1. Nathan Crook, North Carolina State University
2. Miles Rogers, Raytheon BBN
3. Christopher Voigt, Massachusetts Institute of Technology
4. Mark Blenner, Clemson University
5. Enoch Yeung, University of California, Santa Barbara
6. Gary Vanzin, Colorado School of Mines
7. Babetta Marrone, Los Alamos National Laboratory
8. Brajendra Sharma, Illinois Sustainable Technology Center
9. Meltem Urgun-Demirtas, Argonne National Laboratory
10. Gregg Beckham, National Renewable Energy Laboratory
11. John Dorgan, Michigan State University
13. Jay Keasling, University of California
14. Paul Tryan, Sandia National Laboratories
15. Deepti Tanjore, Lawrence Berkeley National Laboratory
16. Katy Christiansne, Lawrence Berkeley National Laboratory
Nathan Crook, North Carolina State University

**Project Overview**

- Biological “upcycling” of mixed waste is common in Nature.
- The ReSource Program requires microbes to satisfy multiple constraints:
  - Degrade diverse materials.
  - Tolerate high concentrations of inhibitors.
  - Outcompete competitors (microbes/phage).
  - Produce high titers of desired products.
- We have two complementary experimental technologies to satisfy TA1:
  - Rapid functional bioprospecting of metagenomic pathways.
  - Rapid directed evolution of large (10-100kb) DNA segments in diverse bacteria.

**Teaming Overview and Objectives**

- Existing team members: Nathan Crook (PI) and Ibrahim Al’Abri (Graduate Student).
- Expertise in high-throughput engineering method development for complex phenotypes.
  - *In vivo* continuous evolution in yeast enables tolerance to butanol and increased xylose consumption (DARPA Living Foundries).
- Developed RNAi screens in yeast to improve butanol tolerance.
- Implemented functional metagenomic screens to improve bacterial fitness in the mammalian gut.
- Seeking collaborators in TA2 and TA3.

**Impact**

**New Techniques/Capabilities**

- Metagenomic DNA -> screening for desired function in 6h.
- Pathways to improved growth in <1 week (3-4 cycles).
- Can’t decide which gene to evolve? Choose all of them!
- Develop ability to self-adapt to new waste streams.
- Multi-microbe consortia to expand breadth of substrates.

**Potential Apps**

- Enable upcycling of currently unrecyclable municipal waste streams. (e.g. single-use plastics, mixed plastic types).
- Enable those without access to waste collection services a means to upcycle waste (e.g. compost).

**Unique deliverables**

- Ability to rapidly swap host strains.
- Evolve only native genes for non-GMO status.

Contact Information – nccrook@ncsu.edu – (562) 714-0448

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**Tagatose consumption by E. coli (5kb)**

<table>
<thead>
<tr>
<th>Strain</th>
<th>OD600 after 27h</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Pathway</td>
<td>0.4</td>
</tr>
<tr>
<td>WT Pathway</td>
<td>0.8</td>
</tr>
<tr>
<td>Cycle 1</td>
<td>1.0</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>1.2</td>
</tr>
<tr>
<td>Cycle 3</td>
<td>1.2</td>
</tr>
</tbody>
</table>

![Graph showing tagatose consumption over cycles](image_url)
BBN is a world technology leader for solving significant and complex problems in a wide range of areas.

Model-based characterization and engineering enables precise control of circuits.

Teaming Overview

- High throughput single cell metabolomics platform
- Machine learning for ‘omics analysis
- Genome-scale, constraints-based modeling
- Computationally-guided metabolic engineering
- Model-based, genetic circuit design
- Protocols and software for absolute quantitation

Impact

- Create synthetic microbial communities with engineered enzymes
- Build metabolic pathways for the breakdown of polymer and cellulosic waste
- Convert to POLs (Focus Area 2)

Seeking Partners with:

- Expertise in additional ‘omics measurements
- Strain engineering ability

Dr. Miles Rogers (miles.rogers@raytheon.com, 617-873-6077), Ms. Helen Scott (helen.scott@raytheon.com, 617-873-7405)
**Christopher Voigt, MIT**

**Project Overview**

**Plastic-eating bacteria**

- **Enzyme optimization**
- **Manipulating difficult environmental bacteria**
- **Transfer capability between species**
- **Pathways to chemicals, food, and materials**

