Conversion of CO_2 and Carbonates to Methane and (bio) Isoprene

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Abstract

We propose a biotechnology to simultaneously convert anthropogenic CO_2 or carbonate minerals from energy generation facilities into a biofuel (methane), and a bioproduct (isoprene),using microbial consortia. Carbon Capture and Utilization Strategies (CCUS) are critical to minimize emissions or remove anthropogenic CO_2 from the atmosphere. Yet, green technologies converting CO_2 to value-added products in addition to biofuels is lagging. To date, 30-40% of emitted CO_2 results from coal fired power plants and technologies have been developed to remove CO_2 from emissions. One of these technologies is the production of carbonate minerals from power plant flue gas carbonate. Methane-producing microbial species in pure culture and in multi-organism microbial consortia are naturally capable of using anthropogenic carbonates or CO_2 for production of isoprene, which they incorporate into branched alkane lipids that constitute cell membranes. Isoprene is a valuable chemical commodity used in production of polyisoprene rubber (the major component of automotive tires), in styrene isoprene-sytrene (SIS) block copolymer adhesives, and as a synthetic intermediate for a wide range of specialty chemicals (cosmetics, vitamins, flavorings, etc). Microbially-derived isoprene is chemically identical to petroleum-derived isoprene, but can be extracted in higher yield and purity. This technology will be developed in two objectives: (Objective 1) quantify conversion of CO_2 and carbonates to methane and isoprene and (Objective 2) engineer methane-producing microbes to overproduce isoprene. These experimental objectives will be initiated in Year One in an effort to augment natural isoprene production in new and existing methane-producing microbial isolates or consortia. This technology will result in an inexpensive, economically feasible method to convert anthropogenic CO_2 and carbonates into an energy source and a commodity with increasing worldwide demand. Successful implementation will also result in a market for carbonates captured by power plants.

Introduction

Methanogens

- Obligate anaerobes found in digestive tracts of animals, rice paddies, and wetlands.
- Produce all bio-methane worldwide
- Isopreniod-based membrane produced via Mevalonate Pathway
- Methanogen lipids used in industrial lubricants and vaccine delivery systems

Organisms:

Methanosarcina acetivorans (*M. ace*)
Methanosarcina barkeri (*M. bar*)

Isoprene

- 2-methyl-1,3-butadiene
- Produced mainly by plants
- Component of synthetic Rubber

A

B

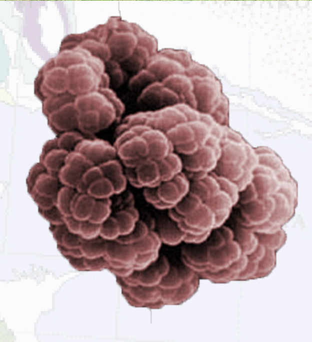
C


Figure 1: Methanogen Habitats: Cattle (A), Rice Paddies (B), and Freshwater Methanosarcina (C)

