Cell lysis and enhanced product recovery from methanotrophic bacteria

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BACKGROUND

Methanotrophic bacteria show great industrial potential as they are able to convert methane from industrial waste streams into useful products such as **biofuels - isoprenoids for bio jet fuels** - and **biomaterials - PHB**. The cost and low efficiencies of current methods for product **extraction** and **recovery** impede the widespread deployment of this technology.

Two technologies which demonstrate potential to overcome these challenges are the use of bacteriophages - viruses that infect bacteria - and switchable polarity solvents - which can be switched between hydrophobic or hydrophilic forms

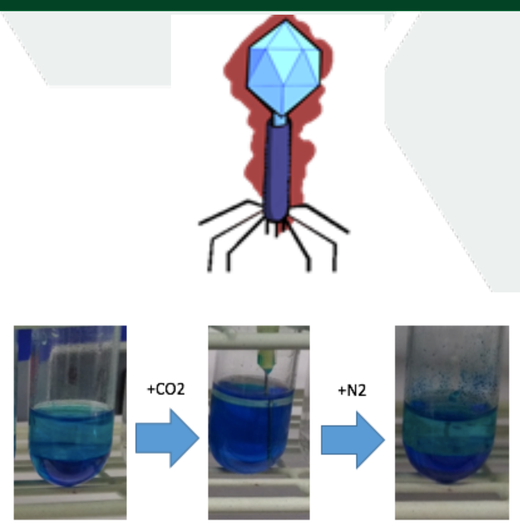


Figure 1a) Bacteriophage; b) Transition of switchable solvent from hydrophobic to hydrophilic and back again

AIMS AND OBJECTIVES

The primary objective is to improve downstream processing and reduce recovery costs and environmental impacts. Specifically, we aim to do this by:

Phages

- Isolating and characterizing a bacteriophage from the environment, which can lyse the bacteria

Switchable Solvents

- Analyzing the interaction between switchable solvents and cellular products and develop methods for removing products from cell debris
- Determine if switchable solvents can be used as a cell lysis mechanism

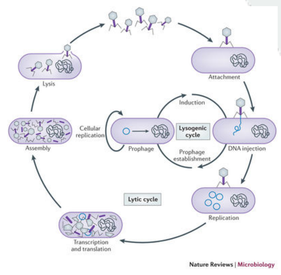


Figure 2: Phage Life Cycle

Salmond *et al*, 2015

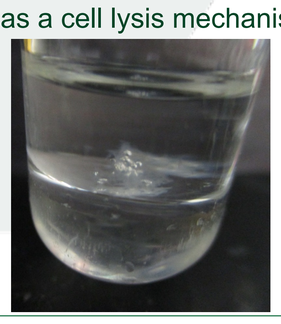


Figure 3:

Organic-phase DMBA floats on top of water, with a PHB gel floating on top of the phase interface.

RESULTS

Bacteriophage Isolation

Phage isolation methodology based on filtration of large volumes of environmental samples – municipal waste water effluent, tailings samples – has been designed and optimized. The system involves coating a cellulose filter with a lawn of bacteria, and subsequently running the sample through the system to allow phages to bind to the host bacteria, resulting in infection

Switchable Solvents

The ability of a candidate switchable solvent, N,N-Dimethylbenzylamine (DMBA) to lyse cells has been tested on 4 different strains of methanotrophs. The non-polar (hydrophobic) form of the solvent was shown to effectively lyse methanotrophs. The polar (hydrophilic) form cannot reliably lyse cells.

The switchable solvent DMBA has been shown to form a gel when added to PHB while in it's nonpolar form. This can be completely reversed by the addition of carbon dioxide and water to the system. PHB is easily separated from the mixture of polar DMBA and water.

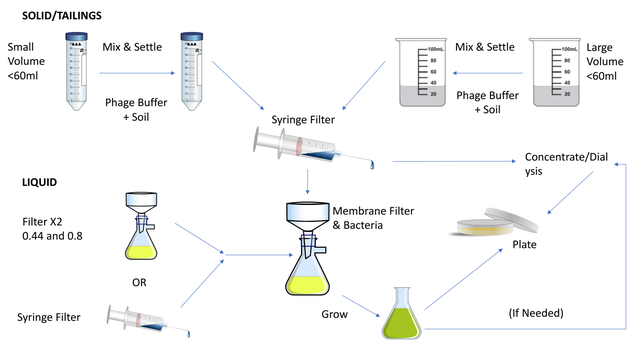
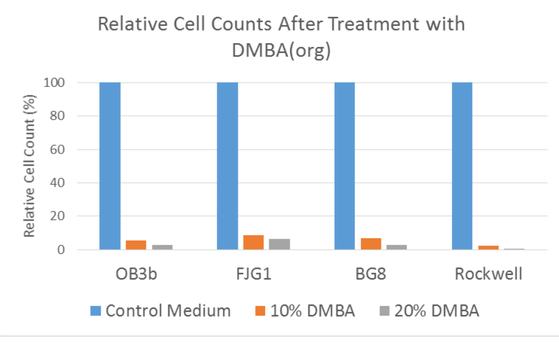


Figure 4: Proposed Isolation Procedure

Figure 5: Cell counts indicative of lysis by solvent

Methanotroph Strains:
Methylosinus trichosporium OB3b
Methylomonas denitrificans FJG1
Methylomicrobium album BG8
Methylocystis sp. Strain Rockwell



FUTURE DIRECTIONS

Bacteriophage Isolation

Once a phage that infects a methanotroph strain is identified, it will be isolated and characterized based on its virulence, physiology, and genetics. The phage will then be used as a basis for the development of a cell lysis processing step to facilitate product recovery. This research also acts as a platform for other investigations, including analyzing the prophage regions of methanotrophic strains.

Switchable Solvents

Work is ongoing to determine the degree to which PHB can be fully dissolved in hydrophobic DMBA under various conditions, as well as the interactions between cellular debris and the different forms of DMBA. Conditions that facilitate complete dissolution or a sufficient difference in the interactions between DMBA and cell debris, isoprenoids, or PHB would allow for a separation process to be developed for product recovery from methanotrophs.

Additionally, the tendency of other intracellular components to dissolve in DMBA will be studied to determine if this solvent will be useful in the extraction of other products.

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FES PROJECT OVERVIEW

T01-P03

The aim of this project is to develop a platform technology for the bioconversion of C1 compounds resulting from forestry activities (fermentation, thermal processing, anaerobic digestion) into biofuels (alcohols, lipids) and biofuel precursors (e.g. isoprenoids). This platform will be integrated in the greater context of biomass conversion by, for example, using by-product streams from other bioconversion activities (e.g. anaerobic digestion and pyrolysis) as feedstock.

Salmond GP, Fineran PC. A century of the phage: past, present and future. Nat Rev Microbiol. 2015;13(12):777-86.

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