

Dioxide Materials[™] The CO₂ Recycling Company[™]

Improved Microalgal Carbon Utilization Efficiency via integrated CO₂ Electro-conversion to Formate and Microalgal Sequestration DE-FE0032186

Rich Masel, Dioxide Materials, Inc. (PI)

Isaac 'Andy'Aurelio (PM)

2023 Carbon Management Research Project Review Meeting August 28 – September 1, 2023



Formate as a carbon source for algae

Properties of formate/formic acid

- Easy to store
- High water solubility

ioxide Materials"

- Enables conversion of electrical energy to cellular energy (i.e., reductant)
- Broadly toxic to many organisms,



Overview



Figure from Josh Bauer and Lukas Dahlin, NREL.

Dioxide Materials^{**}



Project Overview

- Funding \$2,000,000 (DOE) and (\$500,000) Cost Share)
- •2/1/2023-1/31/2026
- •Kickoff 5/22/2023

Organization chart

Rich Masel **Dioxide Materials** ΡI





Technology Background

Dioxide Materials	NREL	AzCati	Algix							
Formic Acid at a power plant	Algae Strain that can grow in formic acid	Existing Algae Ponds	Incorporate Algae in Polymers							
<image/>	FAME Protein									



Goals Of The Program

- •Electrolyzer 5 cm² ⇒1000 cm²
- Productivity on Formate 1-5 gm/m²/day
 ⇒ >20 gm/m²/day
- Pond carbon efficiency 30% ⇒ >50%
- Algae growth 250 mL ⇒ 1000 L pond
- •Use products in Bloom EVA



Technical Approach

 Formic acid electrolyzer scaling New materials Oxygen control •Engineer P. renovo Incorporate Formate Dehydrogenases Adaptive lab evolution Test in indoor photoreactors (BP1) open ponds (BP2)



Background: Electrolyzer Development





Task 2.1.1: Replace beads with solid structure & test in 5 cm² cell



Task 2.1.2 Design 100 cm² cell





Simulate Flow Distribution





Simulate Flow Distribution



Pioxide Materials

Simulate Flow Distribution



Pioxide Materials

Picture Of Device





Milestones 1.1.2, 1.2.3, 5% formic acid from simulated blast furnace gas for ≥24 hours (Oct 2023), ≥ 100 hr (Jan 2024)

Simulated blast furnace gas: 25% CO, 30% CO₂, 3% H₂, $\frac{10 \text{ ppm of SOx}}{10 \text{ ppm of SOx}}$, N₂ bal.



Simulated power plant gas next $-O_2$ is a problem



Background: Strain Development`

- Wild type *P. renovo* intolerant to formate > 5 milli-molar
 - No growth on formate alone
- Need to express formate dehydrogenase (FDH) to enable growth
 - FDH from Cupriavidus or Candida



Results: Cassettes with different FDH's to identify formate tolerant strains





Results Growth on 10 mM formate



Dioxide Materials"

Milestone 2.1.1. March 2024 >50% formate utilization





Status Vs BP1 milestones

Item	Date						
Revised Management Plan 🗸	8-Mar-23 🗸						
100 cm ² cell designed ✓ and parts ordered✓	29-May-23 🗸						
100 cm ² electrolyzer producing 5% formic acid at ≥100 mA/cm ² from simulated flue gas for ≥24 hrs ✓	04-Oct 23 🗸						
100 cm ² electrolyzer producing 5% formic acid at ≥100 mA/cm ² from simulated flue gas for ≥100 hrs	04-Jan 24 🗸						
Design, synthesize, and transform 5 formate dehydrogenase enzymes into P. renovo. Achieve >50% formate utilization	31-Mar 24 🗸						
Acid pretreat biomass and quantify lipid class and fatty acid profile, utilize extant database to predict NIPU performance.	4-May 24						
Generate 0.5 kg of biomass for downstream product testing	31-July 24						
Utilize biomass composition to evaluate expected bioplastic conversion performance	31-July 24						
100 cm ² electrolyzer producing ≥5% formic acid from simulated 31- July 24 flue gas at a current of ≥100 mA/cm ² for ≥250 hours							
© 2023 Dioxide Materials - Non-confidential	2						

Plans for future testing commercialization





- Program moving forward
 - Demonstrated scaling of electrolyzer
 20x
 - Active area from 5 cm² to 100 cm² (100 hr)
 - •Demonstrated *P. renovo* strains that grow on formate (5x than previous)
 - outperform wild type on atmospheric CO_{2,} Illumination 280µmol/m²/sec



This work was supported by the department of energy under contract DE-FE0032186

Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.





Dioxide Materials[™] The CO₂ Recycling Company[™]

Appendix







Gantt Chart BP1

Project	t Lead: Dioxide Materilas	2023 2024																		
		Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug
WBS	Task Name	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7	8
	management				\rightarrow															
1.1	PMP		-																	
1.2	kickoff																			
2	Electrolyzer development			-	-				-							-				
2.1	100 cm2 construction			-	-			-												
2.1.1	Design 100 cm2 cell			•	-	_														
2.1.2	order cell				•		-	_												
2.1.3	cell received						+	-												
2.1.4	build and test BOP						•	-	_											
2.2	New center compartment					_														
2.2.1	Develop at least 5 possible center compartments						-	_												
2.2.2	Test in 5 cm2 cell					+	-													
2.2.3	5% at 200 mA/cm2 for 24 hours						-													
2.3	Testing 100 cm2 cell							-	_					-						
2.3.1	24 hour tests							•		-	_									
2.3.2	5% formic acid for 24 hours									-+	_									
2.3.3	100 hour tests									+			•							
2.3.4	5% formic acid for 100 hours with flue gas												-							
2.3.5	250 hour tests												•						•	•
2.3.6	5% formic acid for 250 hours with flue gas																		-+	
	Strain development		-								-									
3.1	Develop formate dehydrogenase variants		•	•	-															
3.2	5 formate dehydrogenase variants			-																
3.3	Generate unique algal strains				L.		•	_												
3.4	5 unique algal strains						•-													
3.5	characterize growth and uptake						l	•		4		1								
3.6	strain down select									•										
3.7	develop photobioreatcor		•											-						
3.8	Photobioreactor approved and working												•		_					
3.9	optimize performance										•					-	-			
3.10	50% formate utilization												+							
3.11	Best three strains																			
3.12	determine optimal growth conditions																•		-	-
3.13	Optimal growth conditions																		\ -	
	Cultivation and Outdoor Deployment																			
4.1	Formic acid delivery to indoor photobioreactors												l	•		-				
4.2	grow algae for testing															•			-	- I
4.3	0.5 Kg of biomass produced																		\ -	
5	Product development																			
5.1	Characterize algal products									l	•								-	-
5.2	Algal products characterized																		-	