BIOCONVERSION OF SINGLE-CARBON EFFLUENTS INTO BIOFUELS AND BIOFUEL PRECURSORS

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**BACKGROUND**
- Methanotrophic bacteria grow on single-carbon substrates as their sole source of carbon and energy.
- Fugitive emissions of methane and other single-carbon molecules (e.g., methanol, CO₂) from participating industries are captured and fed into bioreactors to grow methanotrophic bacteria.
- The bacteria convert the methane into biofuels (e.g., butanol, isoprene) at site.
- Efficiency of carbon removal and biofuel production is dependent on scale-up.

**SHORT-TERM OBJECTIVES**
- Protocol for optimized growth of methylotrophs
- Implementation of SCF operation for methylotrophs
- Generation of metabolic maps using RNAseq, metabolome and proteome data under different growth conditions
- Identification of target pathways for biofuel and precursor production in methylotrophs
- Genetic system for development of chassis methylotroph
- Isolation of phages infecting methanotrophs (for lysis and recovery of products)
- Identification of processing conditions from forestry effluents

**PROJECT OVERVIEW**

**PROJECT 1: OPTIMIZATION OF METHANE CONVERSION**
- Screening methanotrophs for naturally produced biofuels, precursors, and pathway identification.
- Mapping metabolic pathways in methanotrophs of interest.
- Optimization of growth and conversion of methane into biofuels and precursors.

**PROJECT 2: DEVELOPMENT OF MOLECULAR CHASSIS**
- Developing genetic tools for creation of a chassis methanotroph for production of advanced biofuels and precursors.
- Expanding the biofuels portfolio in methanotrophs.

**PROJECT 3: ADVANCED FERMENTATION TOWARDS BIOFUEL PRODUCTION & RECOVERY**
- Investigating the impact of self-cycling processing on global regulation of methanotrophs and engineered strains towards the optimization of biofuel production.
- Develop efficient methods for bioproduct recovery from methanotrophs.

**PROJECT 4: STRAIN ADAPTATION, SCALE-UP AND IMPLEMENTATION**
- Adaptation and implementation of strains to industrial conditions.
- Scale-up and integration with point source emissions of methane (and methanol, CO₂).
- A C1 chassis microorganism for enhanced conversion of C1 compounds from industrial effluents to biofuels and precursors.
- An expanded biofuel portfolio from methylotrophs (C1 microorganisms) including natural and engineered products.
- A modular platform technology for efficient bioconversion of C1 feedstocks into biofuels and precursors that can interface at site with any C1-producing process for improved energetic, economic, and environmental outcomes.
- An alternative strategy to mitigate fugitive methane emissions and provide a new, non-sugar based, biofuel production chain that will introduce new revenue streams to industries (e.g., forestry).

**EXPECTED OUTCOMES**
- Methanotrophic bacteria grow on single-carbon substrates as their sole source of carbon and energy.
- Fugitive emissions of methane and other single-carbon molecules (e.g., methanol, CO₂) from participating industries are captured and fed into bioreactors to grow methanotrophic bacteria.
- The bacteria convert the methane into biofuels (e.g., butanol, isoprene) at site.
- Efficiency of carbon removal and biofuel production is dependent on scale-up.

**THEME OVERVIEW**

**BIOMASS**
We already know how to create fuels from certain types of biomass, but many other feedstocks can potentially be transformed in a similar manner. In order to identify new viable sources, we must develop a more sophisticated understanding of the technological processes that might be used to convert biomass to fuel, and assess the potential business cases for adopting certain sources that might have other economic uses, or compete with established cash crops. We can also explore the potential for tailor-made fuels for the transportation sector, developed from biological sources.

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