**Impact**

- Enzymes for industrial biodegradation of complex waste mixtures
- Engineered organisms that can either grow on the materials themselves or can process the carbon sources provided by an upstream chemical process
- Broad capabilities to generate many diverse products

**Teaming Overview and Objectives**

- Antony Sinksy (MIT), Ben Gordon (MIT-Broad Foundry)
- Relevant Experience:
  - Have optimized PETase to almost glass transition temperature of PET
  - Engineered Marinobacter, including metabolic flux manipulation
  - Can rapidly engineer many environmental bacteria
  - Extensive rapid pathway engineering experience, including food, difficult chemicals, pharmaceuticals, and materials
  - Fermentation and bio-production experience
- Synthetic Biology Center at MIT
- MIT-Broad Foundry) for rapid strain engineering
- Seeking collaborators for waste pre-processing, to scale fermentation and purification

Contact Information – cavoigt@gmail.com - 617-324-4851
Project Overview

Our team intends to partner with other teams possessing expertise in TA1 and TA3 to generate engineered non-model bacteria and yeast capable of making DoD relevant products, inspired by natural consortia and combining into synthetic consortia. We plan to achieve highly productive strains to make products from simple organic substrates derived from deconstructed waste, with a strong preference for C2 and larger substrates.

We are positioned to pursue **TA2: “Buildup”**. Our team has capabilities to develop individual rugged microbes and consortia that can produce a variety of products needed in either HADR or expeditionary scenarios. Product preference is geared towards oil/lubricant applications and fatty acid/amino acid/vitamins for food.

Phase 1: Install product pathways using anticipated intermediates from Breakdown TA1; Creating more rugged strains for anticipated inhibitory compounds. Phase 2: Integration with TA1 and TA3; preparation for confounding factors; size reduction through engineering high titer pathways. Phase 3: System optimization for various exacerbating factors.

Teaming Overview and Objectives

**Blenner** has several funded projects using waste as feedstocks for microbial production of biochemicals. NASA-funded project focuses on the use of **astronaut wastes** and **bioprocessing wastes** as a feedstock microbial production of mission critical products. Blenner is an expert in **Metabolic Engineering** and **Synthetic Biology** of **non-model yeast/fungal systems** (rugged enough for ReSource applications). Karig is an expert in **microbial communities, synthetic biology, and cell-free systems**

Blenner is currently a visiting scientist at NASA, winner of PECASE award for utilizing astronaut wastes for biomanufacturing. Karig is the head of the Synthetic Biology lab and PI of a skin microbiome DoD MURI

Impact

- Metabolic engineering and synthetic biology will enable us to fulfill the role of making a **range of products** – providing flexibility for teaming.
- We develop capabilities to engineer yeast/fungal with rugged properties far exceeding the state of the art for conventional hosts.
- We anticipate leveraging the natural tolerance and metabolism of normally toxic substances to enable the use of waste and otherwise recalcitrant feedstocks through **evolutionary methods**.
- Leveraging natural consortia and develop synthetic consortia to enable flexibility in the use of feedstock with unpredictable variations.
Teaming Overview and Objectives

- PI: Enoch Yeung, Team: Dennis Joshy (PhD student, bioengineering and synthetic biology), Shara Balakrishnan (PhD student, bioengineering & optimization), Aqib Hasnain (PhD student, synthetic biology & machine learning), Andy Cai (lab technician, experimental design & fabrication)
- Expertise engineering soil bacteria e.g. Acinetobacter (WIP), Pseudomonads (1 in 2019), Burkholderia, Vibrio strains (1 in 2019):
  - Expertise with data-driven biological design, emergent dynamics (6 in 2019)
- Spectral flow analyzer, GC-MS, LC-MS, QuantStudio6 for RT-qPCR, 3 monochromator plate readers with variable temperature incubation, 4 deep in-house learning workstations with V100 GPUs for data-driven analysis
- We seek additional collaborators with access to military waste sites (we are planning on leveraging proximity to the Casmalia superfund site)

Project Overview

- **Aim**: Optimize natural microbes and engineer microbial consortia with enhanced waste-to-resource conversion properties.
- **Approach**: We aim to engineer microbial communities, with species-specific division of labor to convert waste products to intermediate compounds to cleaning compounds and emulsifiers.
  - For each step in the waste-to-resource conversion process: individual species are optimized using a combination of genetic editing, multi-omics analysis, and data-driven optimization
  - Data-driven analytics and systems analysis tools for multi-step design and optimization of consortia dynamics
- **Challenges**: Emergent complexity arises rapidly in multi-species systems, we propose data-driven analytics that allow real-time learning of emergent dynamics.