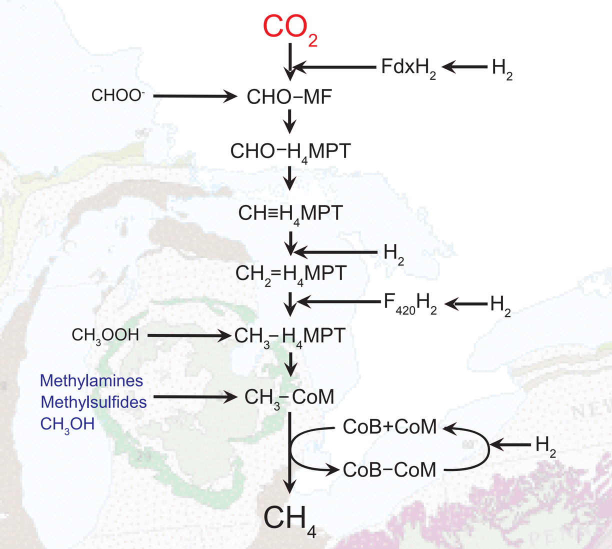


Figure 2: Overview of Methanogenesis Pathways

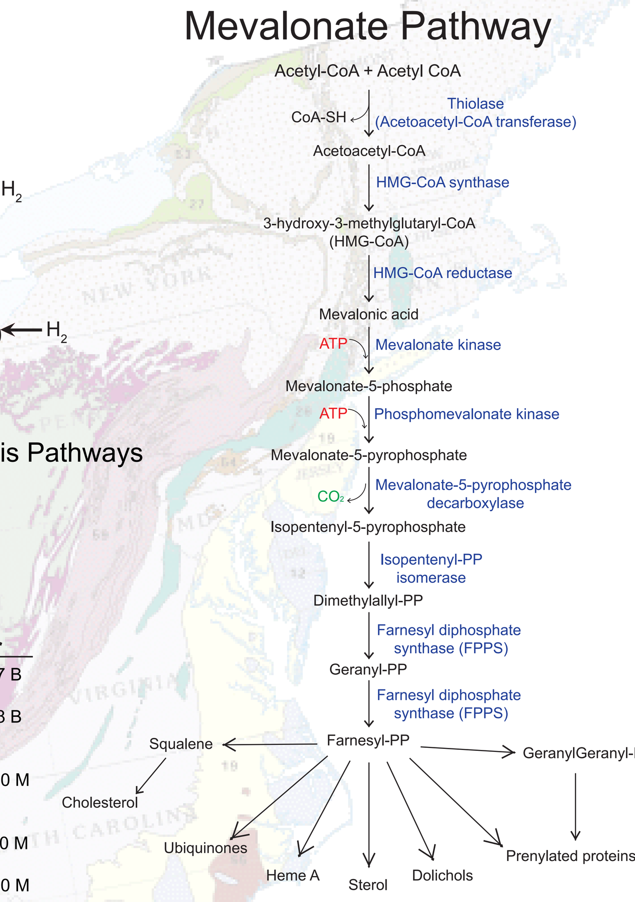


Figure 6: Overview of Mevalonate Pathway

Table 1: Uses of Isoprene

Chemical Form	Industry use	mton/yr	$/yr
Natural rubber	Various	520,000	$1.7 B
Polyisoprene rubber	Tires	540,000	$1.8 B
SIS block copolymers	Adhesives	150,000	$530 M
Butyl rubber	Tires, tubes	31,000	$110 M
Isoprene Synthetic Intermediate	Vitamins, pharmaceuticals, flavorings, Cosmetics, Epoxy hardeners	46,000	$160 M
Total		1,300,000	$4.3 B

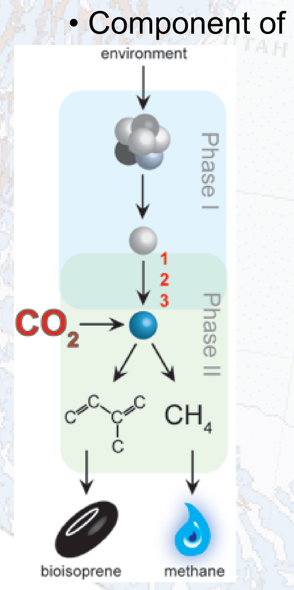


Figure 3: Project Overview

Figure 4: Theresa Street Wastewater Treatment Plant Lincoln, NE. Pictured are large anaerobic digesters which have the capacity to facilitate methanogen growth and biomethane production.

Figure 3: Gerald Gentleman Station. Located in Southerland, Nebraska, this coal fire electrical plant is the largest generating plant in the state. Operating at full capacity this facility could produce up to 20 million tons of CO_2 a year. The carbonate from this output is a potential source of carbon for methanogen growth.

