



BETO 2021 Peer Review

5.1.3.103 Novel Cell-Free Enzymatic Systems for CO₂ Capture

NC STATE UNIVERSITY

Wilson College of Textiles



March 11, 2021

Conversion Technologies: Carbon Dioxide Utilization

PI: Min Zhang

National Renewable Energy Laboratory (NREL)

Sonja Salmon, North Carolina State University (NCSU)

Jesse Thompson, University of Kentucky - Center for Applied Energy Research (UK-CAER)

Project Overview

What are you trying to do?

Develop, innovate, and reduce costs of CO₂ separations

- using enzyme-based technology to accelerate CO₂ capture
- alternative solvents with low regeneration energy

How is it done today?

Current Monoethanolamine (MEA) based Carbon Capture Utilization & Storage (CCUS) processes are . . .

- energy intensive and expensive
- Corrosive solvent

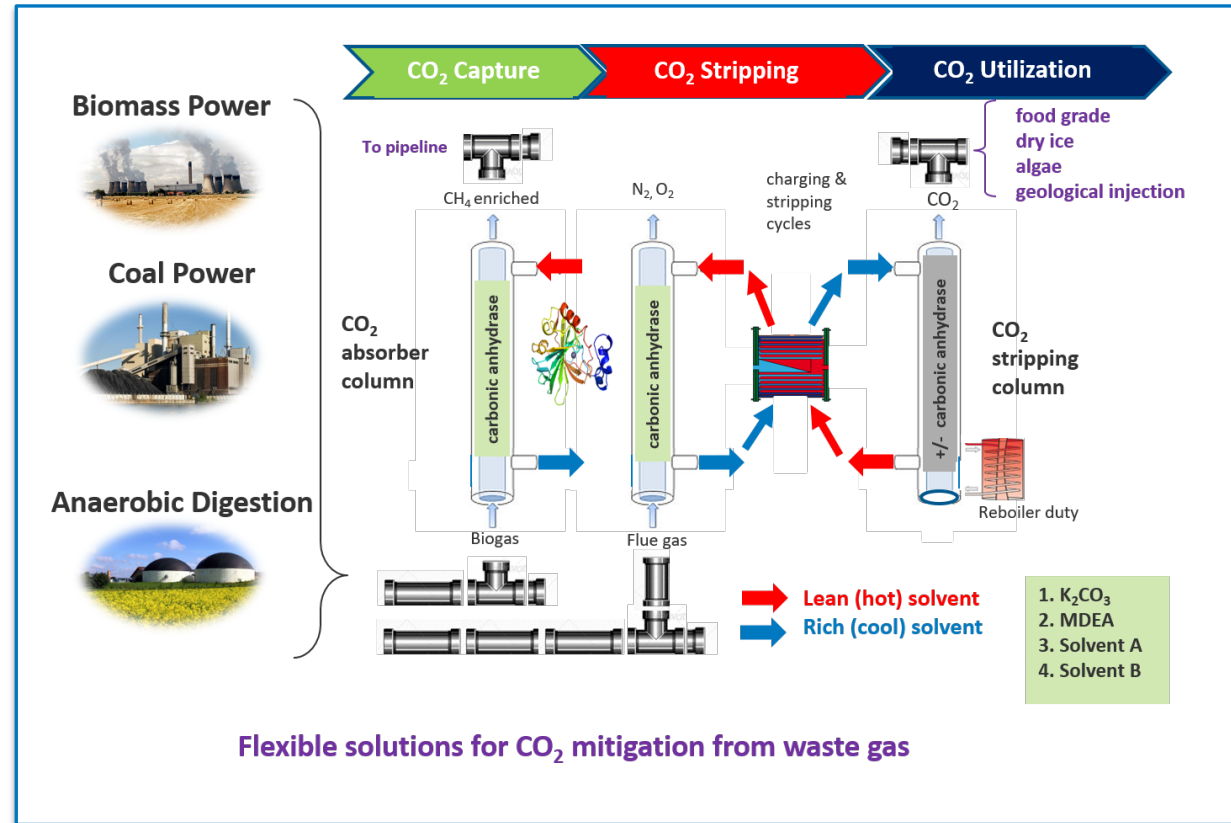
Why is it important?

In order to **reduce the energy requirement** of CO₂ scrubbing for **decarbonizing** the biopower & power sector.

What are the risks?

Technical: Enzyme robustness, process longevity, solvent compatibility, & CO₂ removal efficiency

Commercialization: technology costs, financial incentive, long-term liability risks, & lack of comprehensive policy



Quad Chart Overview

Timeline

- Project start date: October 2018
- Project end date: June 2022 (subcontracts had a late start date due to placement process)

	FY20	Active Project
DOE Funding	(10/01/2018 – 6/30/2022) \$452,084	\$1.45M for 3 years Project cost share: \$550K Novozymes (in kind) \$75K NCSU \$75K UK-CAER

Project Partners (subcontractors*)

- Sonja Salmon (North Carolina State University) *
- Jesse Thompson (University of Kentucky) *
- Novozymes

Barriers addressed

- Ct-D. Advanced Bioprocess Development:
CO₂ mitigation through carbon capture and utilization
- Energy efficiency for CO₂ Capture
 - Efficient BECCS

Project Goal

- Develop more efficient CO₂ waste gas scrubbing technology
- Accelerate CO₂ capture by improving carbonic anhydrase (CA) robustness & longevity
- Employ non-toxic solvents with low regeneration energy

End of Project Milestone





- Enable a 20% energy reduction compared to MEA reference case (90% CO₂ capture) at bench scale
- Favorable sustainability profile
- Potential for operating and capital savings due to use of benign solvent + novel enzymes

Funding Mechanism






FY17 Biopower Lab Call DE-LC-000L045:
requires 30% cost share

Market Trends




Product

-  Anticipated decrease in gasoline/ethanol demand; diesel demand steady
-  Increasing demand for aviation and marine fuel
-  Demand for higher-performance products
-  Increasing demand for renewable/recyclable materials




Feedstock

-  Sustained low oil prices
-  Decreasing cost of renewable electricity
-  Sustainable waste management
-  Expanding availability of green H₂
-  Closing the carbon cycle

Capital

-  Risk of greenfield investments
-  Challenges and costs of biorefinery start-up
-  Availability of depreciated and underutilized capital equipment

Social Responsibility

-  Carbon intensity reduction
-  Access to clean air and water
-  Environmental equity

NREL's Bioenergy Program Is Enabling a Sustainable Energy Future by Responding to Key Market Needs

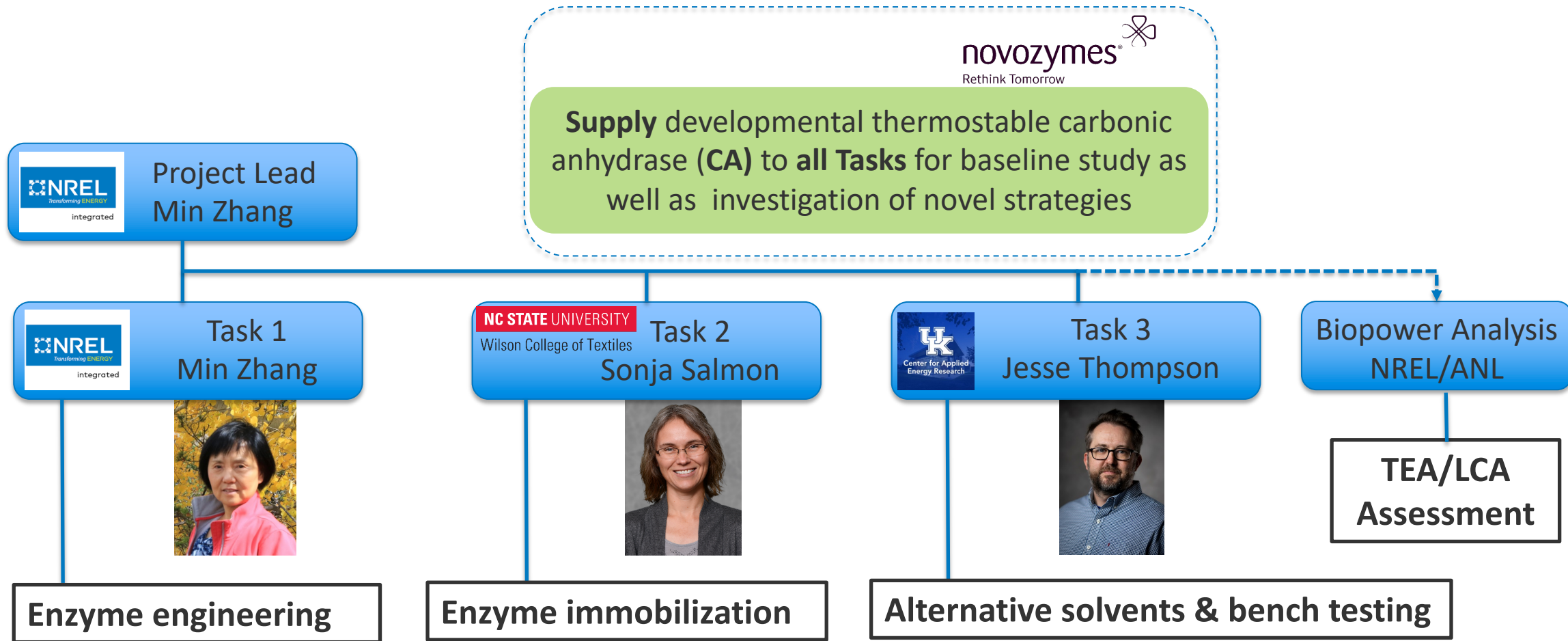
Value Proposition

- Reduction in energy requirement for CO₂ scrubbing
- Unique effort contributing to BETO's carbon efficiency goals
- Bioenergy with carbon capture and storage (BECCS) combines . . .
 - Biopower
 - Greenhouse gas mitigation technology
 - Energy production with net-negative emissions

Key Differentiators

- Target ALL 3 technology improvement pillars on parallel paths:
 - Enzyme engineering
 - Enzyme immobilization
 - Solvent evaluation
- World class team of experts in enzyme engineering, enzyme immobilization, & CO₂ capture R&D
- Partnership with world leader in enzyme production: Novozymes