Impact

- Bacteria are masters of low-power, automated, conversion processes. They provide a genetically malleable bioengineering platform for rapid optimization of resource-to-waste conversion processes.
- Our proposed impact is to increase the conversion yield by at least two orders of magnitude (titer yield) and the rate of conversion by at least an order of magnitude (end-to-end conversion time)
- Live resource-to-conversion systems, live lubricant systems and dynamic phase-shifting living materials
- Unique measure of success: (reduction from 70% to 10% of a category of crude oil sludge/hydrocarbon waste in the Casmalia superfund site)
Gary Vanzin, Colorado School of Mines (CSM), CRED team

- We envision a transformational **Carbon Redirection (CRED)** process where microorganisms efficiently consume organic waste to produce clean water, fungible energy and useful bioproducts. The platform is inexpensive, portable, flexible, and easily operated.
- We will reinvent the wastewater treatment processes to be portable, low cost, low maintenance, and yield fungible energy and/or value added bioproducts. Our technology disrupts the current paradigm by shuttling what others see as disposable waste to our vision of resource reallocation, capturing unexploited energy and carbon to produce valuable materials.
- Technical challenges include removal of inorganic compounds, and upgrading of methane to useful products.

**Teaming Overview and Objectives**

- The team: Diverse group of scientists and engineers including CSM faculty Drs. Junko Munakata Marr, Linda Figueroa, Gary Vanzin, Civil and Environmental Engineering; Lt. Col. Andrew Pfluger, Ph. D., Academy Professor, U.S. Military Academy; Dr. Michael Guarnieri, National Renewable Energy Laboratory.
- Team has authored 10 publications (2015-2019) related to the proposed technology.
- CSM houses a pilot-scale system treating 720 L d⁻¹ raw wastewater. A low cost mobile pilot is being installed at a wastewater treatment plant.
- Collaborator needs include fluid dynamics modeling, bioproduct selection and purification, and diversified waste sources (such as food and municipal solid wastes)

**Current waste treatment infrastructure:**

Waste → Biomass → Anaerobic digestion → Methane

**CRED process:**

Waste → Methane → Bioproducts

**Impact**

- The technology eliminates the need to deal with waste disposal, and instead catalyzes the discussion of what important products can be generated from waste.
- Potential applications include water purification, fungible energy production, and bio-based product creation (biodegradable plastics, ballistic armor materials, petroleum feedstock replacements.) The process is tunable/flexible/scalable for product creation based on needs.
- Milestones/metrics include an optimized system for diverse feed waste, appropriate bioproduct selection and formulation, and a deployable, modular and portable reactor system.
- Transition to deployable status requires target bioproduct selection and optimization under real world scenarios.
Teaming Overview and Objectives

- A multidisciplinary team with broad expertise in biosynthesis, biochemical, chemical and polymer synthesis, computational and data sciences, physics, and engineering
- Broad R&D portfolio funded by DOE (Sc, BER; and EERE BETO); NASA, and LDRD
- “We can measure anything, anywhere”
- Water treatment using supercritical methods, with concurrent recovery of metals & destruction of organics
- 3-D printing formulations, e.g. from processed waste
- Separations, extraction processes
- Experimental and computation facilities, including high performance computing
- Seeking ReSource partners with expertise in conversion technologies and experience in military waste characteristics

Impact

- Completely circularized process for at least 1 waste stream of interest (e.g. plastic packaging) in a cost and energy efficient manner
- Water treatment, food waste, bio-waste treatment, plastics re-use; applied to onsite, on-demand clean water production, 3-D printing, supply production, nutrient, and fertilizer production, and more...
- Final process configuration will be an automated system
- Technical transition will be expedited by early partnerships with military/special operations to build a set of requirements

Contact Information – blm@lanl.gov, 505-695-6096
Kevin OBrien, University of Illinois, Magic Box

**Project Overview**
We propose the integration of several leading-edge technologies for biological and chemical processes for converting wastes to electricity, lubricants, potable water, and food products into a single scalable and deployable system.

