Engineering a Multi-species Fermentation Platform for Biofuel Production

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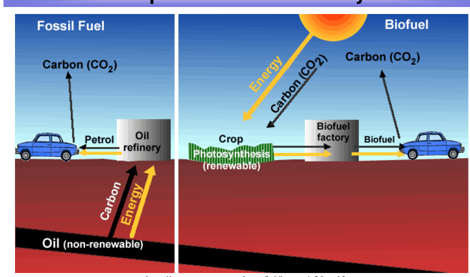
Abstract

Biodiesel production from biomass waste feedstocks is a renewable carbon neutral fuel technology. Direct biosynthesis of biodiesel from biomass is hindered by complex rate limiting pathways. By engineering synthetic consortia, the biosynthetic pathway can be divided-up among multiple microbial populations, reducing metabolic burdens and eliminating rate limiting steps that create optimal production processes. Developing tightly controlled cell-to-cell communication circuits among engineered organisms allows the mixed populations to carryout different portions of the complex biosynthesis simultaneously in a single reactor. When optimized, the synthetic consortia will minimize toxic byproduct accumulation and maximize usable biofuel yields.

Rationale

Combustion of fossil derived fuels is transferring carbon from the geosphere to the atmosphere and hydrosphere at an unsustainable rate. Carbon, once sequestered in the earth's crust for millions of years, is now accumulating in the air and water, causing global warming and ocean acidification.

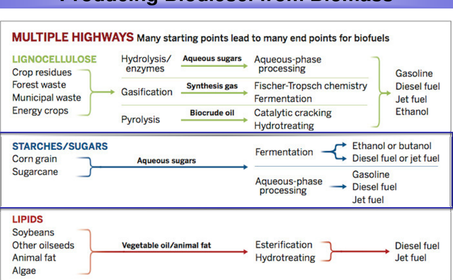
Disruption of the Carbon Cycle



http://www.reec.nsw.edu.au/k6/image/e20a.gif

Carbon neutral renewable energy technologies are essential to reduce the demand for fossil derived fuels. Biodiesel production from biomass has a low carbon footprint and can utilize agricultural wastes as starting materials.

Producing Biodiesel from Biomass

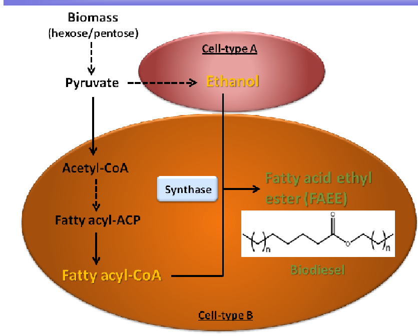


Race to the Pump, C&E News. February 14, 2011

Current methods for generating biodiesel rely on post-processing of plant derived oils, requiring provisional energy and producing unwanted byproducts. Direct biosynthesis of diesel must to be optimized in order to obtain the greatest amount of usable energy in the most cost efficient manner.

Design

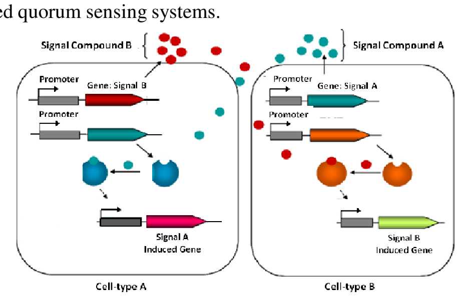
Cell-to-cell Biodiesel Consortia



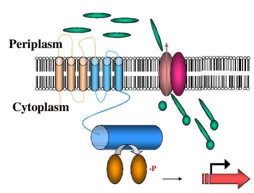
Direct biodiesel synthesis in a single reactor is achieved by dividing the pathway from biomass to biodiesel fuel among multiple engineered cell types in a consortia. This approach limits the metabolic burden on each individual organism, and exploits their relative strengths.

Engineered Cell-to-cell Communication Circuits

Interspecies cooperation and population dynamics are controlled via engineered quorum sensing systems.



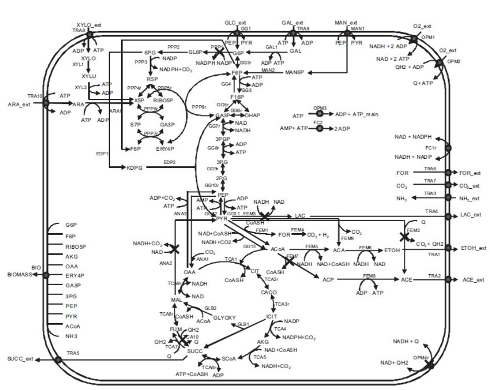
Multiple quorum sensing systems have been identified and characterized, and these mechanisms can be cloned into non-native hosts.



Quorum sensing (QS) systems can be engineered as inductive cooperative circuits (above) or signal responsive repressors (left). These QS systems can be designed to control associated organism function and population dynamics.

Metabolic Pathway Engineering

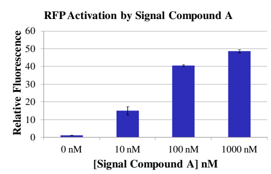
Comprehensive understanding of microbial metabolism can be used to propose and develop elementary metabolic systems optimized for highly specific function. This understanding is applied to engineer highly specialized microbial strains for unique solitary functionality.



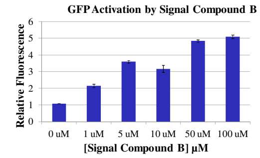
http://aem.asm.org/cgi/reprint/74/12/3634.pdf

Results

Engineered Cell-to-cell Communication



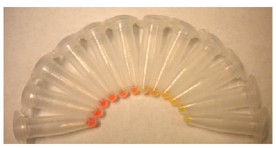
Culture media from Cell-type A is used to induce GPF expression among Cell-type B cells on minimal plate (above).



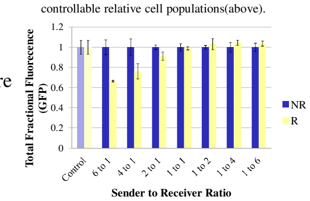
Engineered communication circuits are tested using fluorescence markers.

The results indicate that the signaling compounds activate fluorescence marker expression, and that the fluorescence intensity is a function of the signal compound concentration.

Other engineered cell-to-cell communication systems have been engineered to repress marker expression. Different ratios of receiver/reporter to inducer to cells were measured.



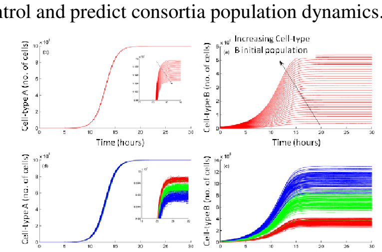
Communicating cells grown in co-culture express different fluorescent markers and exhibit controllable relative cell populations(above).



The data indicate that the relative fluorescence decreases as the number of inducer cells increase because more of the signaling compound is produced and accumulated in the co-culture.

Consortia Population Dynamics

Stochastic modeling is employed to aid design of regulatory schemes, identify needed communication control and predict consortia population dynamics.



Modeled results from our collaborators: Konstantinos Biliouris and Yiannis Kaznessis; Department of Chemical and Materials Engineering, University of Minnesota Twin Cities.

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