Gene A

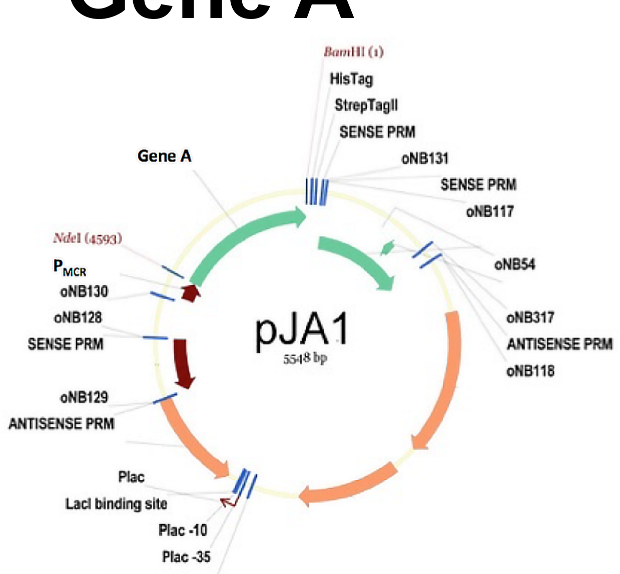


Figure 7: Plasmid Map for pJA1 with Gene A Insert

- Cloned and transformed plasmid into *E.coli* for production of transfectable plasmid
- Transfected plasmid JA1 into *Methanosarcina acetivorans* and *Methanosarcina barkeri* using liposomal transfection
- Verified insertion of gene of interest into genomes by PCR

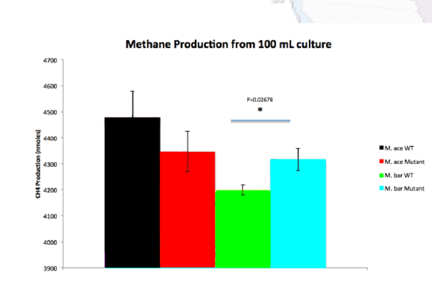


Figure 9: End-Point Methane Production assay

- 100 mL cell cultures
- Cells grown to early stationary phase
- Gas Chromatography analysis of headspace

No significant difference in amount of methane produced between *M. acetivorans* WT and *M. acetivorans* Mutant, however significant overproduction of methane when comparing *M. barkeri* WT to *M. barkeri* Mutant.

Figure 10: Gene A Activity Assay

- Cells grown to mid-/late- exponential phase
- Whole cell lysates
- EnzChek Pyrophosphate Assay Kit
- Increase absorbance at 360 nm

Successful expression of gene A in *M. acetivorans*

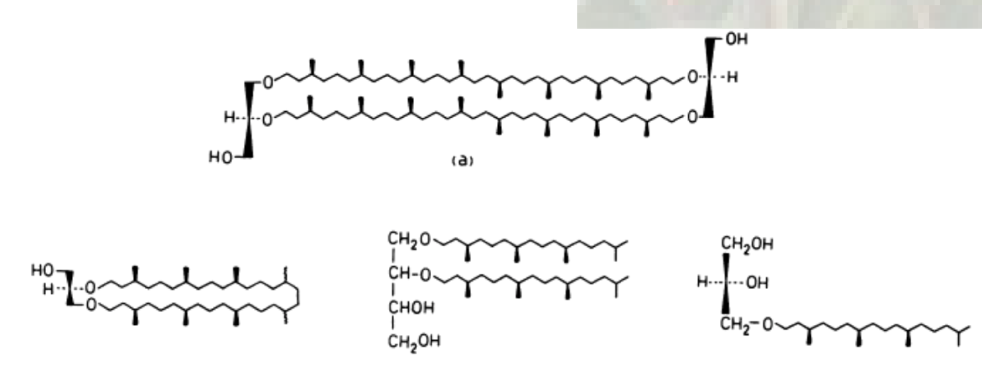


Figure 8: Methanogen Membrane Lipid Structures: soprenoid ethers, backbone of comples lipids of methanogenic archaea. (a) Di-biphytanyl-diglycerol-tetraether; (b) macrocyclic diether; (c) tetritol-diether; (d) 3-O-phytanyl-sn-glycerol

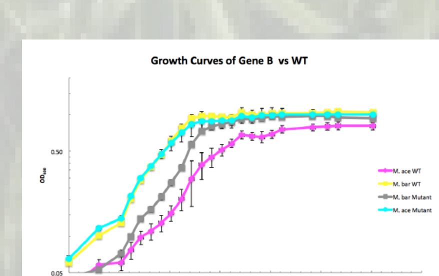


Figure 11: Growth Curve of Methanogen Transfectants

- Cells grown in quintuplicate
- OD_{600} taken every 4 hrs

***M. barkeri* Mutant exits lag phase quicker when growing**
Exhibits different growth profile

Carbonates

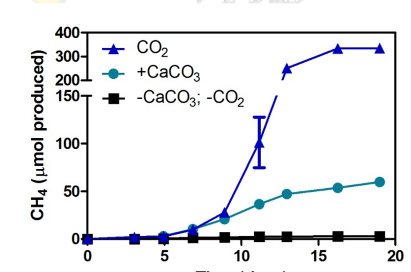


Figure 15: Methane production in anaerobic bioreactors amended with CO_2 or $CaCO_3$ relative to a control where inorganic carbon was omitted. Values represent averages of triplicate reactors. Standard error of measure is denoted by error bars. Error bars not visible are smaller than the symbol.