**Teaming Overview and Objectives**
- Established team with demonstrated expertise in complex project management and technology advancement in most of the relevant areas. Includes members from U of I, U.S. Army, and Mainstream Engineering.
- We seek collaborators who have expertise in (1) direct processing of plant and animal species into high-quality protein (i.e., *Hermetia illucens*), and (2) automated process control systems.

**Impact**
Successful integration of existing technologies into a turn-key system would replace the burden of military waste with mission-ready supplies applicable for combat, peacekeeping, disaster relief, or humanitarian operations. Goal: exceed current energy recovery efficiencies (50-80%).

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**Phase I**
Characterize Waste Streams
- DRY WASTE:
  - Sorted plastics
  - Unsorted trash
- WET WASTE:
  - Food scraps
  - Sewage Processing liquids

**Phase II**
Integrate Waste Conversion Processes
- THERMOCHEMICAL CONVERSION AND DISTILLATION
- ALGAE CULTIVATION
- DRYING AND EXTRACTS
- AQUAPONICS

**Phase III**
Product Optimization
- MATERIALS AND CHEMICALS
  - Lubricants
  - Adhesives
  - Tactical fibers
- PURIFIED, USABLE PRODUCTS
  - Protein and nutraceuticals
  - Vegetables
  - Drinking water
  - Protein meal
  - Fish

**Key**
- Military wastes and products
- U of I and current partner technologies
- Looking for partners
- Waste to product pathways
- Energy

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B.K. Sharma – bksharma@illinois.edu – 217-265-6810
Project Overview

- Decentralization is key for mitigating disasters and military operations when energy generation can be compromised.
- Waste management is critical for public health.
- Argonne has extensive experience in the development, testing, scale-up, design and field validation of waste separation/recovery/utilization and fit for purpose treatment water technologies.
- Development of modular technologies for different application scale.

Teaming Overview and Objectives

- Over twenty years of expertise in developing and scaling up processes in waste and water treatment field.
- Efficient testing capability at multiple scales.
- Bench- and pilot-scale technology demonstration in fully equipped state of art labs.
- Over 40 patents and patent pending applications.

Products, Fuels and Power From Waste Streams

- Design and synthesize switchable membrane modules for specific functions to conduct independent treatment operations.
- Fabricate an exchangeable membrane cassette consist of a stack of switchable membrane modules.
- Configure the new membrane cassettes into a mobile platform.
- Utilize electrochemical separations for cost-effective desalination.

“Fit-for-Purpose” Water Treatment

- Development of a flexible feedstock-blending plan for waste streams produced by the military operations.
- Concepts, technologies and tools to transform waste streams into high value platform chemicals (methane, organic acids, plastics).
- Assess economic and environmental viability and further facilitate its implementation at the full-scale to meet military needs.
- New Modular Processes for waste management and water treatment.

Impact

- Over twenty years of expertise in developing and scaling up processes in waste and water treatment field.
- Efficient testing capability at multiple scales.
- Bench- and pilot-scale technology demonstration in fully equipped state of art labs.
- Over 40 patents and patent pending applications.

Dr. Meltem Urgun-Demirtas --- demirtasmu@anl.gov --- 630-252-6038
Teaming Overview and Objectives

- NREL's Biological and Catalytic Conversion team (40 people)

Relevant experience in:

- Biological funneling – engineered microbes to convert heterogeneous substrates to single products (PNAS 2014)
- Enzyme design for breakdown of plastics and natural polymers (Nature Comm 2018, PNAS 2018)
- Holistic hybrid process design, synthesis, and integration (Science 2017)
- Institutional expertise in biological, catalytic, and thermo-mechanical process design from microscale to pilot scale
- Collaborators in waste sorting, small-scale systems design, small-scale solids handling, 3-D printing, enzyme discovery

Impact

- ReSource system to thermo-mechanically increase reactivity of plastics and biologically breakdown/convert them into on-demand materials including e.g., macronutrients, lubricants, and 3-D printing precursors
- Broadly impactful systems for use in plastics upcycling across scales and industrial sectors
- Potential applications: 3-D printed materials on-site and on-demand, skin graft materials, food, lubricants and other lipid-derived useful materials
- Metrics: Conversion of waste plastics in 1 day or less to useful materials
- Technology Transition: Team with industrial partners to ensure commercial and military applicability and utility
Teaming Overview and Objectives

- **Vision** - mobile, autonomous carbon refinery capable of locating, collecting, and transforming local carbon resources; delivers carbon-based materials on demand.