1. Management- Project Structure

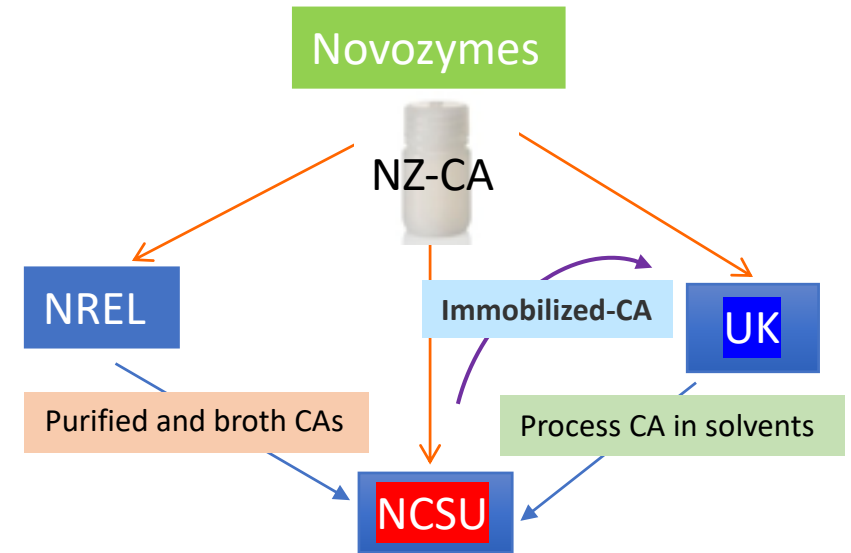


- ✓ **Collaborate with key experts** in the field **with complementary expertise**
- ✓ Microbiologist, molecular biologist, biochemist, chemist, polymer chemist and chemical engineer
- ✓ Enzyme supply by the enzyme producer **Novozymes**

1. Management- Risk Identification and Mitigation Strategies

Effective multidisciplinary team collaboration through:

- Multimember/Bilateral **NDA** and **MTAs**
- NREL-NCSU-UK-Novozymes **Kick-off meeting**
- **Monthly project meetings**
- Weekly technical communications
- **Prioritization** of recourses for targeted activities



CA samples distribution and testing

• Stakeholder & Parallel Project Engagement

- Explore carbonic anhydrase applications in Algae projects
- Stakeholder outreach to seek feedback on technology development and implementation
- Engaged Novozymes, submitted new TCF (Technology Commercialization Fund) proposal to scale up production of improved robust carbonic anhydrase

2. Approach –Background & State of Technology

MEA:

Fast reaction

Efficient CO₂ absorber

High regeneration energy



Alternative solvents:

Slow reaction

Not efficient CO₂ absorber

Low regeneration energy



Alternative solvents + Enzyme:

Fast reaction

Efficient CO₂ absorber

Low regeneration energy

Challenges:

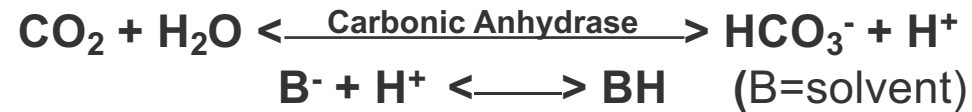
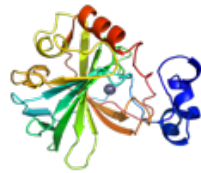
- **Effective Enzymes** in process conditions
- In-process enzyme **longevity** and **post-process sustainability**
- **Compatible solvents**

Critical Success factors:

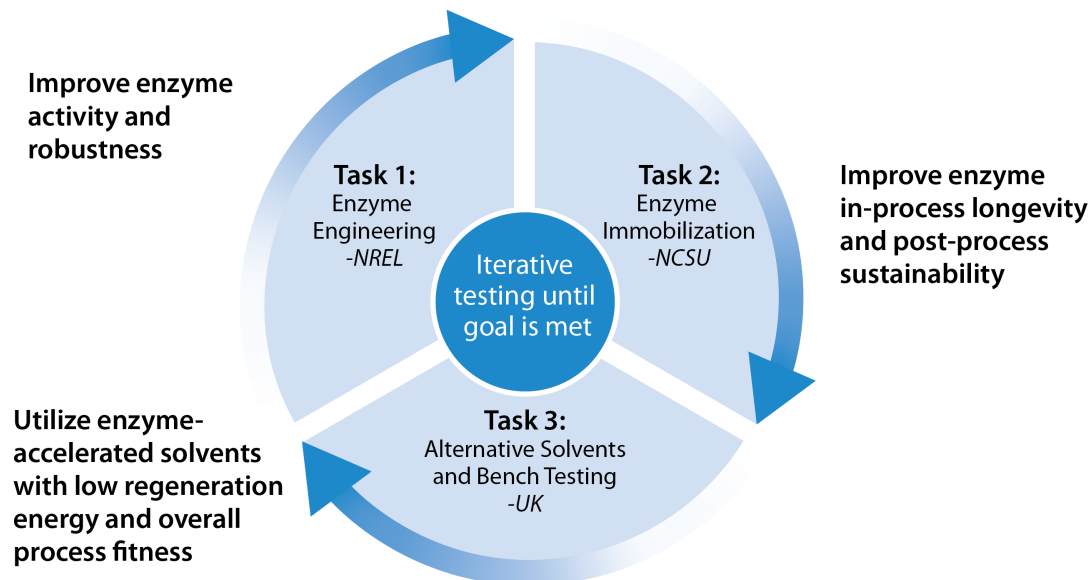
- Enzyme with
 - ✓ Fast Rate
 - ✓ Thermotolerance
 - ✓ Solvent compatibility
- In-process longevity
 - ✓ Immobilization
 - ✓ Effective contactor design
 - ✓ Post-process sustainability
- Enzyme-accelerated solvents
 - ✓ Low regeneration energy
 - ✓ Process friendly
- Integrated system with less overall energy requirement for CO₂ separation

2. Approach - Strategy and Decision

- Carbonic anhydrase (CA) - *fastest known enzymes in nature* - catalyzes CO₂ in low-energy solvents at fast rate:



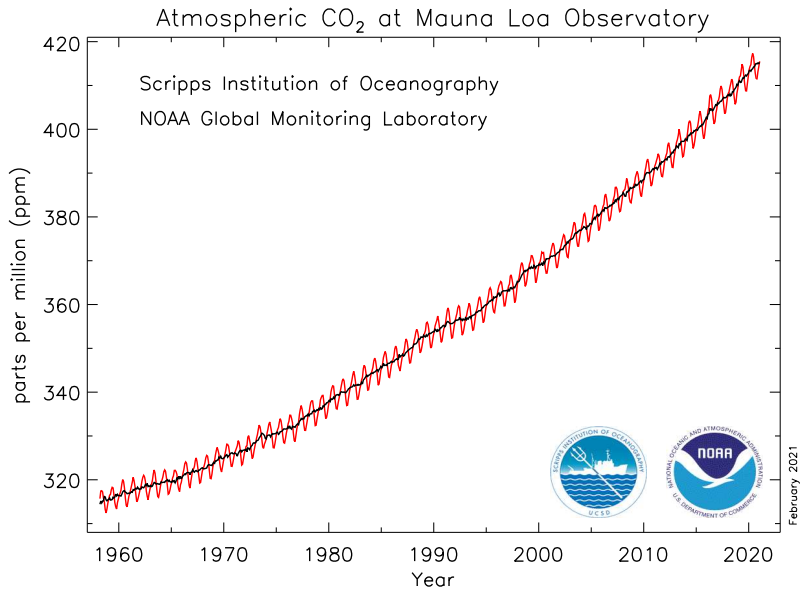
- Three parallel paths with the goal of improving CO₂ capture efficiency:



Go/No-Go Decision Points:

- Natural selection of CAs vs Protein engineering:**
 - CA candidates exceed NZ-CA performance by 25-30%
 - Protein engineering to improve best CA candidate
- Immobilization methods**
 - provide $\geq 50\%$ enzyme activity versus equivalent amount of non-immobilized enzyme

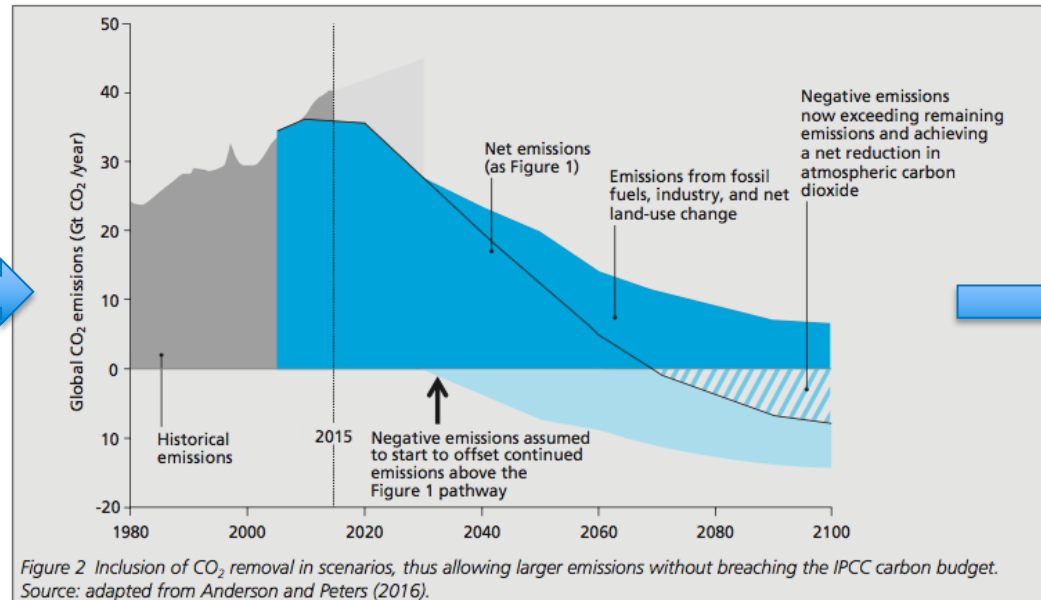
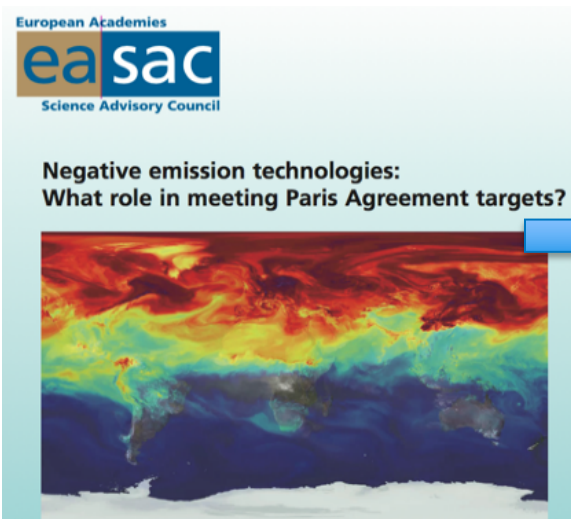
3. Impact – CO₂ mitigation via BECCS



- Reduce the energy required for CO₂ scrubbing
- Enable cost reduction for BECCS technology
- BECCS produces energy with net-negative emissions



BECCS is one of the essential technologies to be considered for meeting the Paris Agreement targets for all the nations.