Figure 16: Calcium Carbonate Mineral in the form of Calcite.

Figure 17: Deep Core Sonic Drill.

Implications

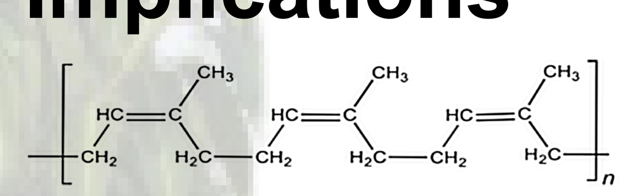


Figure 17: Various renewable industrial products which can be produced by engineered methanogens.

Gene B

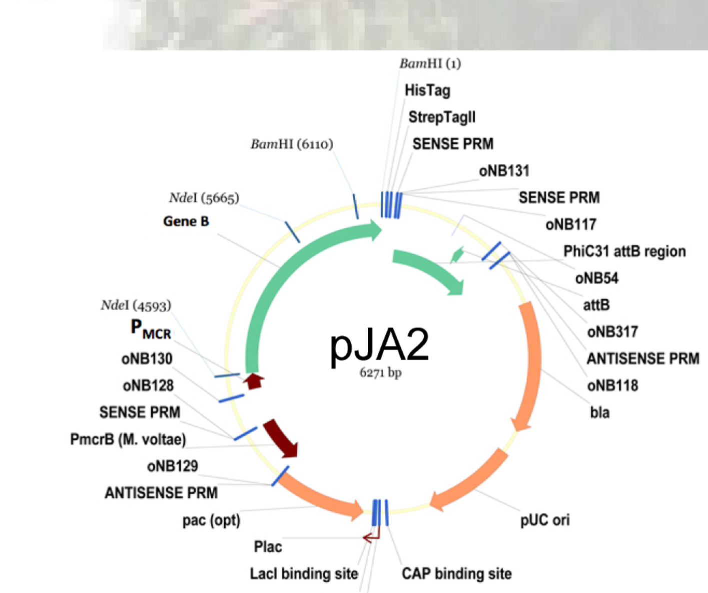


Figure 12: Plasmid Map for pJA2 with Gene B Insert

- Gene of interest codon optimized for methanogen transcription
- Cloned and transformed plasmid into E. coli for production of transfectable plasmid
- Transfected plasmid pJA2 into *M. acetivorans* and *M. barkeri* using liposomal transfection
- Expression of Gene B under control on high constitutive promoter proved to be lethal to both species when plated on HS agar plates
- Transfectants introduced to antibiotic containing HS media produced viable cells containing inserted gene of interest in genome