- Requires integration of satellite data, drone based sensors, AI, machine learning, with *new process intensified refining operations*.

- Technical challenges addressed through transdisciplinary teaming.

- Phase I develops prototypical intensified autonomous refining units. Phase II integrates units under an AI to enable product selection. Phase III adopts and adapts sensor and mobility technologies.

Impact

- Unique, small footprint, refining units capable of converting plastic wastes into edible foods and useful chemicals (lube oils, adhesives, antibacterials, etc.).

- Potential applications enabled by this technology:
  - Sustained operations through reduced logistics.
  - Direct conversion of fossil carbon into foodstuffs.
  - Small scale specialty chemical manufacturing.
  - Economic plastics recycling.

- Unique metrics and milestones.
  - First ever demonstration of polyethylene to food.
  - Prototypical multifunctional unit operation weighing less than 500lbs that is easily assembled in the field

- Technology transfer infrastructure is very well established.
Teaming Overview and Objectives

**LanzaTech:**

- **Established multidisciplinary team:** Dr. Sean Simpson (PI; Co-Founder & CSO), Dr. Michael Köpke (Director, Synthetic Biology), Dr. Chris Mihalcea (Director, New Applications), Dr. Robert Conrado (VP, Engineering, Design & Development)
- **Leading in feedstock & product flexibility:** Global leader in Gas Fermentation; Diverse gas compositions; Waste/plastic conversion; Gas treatment/ handling; Scale Up/Down; Modular units; Synthetic Biology Platform; Microbe manufacturing
- **Global Operations:** HQ and State-of-the-art R&D facility in Skokie, IL; US site for large-scale field demonstrations; In field commercial deployment globally; Technical freedom to operate

- Seeking collaborators in areas of:
  - Gasification
  - Nutrition/ Material sciences

Impact

- Readily deployable process for conversion of plastic and/or mixed waste inputs to food, tactical materials and water
- Modular, integrated, automated unit
- Integrated operation with real waste in field demonstrated

End of Project Goal: *Technology ready for final engineering, manufacturing and deployment*
Project Overview

- Our team will work to build a library of organisms capable of building a variety of products to sustain military operations as well as humanitarian relief efforts. These organisms will be engineered to catabolize a variety of waste products (e.g., wood, paper, plastic, organics) and build mission-appropriate products from central metabolic intermediates. We will use microbial strains that sporulate or can be lyophilized in order to provide a final product that is robust and cold chain independent.
- The Keasling lab has engineered a variety of microorganisms to produce small molecules that include:
  - Fuels, polymer precursors, commodity chemicals
  - Pharmaceuticals, nutritional supplements, flavors, and fragrances
- We use a combination of computational design tools and a high throughput strain construction and analysis pipeline to generate our proposed library of organisms.
- Our sweet spot is engineering microorganisms. For example, have demonstrated the production of biodiesel from lignocellulose in a single organism.

Teaming Overview and Objectives

- The Keasling lab is currently composed of the PI, 15 Graduate students, 10 postdocs, and 8 professional career scientists and visiting scholars.
- We also have a strong local network of collaborators to draw upon for additional expertise in catabolism and protein design.
- Technology developed in the Keasling lab has been used to launch 8 companies
- Institutional assets include world class microbial strain construction and analysis facilities.
- We are seeking collaborators that can build the hardware and chemical processes needed to convert starting materials into microbial feed (TA1) and release and recover products of the process (TA3A&B).

Impact

- The long term impact of this program will be a fundamental change in municipal waste handling and overall reduction in landfill usage.
- Potential applications include: on demand medicine and nutrition, on site production of feedstocks for 3D printing, reduction of burden on the warfighter and sustained operational capabilities.
- Initial milestones will include the conversion of a waste stream feedstock into desired product. Ultimately, success will be beating the incumbent cost of delivering supplies.
- The Keasling lab has a strong track record in commercializing technologies. As many of these strains will have consumer applications, we are confident that one or more startups will be generated to transition this project.