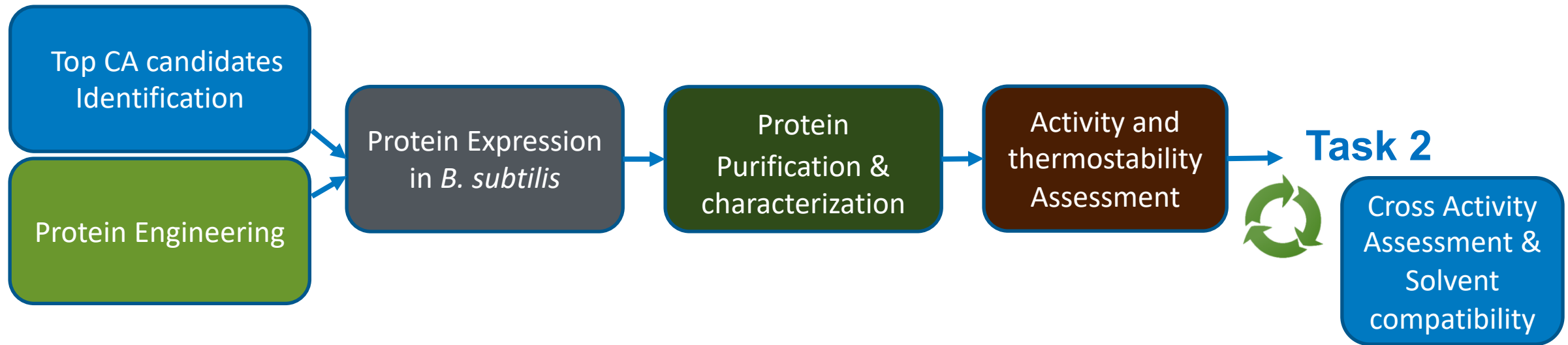


“the role of bioenergy production with carbon capture and storage (BECCS) and direct air capture with carbon storage (DACCS) and found that 7.9–10.6 GtCO₂ (2.1–2.9 GtC) removal by BECCS combined with 8–32 GtCO₂ (2.2–8.7 GtC) removal by DACCS by 2100 would be required to hold or return average temperatures to below the 1.5 °C level.” *Millar et al. (2017)*

3. Impact: CO₂ Mitigation and beyond

- **Low energy CO₂ scrubbing technology** not only **lowers the production cost for biopower** but also can provide **cost savings for fossil fuel production**:
 - CO₂ scrubbing of flue gases from **power plants**
 - **Impact** on many sectors including **biopower industry, biogas industry** as well as **CO₂ utilization**
- **Active discussions** with potential industrial stakeholders in different sectors
- **Two patent filings in progress** (Novel Contactor & Improved carbonic anhydrases)
- The **project team and Novozymes (providing 50% cost share)** submitted a **TCF** (Technology Commercialization Fund) proposal to DOE's Office of Technology Transitions on "Production of Robust Carbonic Anhydrase"
- **Relevant to BETO's efforts** to develop innovation solutions in **carbon efficiency**

4. Progress and Outcomes: Task 1 Enzyme Engineering



Objectives - Identify carbonic anhydrases (CAs) with higher activity & improved robustness

1. Conducted side-by-side activity comparison of top CAs including Novozymes CA (NZ-CA)
2. Protein engineering via comparative structure analysis to identify mutations to improve enzyme robustness
3. Prepare sufficient CAs for thermostability and solvent capability test as well as enzyme immobilization and characterization in Task 2

4. Progress and Outcomes: Task 1 Activity Improvement & Process Robustness

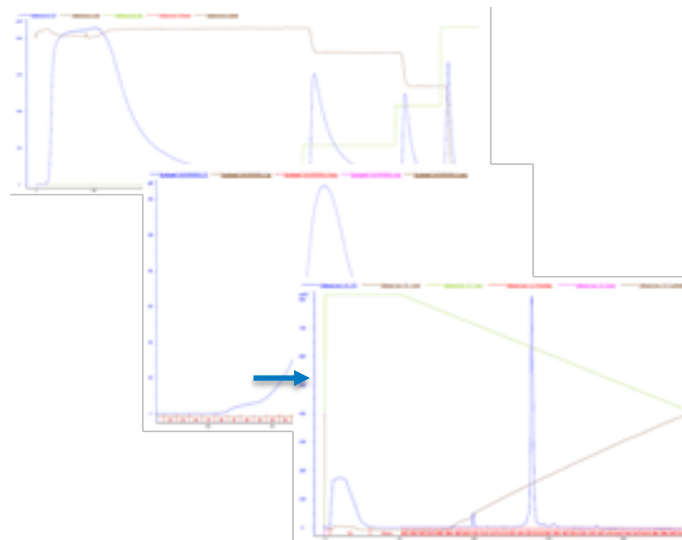
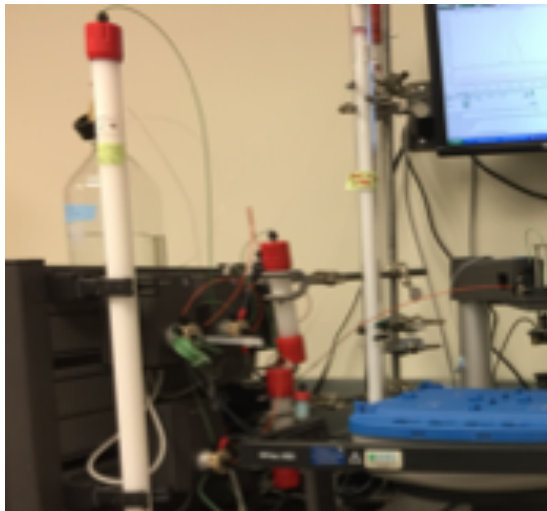
New isolated CAs with > 30% activity improvement as compared to baseline NZ-CA - meeting milestone target

Expression top candidate carbonic anhydrases (CAs)

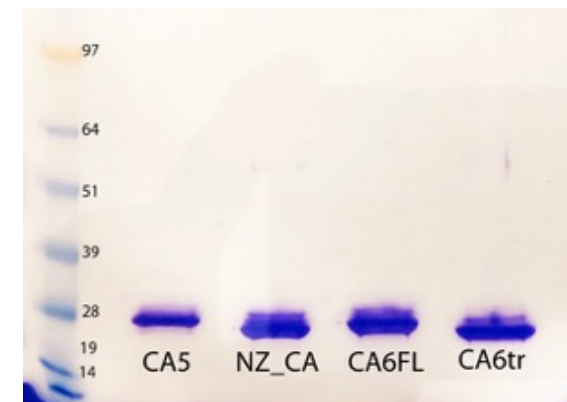
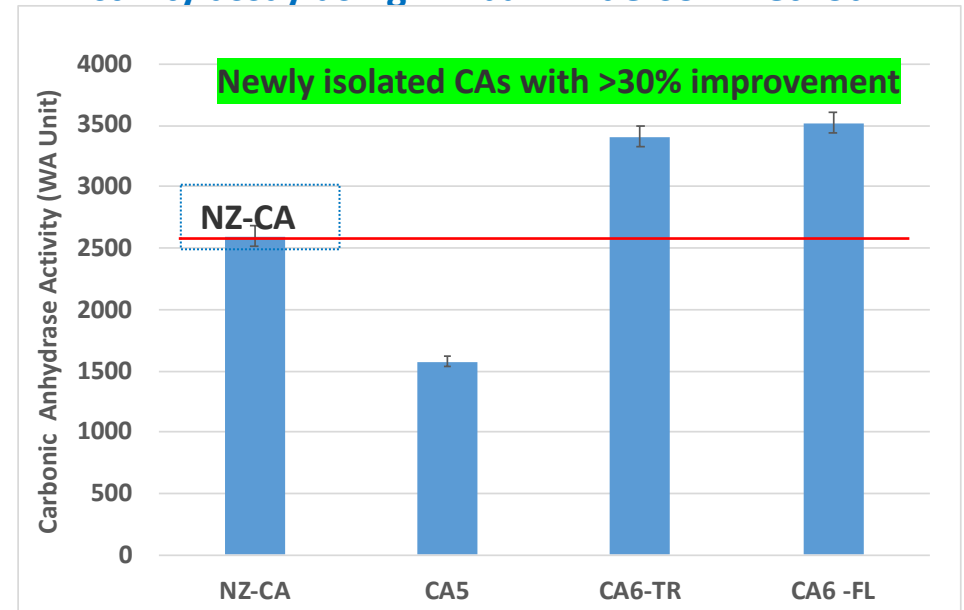
Bacillus subtilis

Testing activity in broth

Protein purification



Activity assay using Wilbur-Anderson method



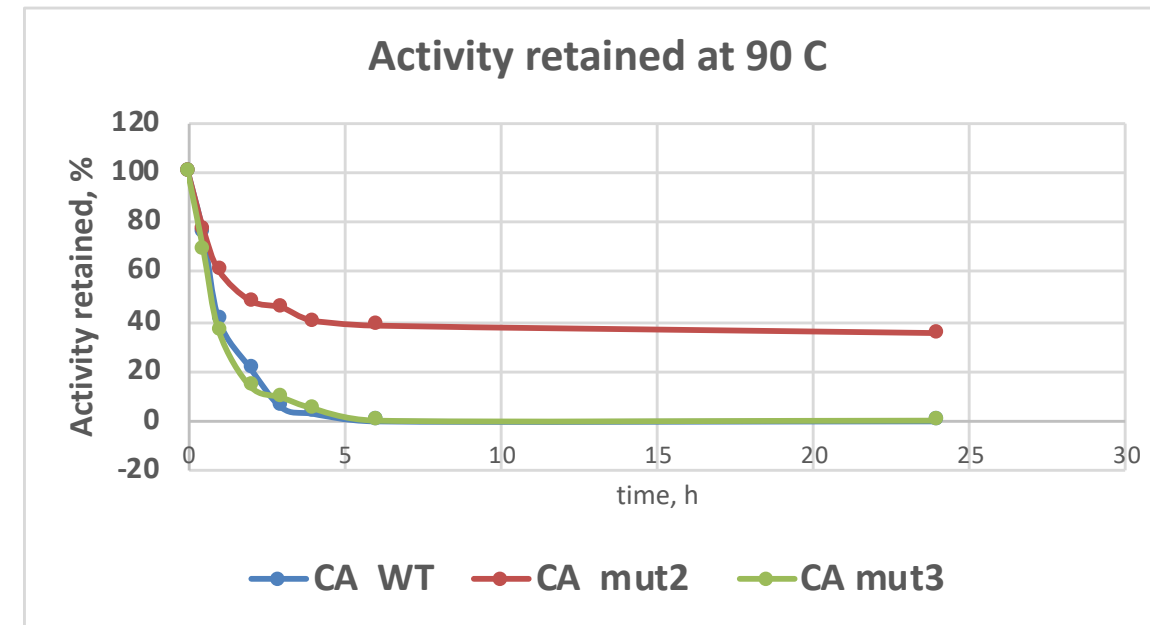
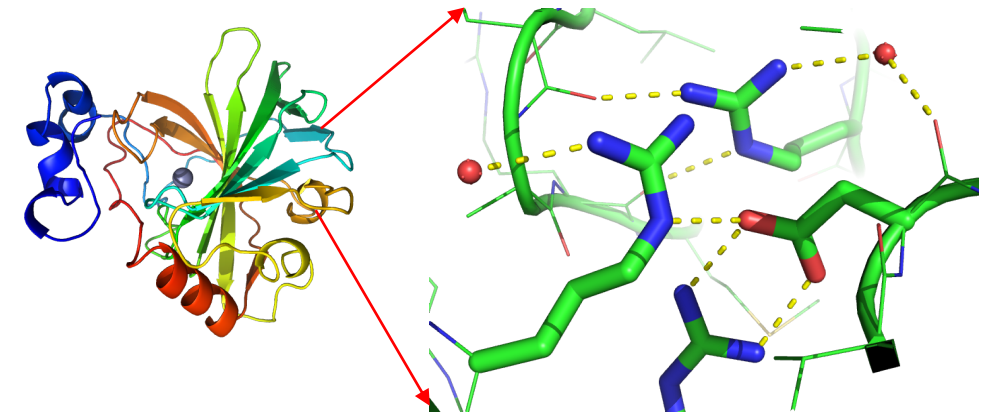
SDS Gel with isolated CAs

4. Progress and Outcomes: Task 1 Thermostability Improvement

mut2 CA retained ~40% activity at 90°C for more than 24 hours!