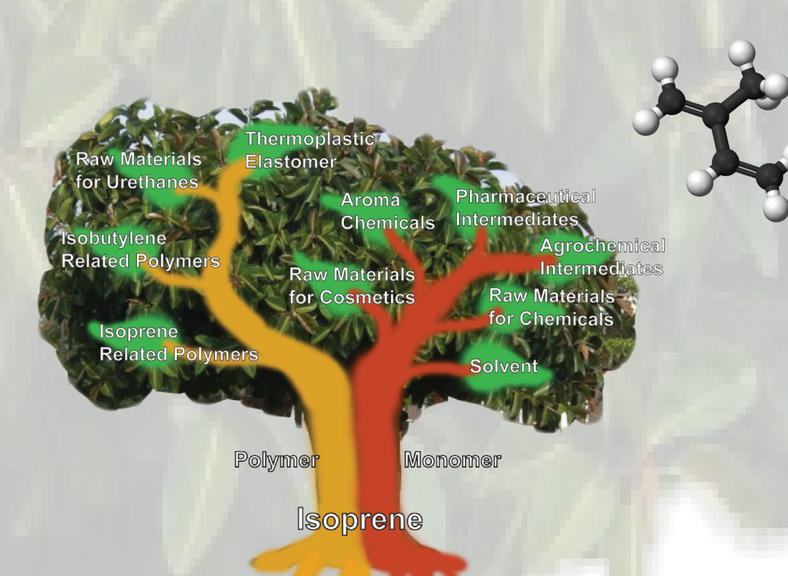


Figure 14: Diagram of Isoprene Applications

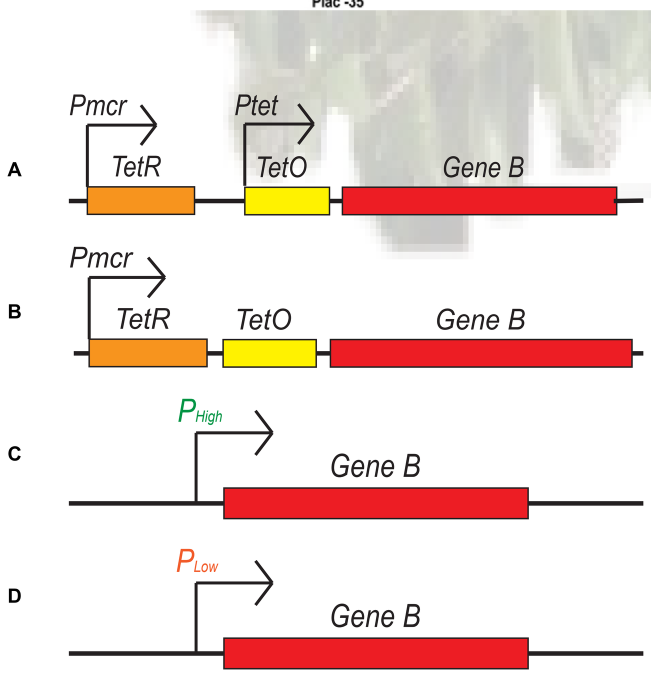


Figure 13: Transcriptional Control using Various Inducible Promoters (A & B) or Constitutive Promoters (C & D)

A & B
- Tetracycline inducible promoter system
- TetR binds to *tetO* operon preventing transcription factors binding
- Introduction of tetracycline releases tetO, promoting transciption

C & D
C: High expression --> 44,000 Miller Units
D: Low expression --> 2,500 Miller Units

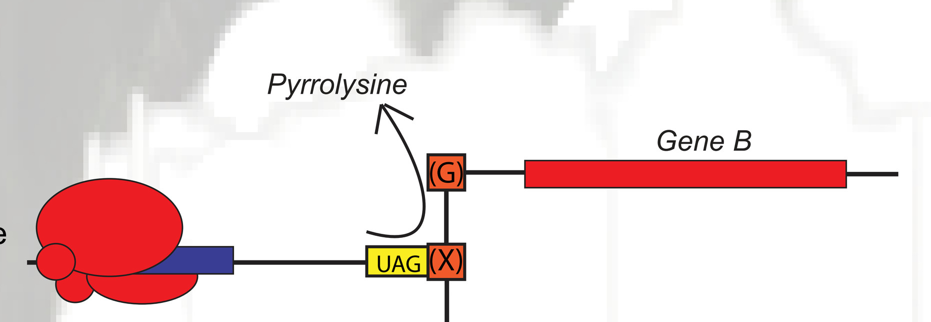


Figure 15: Translcriptional control via UAG codon

- Encodes for stop as well as pyrrolysine
- Subsequent nucleotides determine stop read or pyrrolysine read

A or T--Stop codon
G or C--Pyrrolysine

- Transcriptional control tested against reporter gene, uidA

Future Directions

Gene A
- Methane Rate Assay
- Quantitative Lipid Profile analysis using HPLC
- Full characterization of enzymatic activity

Gene B
- Obtain stable transforms
- Isoprene detection using Gas Chromatography
- Characterization of promoter systems utilizing Western blots
- Characterization of pyrrolysine control using Western blots

Carbonates
- Single-cell genome sequencing
- Metagenomics
- Meta-transcriptomics
- Mechanism of carbonate dissolution

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