Contact Information – keasling@Berkeley.edu – 510-486-5462
Sandia:

- Exceptional Service in the National Interest
- National Security Focus:
  - Defense
  - Energy
  - Nuclear Deterrence
  - Non-Proliferation
- Key Core Capabilities:
  - Biosciences
  - Combustion Technol.
  - Systems / Device Engineering / Analysis
  - Milli / Micro / Nano Fabrication
  - Materials Science

ReSource Teaming Interests:

- Deconstruction and Conversion of Biological and Polymeric Wastes
- Deconstruction by Gasification or Liquefaction
- Conversion / Upgrading by Biological and/or Chemical Technologies
- Reduction by 10 – 100X in Residual Waste Volume and Mass
- Displacement of High-Volume, Logistically-Challenged Resources (Food, Fuel, Water, Energy)
- Development and Scale-Up to Integrated Engineered Systems:
  - Custom Proof of Concept
  - Production-Ready Prototypes
  - Production (typ. <50 units)
Deepti Tanjore, Berkeley Lab, ABPDU

Project Overview

Making biomanufacturing cost-effective by understanding culture aging and automation

Vision to accomplish technical areas: Understand culture heterogeneity (mutations) occurring during aging in biomanufacturing is necessary to automate process optimization to minimize such mutations.

Technical Challenge: Each bioreactor study costs ~$5000 and miniaturizing process optimization has been ineffective. Fewer shots at optimization leads to higher risk in scale-up of bio-based technologies.

Approach to overcome technical challenges:
• Studying cell cultures at varying levels of process stresses through –omics, morphology, and productivity levels in bioreactors to predict future changes.
• Identifying physical and/or chemical changes representative of culture heterogeneity.
• Developing real-time sensors that can measure heterogeneity spatially in a bioreactor.

Teaming Overview and Objectives

• Deepti Tanjore, Director ABPDU, Berkeley Labs
• The ABPDU has worked with 40+ companies and scaled-up dozens of technologies using the design of experiments approach.
• ABPDU is equipped with 2mL – 300L bioreactors and 10L-210L reactors.
• Cellular imaging, image processing, and machine learning.

Impact

• It will reduce bioprocess scale-up time by 5X and minimizes biomanufacturing risk and thereby costs.
• Microbial imaging, spatial sensing in bioreactors, predictive microbial heterogeneity in bioreactors.
• Separating biological stochasticity from culture heterogeneity occurring from process conditions.
• By working with 6 partners that provide instrumentation to bioreactor owners.

dtanjore.lbl.gov  abpdu.lbl.gov  510-495-8037
**Project Overview**

Developing advanced biomass conversion technologies for the production of biopower, biogas, biofuels and bioproducts from a wide range of input feedstocks, such as DoD relevant waste resources.

World-class expertise in synthetic biology, bioinformatics, machine learning, pan-omics, biomass deconstruction, microbial communities, thermochemistry, chemical catalysis, techno-economic modeling, and life-cycle analysis.

Interest: Developing a mobile, deployable conversion unit that can transform local feedstocks into biopower and biofuels for a wide range of deployment scenarios.

Approach to overcome technical challenges:
- Systems engineering and integration of chemical-thermochemical-biochemical conversion technologies.
- Identifying key bottlenecks and opportunities through techno-economic and life-cycle analysis.
- Fully instrumented conversion reactor to enable dynamic control and optimal conversion efficiency.

**Teaming Overview and Objectives**

- Katy Christiansen, Biomass Program Lead, Berkeley Lab
- LBNL has worked with >60 companies for the commercialization of biofuels and bioproducts.
- World-class capabilities in synthetic biology, biomass conversion, and HPC modeling of complex systems
- Cellular imaging, image processing, pan-omics and machine learning.

**Impact**

- Mobile and scalable conversion technology capable of rapid deployment and continuous operation.
- Fully integrated system consisting of feedstock processing, deconstruction and conversion.
- Capable of generating tunable outputs of biopower, biogas, biofuels and bioproducts.
- Full diagnostic and analytical readouts.
- HPC enabled reactor and system design.

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