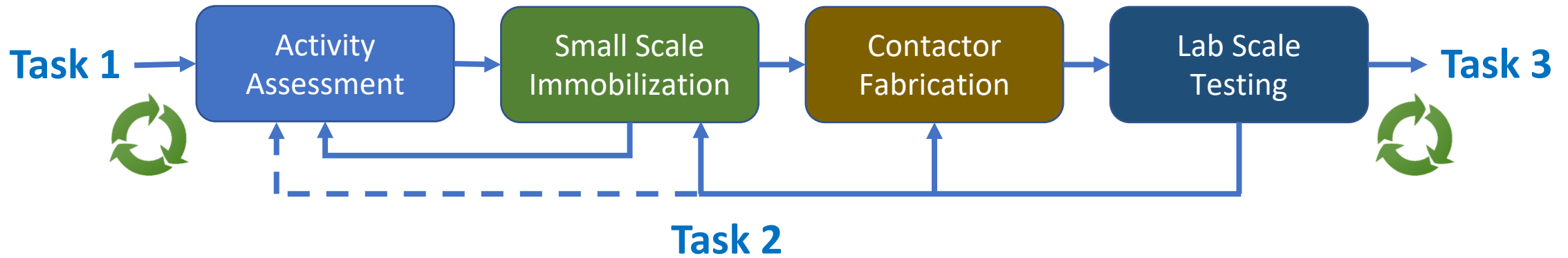
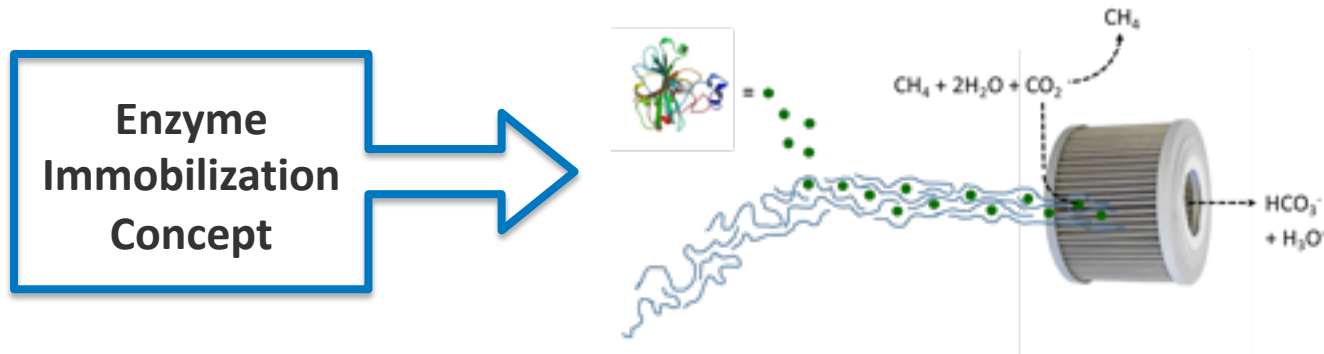
Thermostability Improvement via Rational Design

- Careful selection of residues to mutate with intent to provide **additional stabilization** via:
 - Formation of additional H-bonds or salt bridges
 - Reducing internal voids and improving packing
 - Improving hydrophobic interactions like stacking
 - Reducing internal strain
- **Three mutants created** for one candidate carbonic anhydrase (CA) and expressed in *B. subtilis* for testing
- **Evaluating** the mutations in other candidate CAs
- **Testing** the effect of the combination of mutations



4. Progress and Outcomes-Task 2 Enzyme Immobilization

NC STATE UNIVERSITY
Wilson College of Textiles

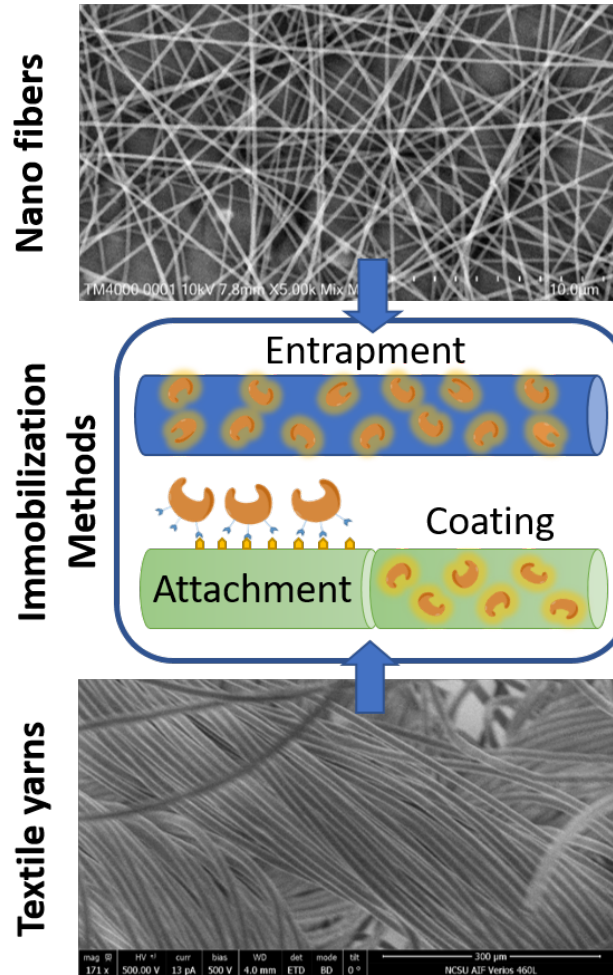


Objectives

1. Successful enzyme immobilization with high level of biocatalytic activity
2. Fabricate prototype contactors and demonstrate performance in lab scale
3. Demonstrate longevity and compatibility with solvents at benchmarking conditions

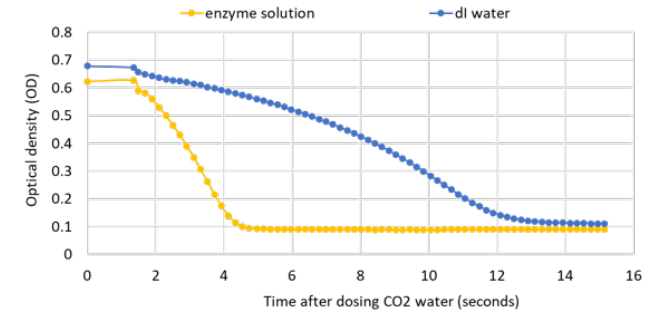
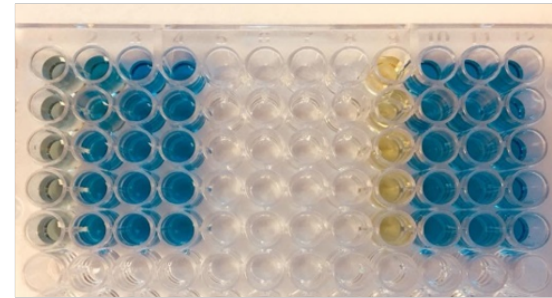
4. Progress and Outcomes-Task 2 Enzyme Immobilization & Assay Development

- Developed novel enzyme immobilization methods with active carbonic anhydrase
- High-throughput assays for assessment of CA samples and immobilized enzyme



(a)

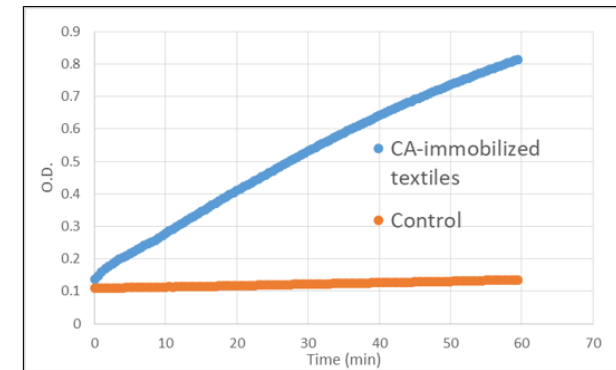
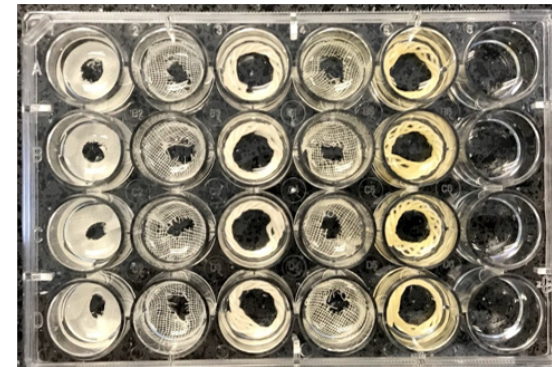
Dissolved Enzyme Activity Assessment



CO₂ hydration (pH change) → Color change of indicator (Bromothymol blue)

Active enzyme has been immobilized by different methods on textile materials

Immobilized Enzyme Activity Assessment



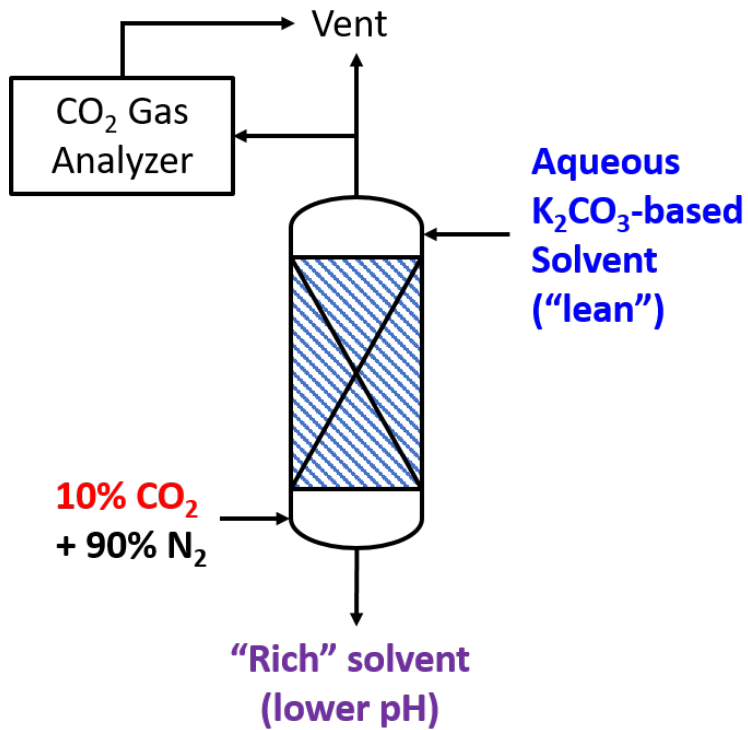
Ester hydrolysis → Color change of substrate (Colorless to yellow)

