanoscale materials.** If you checked “Yes,” please provide a detailed description of: (1) the nanoscale materials used, (2) potential risks those materials may pose to the public or environment, and (3) how the materials will be disposed of.

Yes | No

- d. **Use, handling storage, transport, or disposal of solid wastes.**² If you checked “No,” please explain why no waste will be generated under the proposed project.

Yes | No

- e. **Generation, use, handling, storage, transport, or disposal of radiological materials.** If you checked “Yes,” please provide a detailed description of: (1) the materials, (2) their role in the project, and (3) storage, handling, transport, and disposal procedures for each material.

Yes | No

- f. **Any emissions into the ambient air, including but not limited to, greenhouse gas emissions, particulate matter, and airborne pollutants.** If you checked “Yes,” please describe all emissions that may result from project activities.

Yes | No

- g. **Release of pollutants or contaminants into water resources (e.g., lakes, rivers, creeks, wetlands, or ground water).**³ If you checked “Yes,” please provide a detailed description of: (1) the emissions that will be released and (2) the water resources that may be affected.

Yes | No

¹ Hazardous chemicals and materials include those which, because of their quantity, concentration, or physical, chemical, or infectious characteristics, may increase the risk of mortality or pose a substantial threat to human health or the environment when improperly stored, transported, disposed of, or otherwise managed.

² Solid waste includes any (1) garbage, (2) refuse, (3) sludge from a waste treatment plan, water supply treatment plant, or air pollution control facility, or (4) other discarded material, including solid, liquid, semisolid, or contained gaseous materials resulting from industrial, commercial, mining and agricultural operations.

³ In this context, the term “pollutant” means dredged spoil, solid waste, incinerator residue, sewage, garbage, sewage sludge, munitions, chemical wastes, biological materials, radioactive materials, heat, wrecked or discarded equipment, rock, sand, cellar dirt and industrial, municipal, and agricultural waste discharged into water. The term “contaminant” means any physical, chemical, biological, or radiological substance or matter in water.

- h. Adverse community-based environmental impacts, such as visual impacts, noise, or socioeconomic impacts. If you checked "Yes," please provide a description of the nature of the particular impact(s), the communities that may be affected, and the effects the proposed project will have on those communities.

Yes | No

Q6 Explanation:

6a.

(1) toxic material: methanol

(2) role in the project: essential starting material of the product

(3) storage: in flammable-compatible cabinet and all the waste methanol will be transferred to and handled by UCLA EH&S (Environment, Health, and Safety) department officer.

6b.

(1) genetic modifications: we will genetically engineer one yeast (*Pichia pastoris*(ATCC 20864, biosafety level 1), and two bacteria strains (*E.coli* K-12 and *Bacillus methanolicus*(ATCC 51375), both biosafety level 1) to produce biofuels ethanol and butanol. We will introduce non-native genes (either genome integration or on the plasmids) to the above micro-organisms using electrophoration transformation method:

mdh (methanol dehydrogenase, reaction: methanol -> formaldehyde)

hps (hexulose-6-phosphate synthase, hexulose 6-phosphate \rightleftharpoons D-ribulose 5-phosphate + formaldehyde)

phi (6-phospho-3-hexuloisomerase, hexulose 6-phosphate \rightarrow D-fructose 6-phosphate)

fpk (phosphoketolase, D-fructose 6-phosphate + phosphate \rightleftharpoons D-erythrose 4-phosphate + acetyl phosphate)

atoB (acetyl-CoA acetyltransferase, 2 Acetyl-CoA -> acetoacetyl-CoA)

hbd (hydroxybutyryl-coA dehydrogenase, acetoacetyl-CoA -> 3-hydroxybutanoyl-CoA)

crt (3-hydroxybutanoyl-CoA -> crotonyl-CoA)

ter (crotonyl-CoA -> butanoyl-CoA)

pduP (butanoyl-CoA -> butyraldehyde)

adh (butyraldehyde -> butanol)

For *E.coli* and *P. pastoris*, we will introduce: mdh, hps, phi, fpk, atoB, hbd, crt, ter, pduP, adh

For *B. methanolicus*, we will introduce: fpk, atoB, hbd, crt, ter, pduP, adh

PI for this project will obtain UCLA EH&S IBC approval prior to initiation of experiments involving recombinant DNA.

(2) All the safety procedures will be in accordance with NIH Guidelines for Recombinant DNA experiments.

(3) All the genetically-modified strains and their associated materials will be fully sterilized via autoclave or other EH&S approved method then disposed of based on EH&S regulation.

6d.

The solid waste generated by this project will be containers from bacteria culturing and solid media plates and will be fully sterilized via autoclave or other EH&S approved method then disposed of based on EH&S regulation.