(b)

(c)

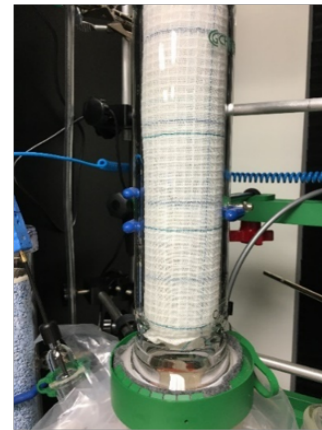
4. Progress and Outcomes-Task 2 Lab Scale Contactor Testing

Small gas-liquid absorber column set-up to screen contactor design effectiveness



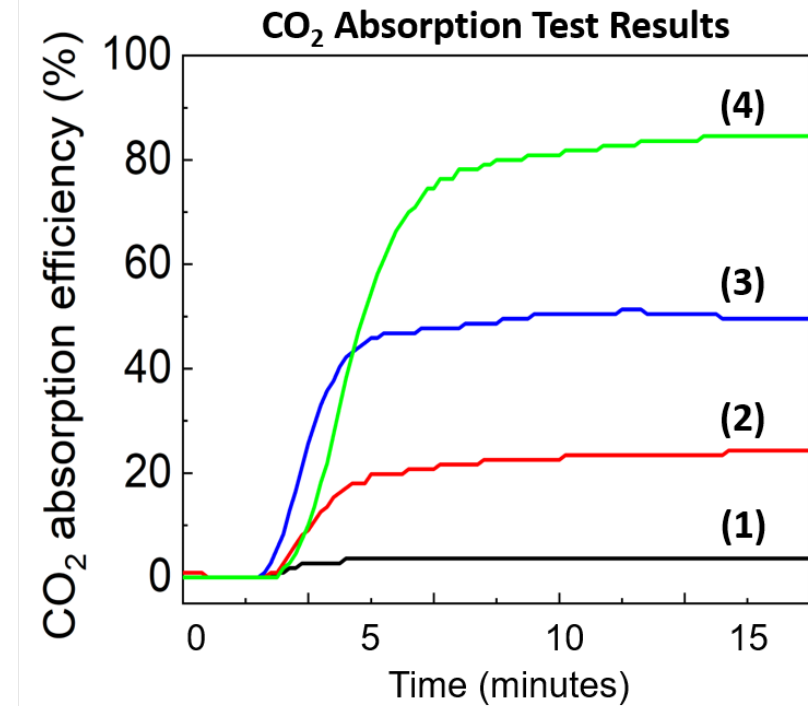
Flow-through Absorber Schematic

(a)



Prototype
Textile-based
IM-CA Contactor

(b)



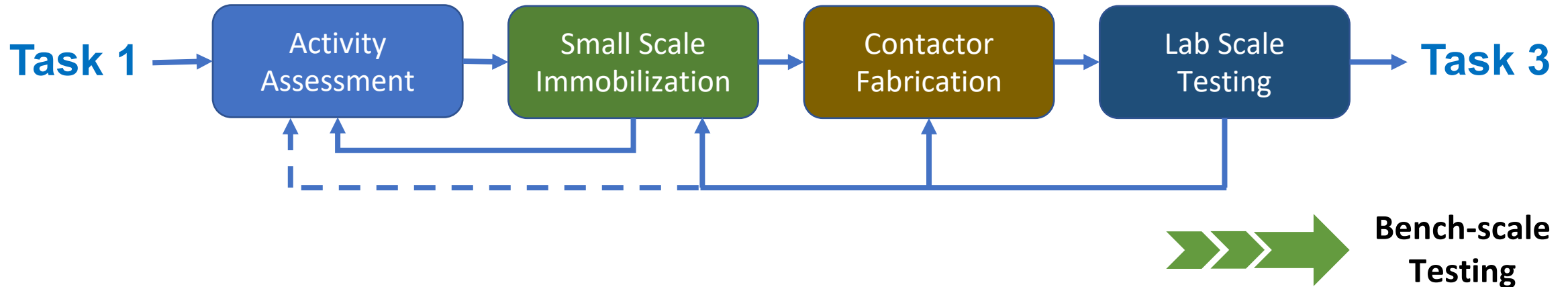
1. Non-catalyzed Rasching ring packing; 2. Non-catalyzed textile-based contactor; 3. Immobilized CA Contactor
4. Textile contactor with non-immobilized enzyme

(c)

4. Progress and Outcomes-Task 2 Lab Scale Contactor Testing

NC STATE UNIVERSITY

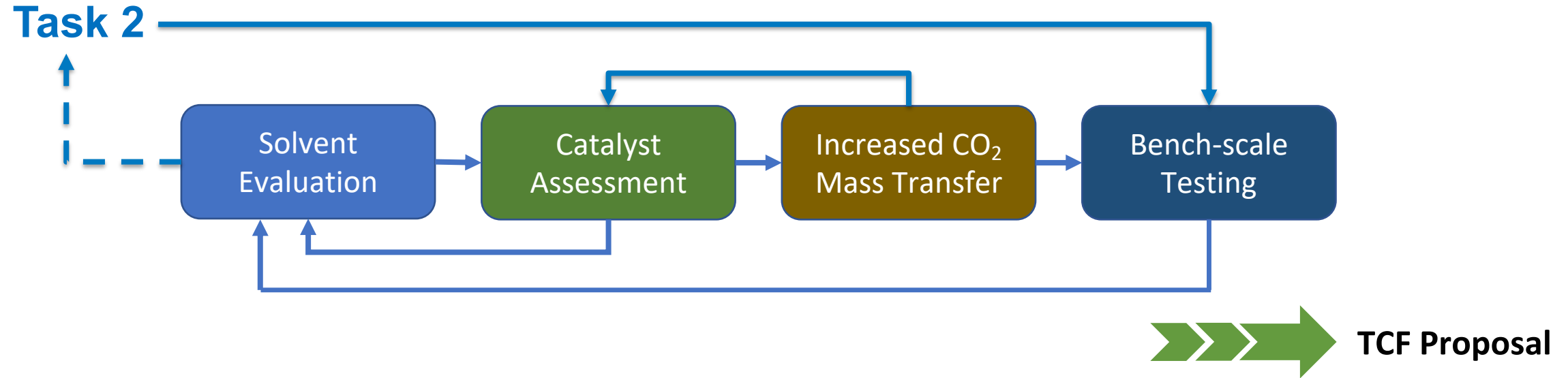
Wilson College of Textiles



Milestones

1. **Immobilized CA provides $\geq 50\%$ enzyme activity** versus equivalent amount of non-immobilized enzyme
2. Textile-based **prototype contactors perform well** in short-term lab scale tests
3. Longevity testing, solvent compatibility testing, and preparations for bench-scale contactor fabrication are in progress

4. Progress and Outcomes: Task 3 Solvent/Catalyst Bench Testing

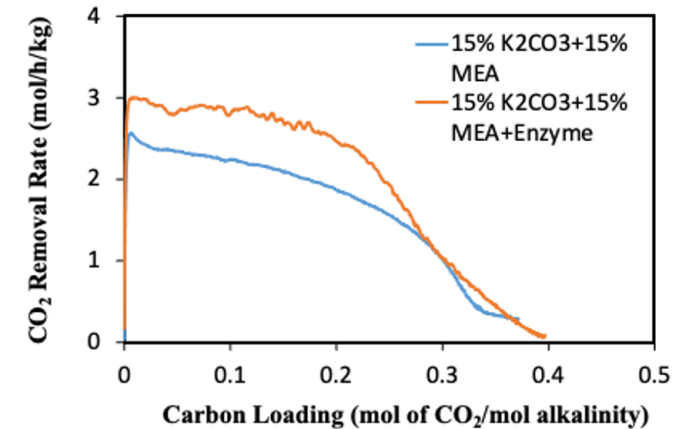
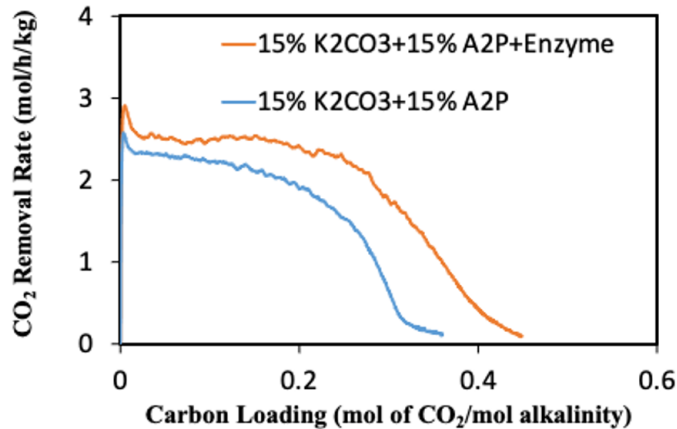
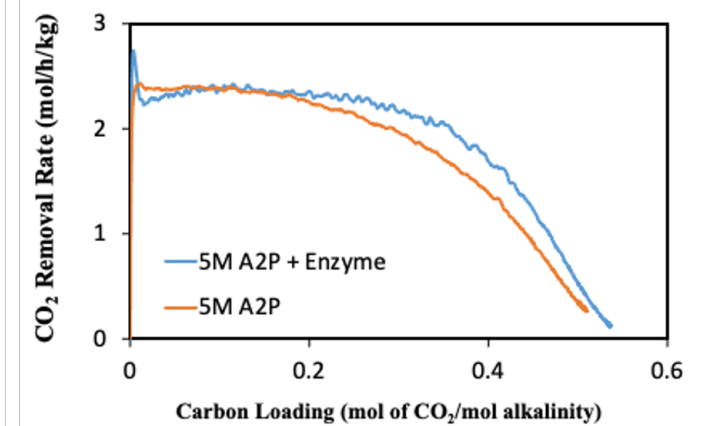
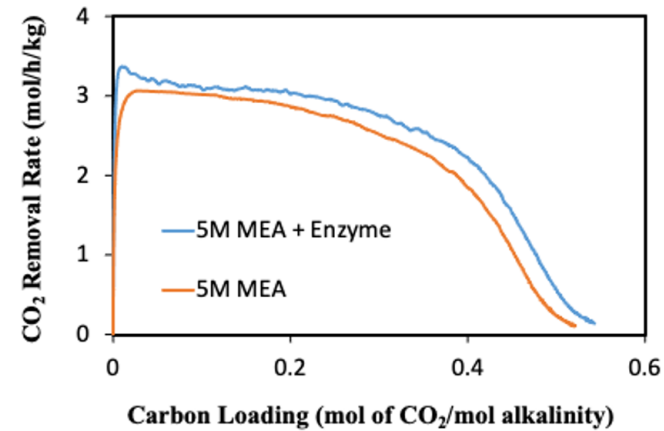
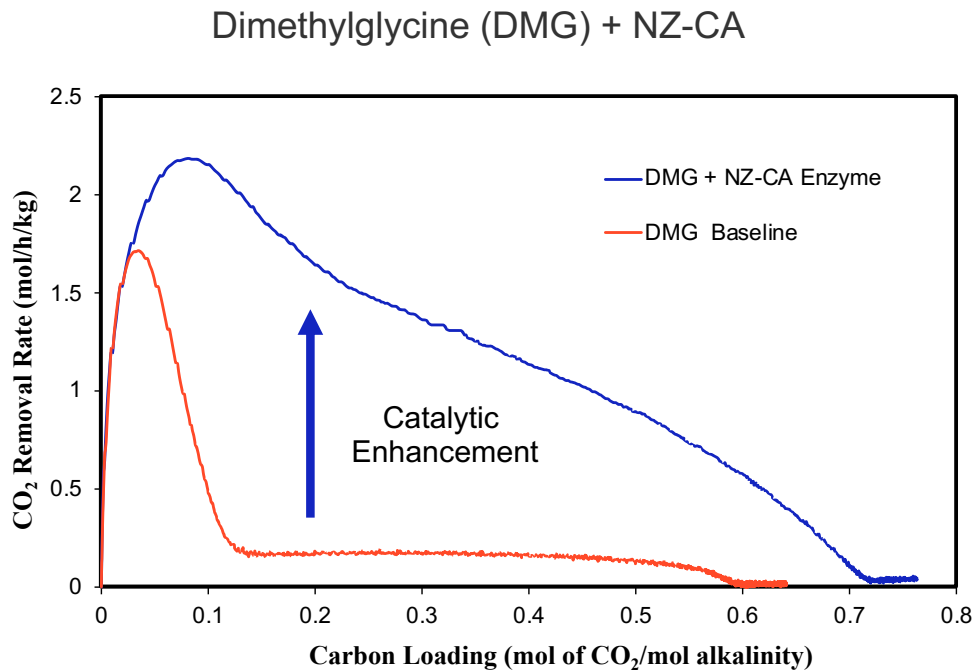


Objectives

1. Lab-scale solvent/free enzyme testing to enhance CO₂ mass transfer
2. Verify compatibility of solvent and enzyme
3. Bench-scale testing of solvent/immobilized enzyme to achieve 20% reduction in regeneration energy

4. Progress and Outcomes: Task 3 Solvent Screening for Catalytic Enhancement

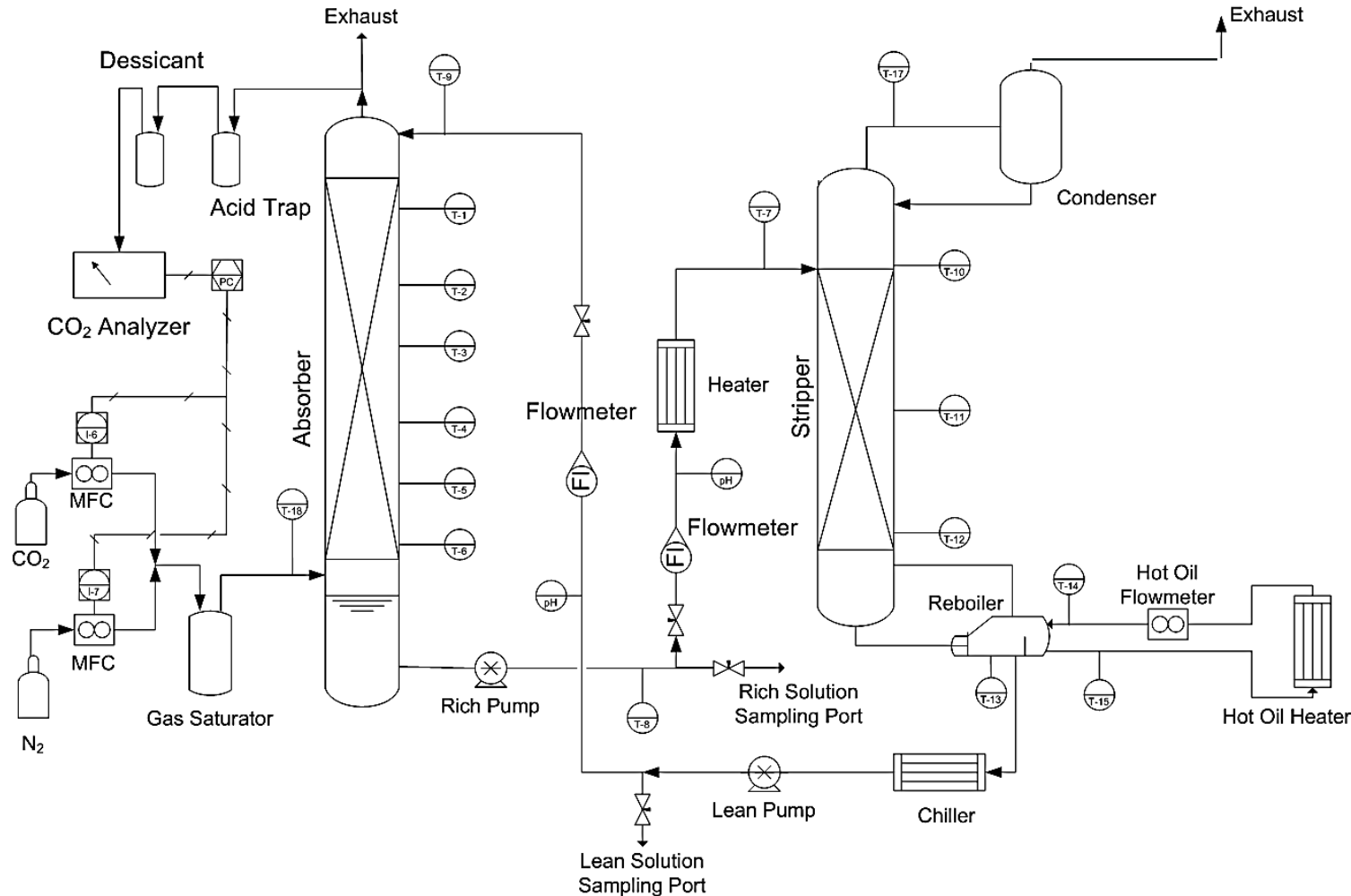
Several solvents were screened for catalytic enhancement with the NZ-CA enzyme; all showed some level of enhancement in CO₂ removal with the NZ-CA enzyme.



4. Progress and Outcomes: Task 3 Unique Facility

Small Bench Integrated CCS

UK-CAER small bench integrated CCS - a fully integrated absorber/stripper system that operates at L/G ratios that are scalable to larger pilot systems.



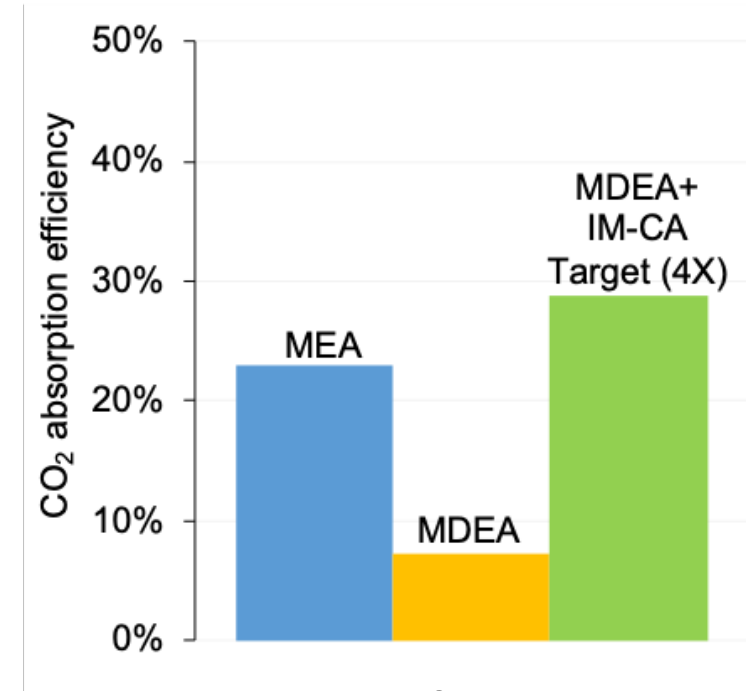
Small Bench Integrated CCS

Testing of the baseline solvent MEA and two NZ-CA enhancement solvents, SB (a solvent blend); & MDEA (Methyl diethanolamine) have been completed and will be used as comparison for the immobilized CA testing:

- The attrition rate of the NZ-CA was significant as it traveled through the high temperature stripper.
- The NZ-CA enzyme added directly to the solvent can cause foaming in certain solvents, contributing to poor CO₂ capture performance.
- Need for enzyme stabilization by protein engineering (Task 1) and/or immobilization (Task 2) to keep the enzyme in the absorber as well as to prevent foaming.



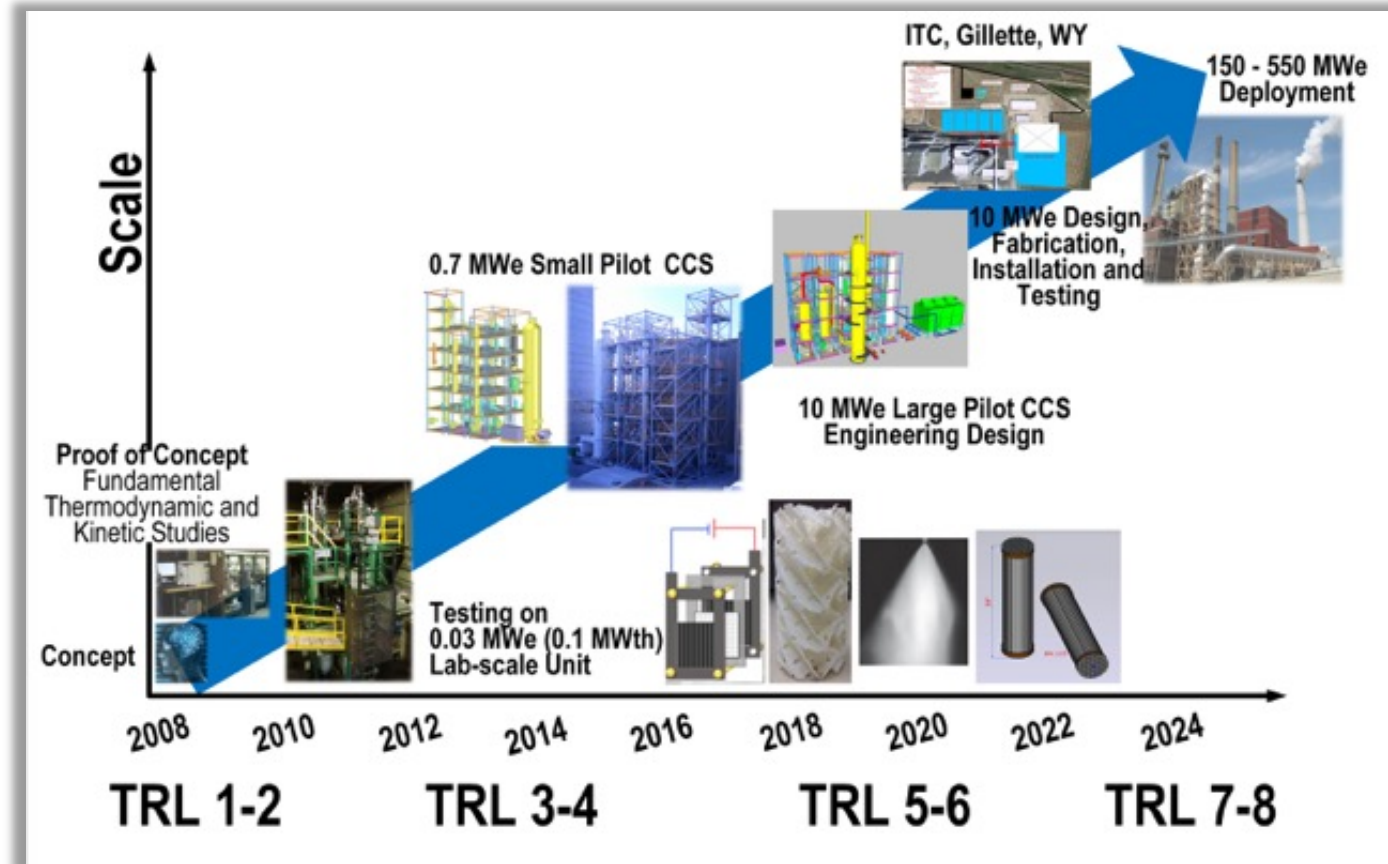
Next step



Target performance vs non-catalyzed solvents

4. Progress and Outcomes: Task 3 Milestones/Future Work

1. Bench-scale testing with target of 20% reduction in regeneration energy with immobilized CAs
2. Provide data to perform high level Techno-Economic Assessment (TEA)



- Our ultimate goal is to have **improved enzyme/immobilization** system sustaining the process **longevity TOGETHER WITH** a solvent that operates with **lower net regeneration energy** with a novel contactor design which can potentially reduce capital costs. We aim to **improve the whole system (OPEX and CAPEX)** to bring down the total cost.

Summary

- **Management**

- Three tasks staffed by **key experts** in the field with complementary experience
- Risk Mitigation: Effective **multidisciplinary team** with submission of new TCF proposal to scale up production of improved robust carbonic anhydrase

- **Approach**

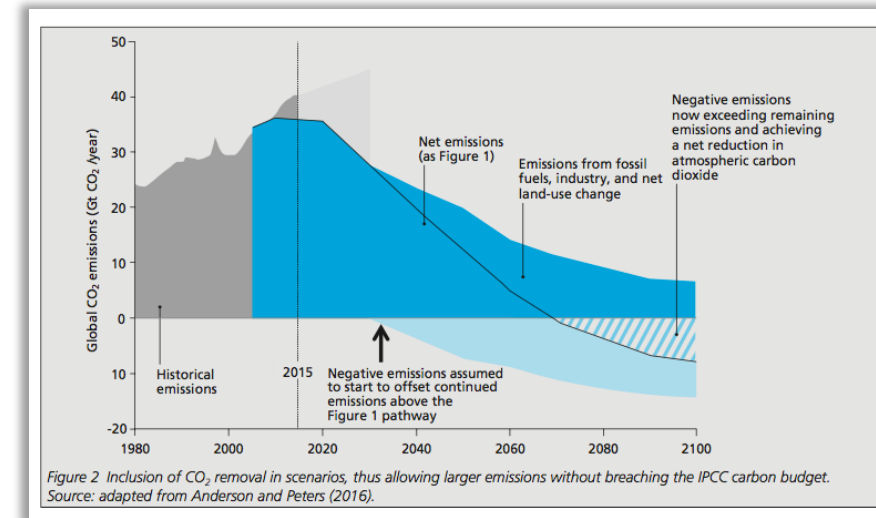
- Parallel focus on **all 3 important pillars**: enzyme engineering, enzyme immobilization, and solvent evaluation
- **Engaged with industrial partners & stakeholders**

- **Impact**

- **Reduce the energy required for CO₂ scrubbing in traditional power and BECCS, leading to reduced and net-negative emissions supporting Paris Agreement targets.**

- **Outcome**

- **Improved enzyme activity (>30%) and process robustness (thermostability at 90°C)**
- **Novel immobilization/fabrication method with > 50% retained enzyme activity**
- Novel contactor testing in lab-scale shows **promise for improving CO₂ absorption**
- Benchmark testing in integrated bench-scale system emphasizing **need for enzyme stabilization by protein engineering and/or immobilization**



Acknowledgements

Funding:

U.S. DOE EERE Bioenergy Technology Office (BETO)



Special Thanks:

Ian Rowe

Novozymes provided in kind cost share



NREL LPM and Platform Lead: Zia Abdullah, Rick Elander

NREL:

Min Zhang
Vladimir Lunin
Hui Wei
Michael Himmel

NCSU:

Sonja Salmon
Jialong shen
Yue Yuan

UK-CAER:

Jesse Thompson
Moushumi Sarma

Novozymes:

Mike Elder
Alan House

Thank You!

www.nrel.gov



Additional Slides

Background State of the Technology

- **Fast-reacting MEA** CO₂ absorption solvent requires **high regeneration energy** due to high heat of absorption (~84 kJ/mol)
- Alternative, more **benign and sustainable solvents** have lower heat of absorption, but react slower (e.g. 59 kJ/mol MDEA, 27 kJ/mol K₂CO₃)
- Bench-scale and pilot testing in previous studies has **proven carbonic anhydrase (CA) ability to accelerate CO₂ absorption** in alternative solvents in both dissolved-enzyme and immobilized-enzyme forms
- Several **thermostable CAs** reported worldwide but Lack of the **side-by-side comparison** among the **CA** candidates- **lack of knowledge**
- **Limited longevity** of the CA in the process
- Process improvements are still needed to **achieve CO₂ capture efficiency with energy reduction** versus benchmark MEA
- Preliminary TEA showed sustained **effective enzyme** and solvent **regeneration temperature** are critical.

Challenges:

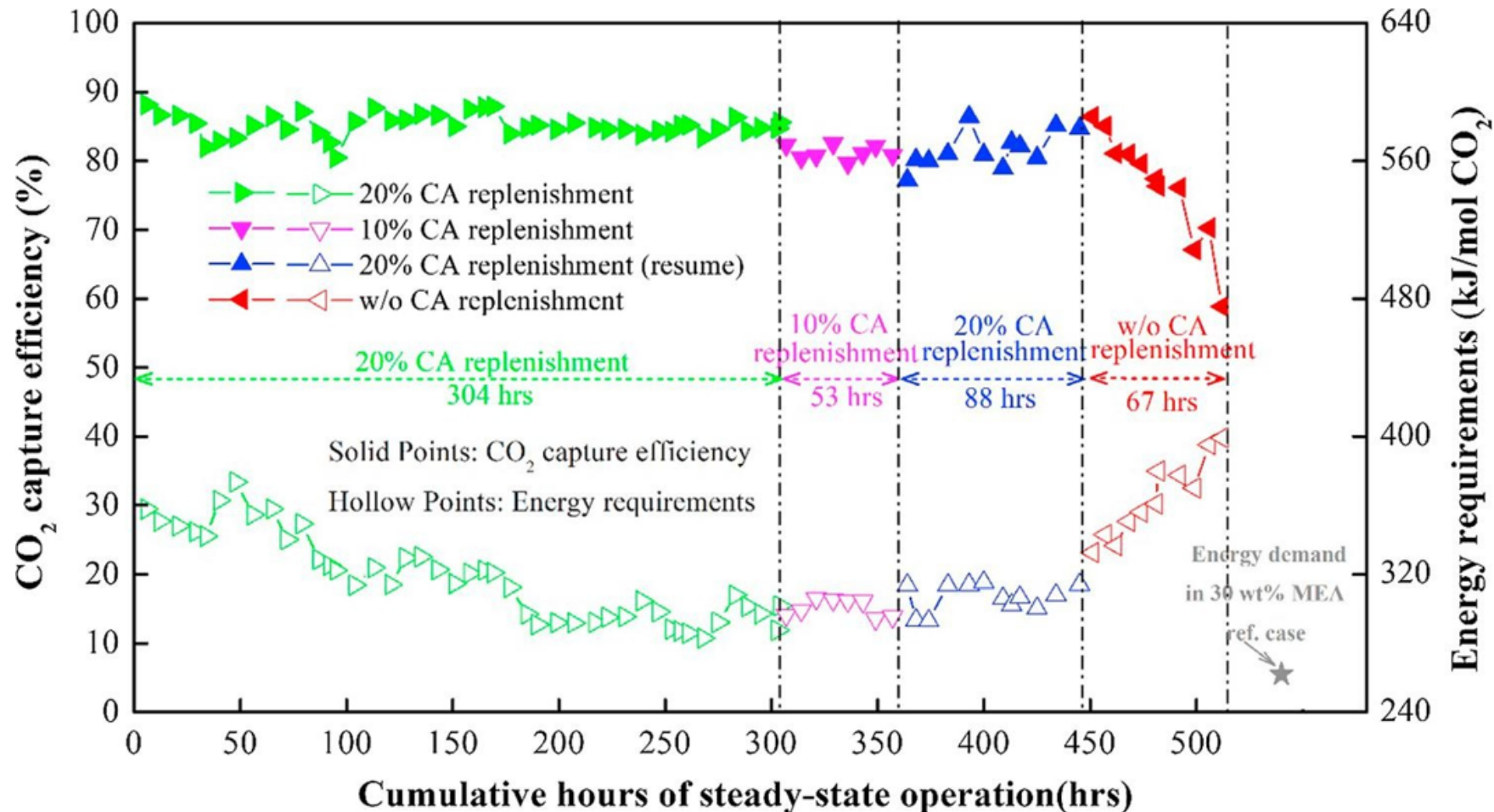
- **Effective Enzymes** in process conditions
- In-process enzyme **longevity** and **post-process sustainability**
- **High energy requirement for solvents**

Critical Success factors:

- ✓ Improved thermotolerance of CA enzymes with fast CO₂ absorption rate, as well as solvent tolerance
- ✓ Improved enzyme in-process longevity and post-process sustainability with immobilization
- ✓ Identified enzyme-accelerated solvents with low regeneration energy
- ✓ Successfully integrated system with overall less energy requirement for CO₂ separation in environmentally more friendly solvents and processes.

Background: Prior UK-CAER Bench Scale Demo

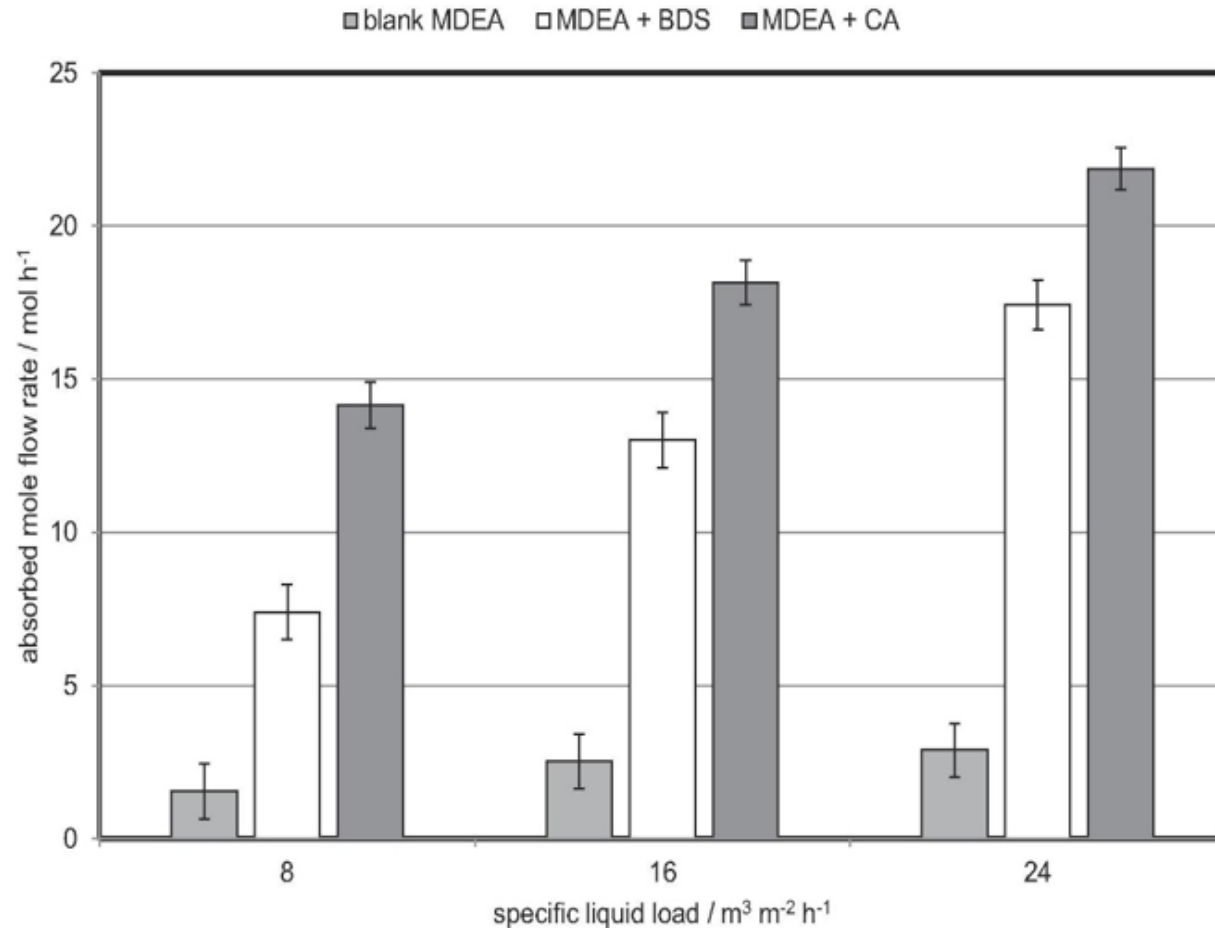
- CA enhancement leads to lower energy versus uncatalyzed K_2CO_3
- Improving enzyme stability could reduce replenishment rate



Qi, Liu, House, Salmon, Ambedkar, Frimpong, Remias, Liu, *Applied Energy*, 2018, 209, 180-189.

This publication is part of work conducted in project DE-FE0007741 by Novozymes, PNNL, UK-CAER and Doosan Power Systems.

Background: Prior Bench Scale Absorption Tests



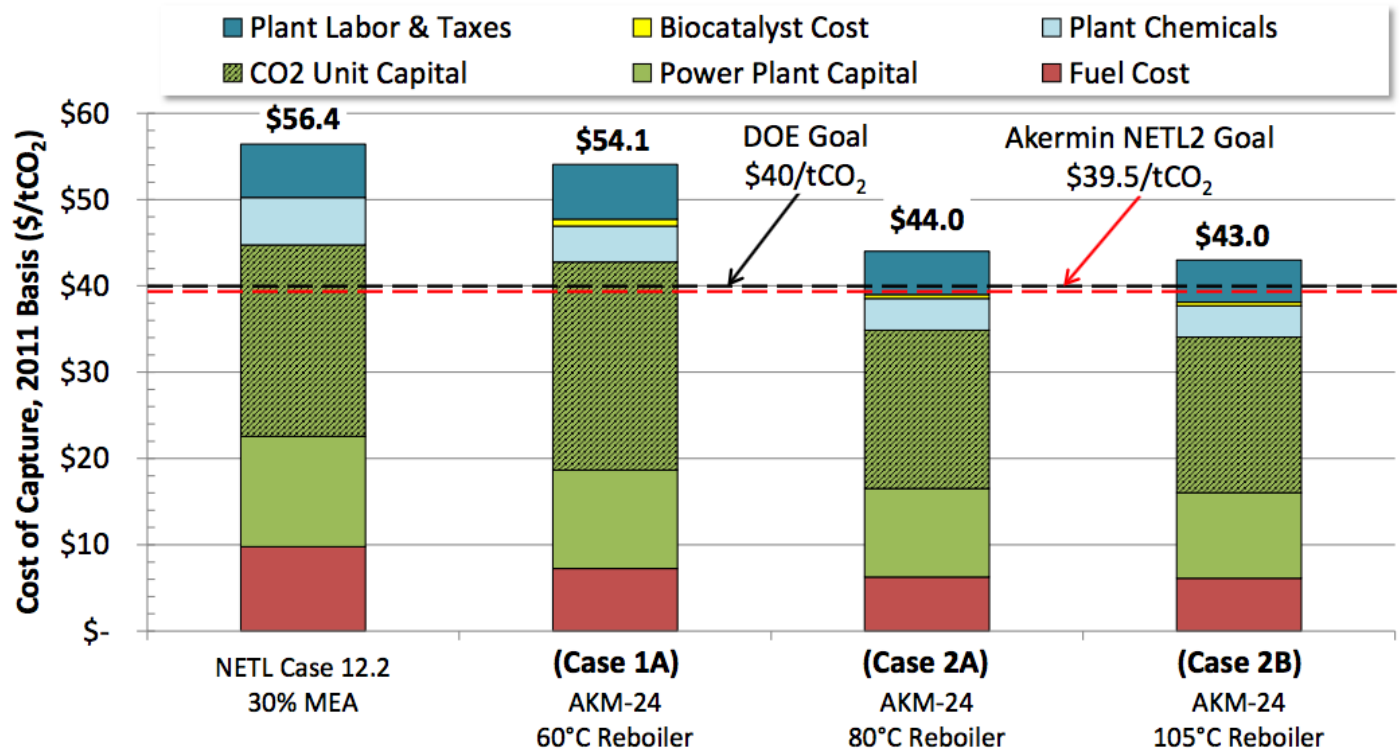
Leimbrink, Nikoleit, Spitzer, Salmon, Bucholz, Gorak, Skiborowski, *Chem Eng Journal*, 2018, 334, 1195-1205.

- Both dissolved CA and immobilized CA particles (BDS) give significant CO₂ absorption enhancement in MDEA solvent.
- These bench-scale tests were run in flow-through absorber-only mode at 40°C.
- Although IM-CA CO₂ absorption performance was slightly lower, the IM technology would protect enzyme from inactivation in a hot desorber.

Background: Akermin –NETL project's cost breakdown for CO2 capture



COST OF CAPTURE FOR VARIOUS CAPTURE SYSTEMS



~20% reduction in cost of capture appears achievable

LOW ENERGY CO₂ CAPTURE
ENABLED BY BIOCATALYST DELIVERY
SYSTEM

Akermin

2015 NETL CO₂ Capture Technology
Meeting Pittsburgh, PA.
Project: DE-FE0012862
June 24, 2014



Responses to Previous Reviewers' Comments -1

- Use of expensive/fragile enzyme?
 - The enzyme cost was <5% of the projected total cost of capture assuming enzyme operates for a long time without replenishment by Akermin's NETL project (<https://netl.doe.gov/sites/default/files/event-proceedings/2015/co2captureproceedings/A-Zaks-Akermin-Biocatalyst-Delivery-System.pdf>; slide 30). Enzyme was supplied by Novozymes in the study.
 - Two patent filings on "Novel Contactor" and "Improved carbonic anhydrases" are in progress in the project which indicating novel improvements have been identified.
 - The patents are dealing both with enzyme fragility AND capital cost issues. Effective performance of the enzyme in the process with longevity and activity by either immobilization and/or improved robustness can reduce the amount of the enzyme use, therefore reduce the cost. Our novel contactor alone provides benefits vs conventional packing, as illustrated by the higher performance of curve (2) vs curve (1) in (c) on slide 16.
 - Novozymes is our partner and provided their developmental enzyme for current project for base line study. Novozymes is our cost-share proposal partner for a TCF, and immobilized enzyme performance is meeting the milestones needed to proceed to bench-scale testing.
- TEA Analysis needed at early and later stages.
 - TEA Analysis is planned to be conducted in FY21Q3 by the Biopower Analysis Project (by NREL/ANL) which are conducting TEA/LCA analysis for all the Biopower projects under BETO's Biopower lab call.

Responses to Previous Reviewers' Comments-2

- Provide better state-of-art, e.g. a similar project at PNNL went unmentioned.
 - This project certainly stands on the shoulders of work by Akermin (NCCC), Codexis (NCCC), CO2 Solutions (with Husky Energy), University of Illinois-UC, and others, globally.
 - PNNL's important role and contributions in the NETL-funded project led by Novozymes are reflected in the co-authored preliminary, environmental, and final technical reports (OSTI Identifiers: 1212665, 1212663, and 1222645).

Publications, Patents, Presentations, Awards, and Commercialization

- Two patent filings in progress
 - Improved CA enzymes
 - Novel contactor
- Background publication/patent applications issued
 - Yue Yuan, Yuxuan Zhang, Hassina Bilheux, Sonja Salmon, Biocatalytic yarn for peroxide decomposition with controlled liquid transport, *Advanced Materials Interfaces*, 2021, 2002104: <https://onlinelibrary.wiley.com/doi/full/10.1002/admi.202002104>
 - Sonja Salmon, Yue Yuan, Chitosan materials with entrapped enzyme and biocatalytic textiles and other biocatalytic materials comprising same, US20200276057
- Review manuscript in preparation
 - Yue Yuan, Jialong Shen, Sonja Salmon, “Enzyme immobilization with fibrous supports”
- Original research manuscript on lab-contactor testing in preparation
- Commercialization - The project team and Novozymes (providing 50% cost share) submitted a TCF (Technology Commercialization Fund) proposal to DOE’s Office of Technology Transitions on "Production of Robust Carbonic Anhydrase"