INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI

SHORT ABSTRACT OF THESIS

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Thesis Title: **Biological conversion of methane and carbon dioxide into methanol using *Methylosinus trichosporium* NCIMB 11131**

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SHORT ABSTRACT

The emission of greenhouse gases, such as methane and carbon dioxide, has been increasing globally at an alarming rate. Conventional chemical methods to transform methane and carbon dioxide into useful chemicals are plagued by the requirement for expensive catalysts and extreme operating conditions. The exploitation of microorganisms as biocatalysts is an attractive alternative to sequester these greenhouse gases and convert them into value-added chemicals or liquid fuels through their inherent metabolic pathways. Microbial biocatalysts are advantageous over chemical processes as they require mild-operating conditions and do not release any toxic by-products. From a contextual perspective, methanotrophs, a class of Gram-negative, methane oxidizing bacteria, are being explored as novel platform to produce methanol – a potential fuel. Methanotrophs uptake methane as an obligate source for the derivation of carbon and energy. Besides, methanotrophs are capable of capturing carbon dioxide and enzymatically reducing it into methanol under specific conditions. However, the state-of-the-art technology of methanotrophic methanol production is primarily constrained by: (i) low titer of methanotrophic biomass and methanol owing to (ii) limited mass transfer and solubility of methane and CO_2 in liquid medium. Current rising energy demand and rapid depletion of non-renewable fossil fuel reserves have shifted the research focus towards exploring non-petroleum based cleaner and renewable alternate transportation fuels. To that end, oxygenated fuels (or alcohols) such as methanol, are advantageous in terms of improving the combustion, emission and performance characteristics of engine. However, a major concern is the dependency of alternative fuel technology on chemical methods for methanol synthesis which are energy-intensive, as well as economically and environmentally non-sustainable. The present study was designed to address the existing challenges and gaps through the development of a sustainable bioprocess for methanol production.

The present study demonstrates a two-stage integrated process for bio-methanol production using *Methylosinus trichosporium* NCIMB 11131, coupled with the sequestration of methane and carbon dioxide. The first stage involved generation of methanotrophic biomass via sequestration of methane, which was utilized as biocatalyst in the second stage to reduce CO_2 into methanol. At the onset of the study, the organism was characterized under various physicochemical parameters, to identify the optimum conditions for obtaining high biomass titer, biomass productivity, methane fixation rate and methanol titer. Maximum biomass titer of 4.45 g L^{-1} and productivity of 0.872 g L^{-1} d^{-1} were achieved in a semi-batch stirred tank reactor, with methane concentration in the inlet gas mixture of 2.5% v/v, gas flow rate of 0.5 vvm, in optimized medium containing 0.75X unit concentration of trace metals, in the first stage. The corresponding methane fixation rate was estimated to be 0.476 g L^{-1} d^{-1}. Maximum methanol titer of 0.58 g L^{-1} was achieved at headspace carbon dioxide concentration of 50% v/v and liquid to headspace volume ratio 10:90 in an air-tight batch reactor in the second stage.

Furthermore, process engineering strategies were developed to address the key challenges associated with inferior mass transfer rates and limited solubility of methane and CO_2 in liquid media leading to lower titers of biomass and methanol. In the first stage, combinatorial process engineering approach of design of micro-sparger, engagement of draft tube and addition of mass transfer vector was employed to enhance production of biomass. Maximum biomass titer of 7.68 g L^{-1} and productivity of 1.459 g L^{-1} d^{-1} were achieved in an airlift reactor equipped with a micro-sparger of 5 μm pore size, in the presence of draft tube and 10% v/v silicone oil as mass transfer vector. Maximum methane fixation rate was estimated to be 0.795 g L^{-1} d^{-1}. Process engineering strategy for the second stage involved customization of a high-pressure stirred tank reactor to enhance the solubility of CO_2 under elevated operating pressure for consequent increase in methanol titer. Maximum methanol titer of 1.98 g L^{-1} was achieved under headspace pressure of 4 bar, which was an increment of 235.6% relative to atmospheric pressure. Subsequently, methanol production was demonstrated through recycling of *Methylosinus trichosporium* biomass to curtail time and resources incurred towards recurrent biomass generation. Following three cycles of biomass reutilization, a maximum cumulative methanol titer of 3.6 g L^{-1} was achieved, a value significantly higher than other studies reported in literature. Methanol was recovered from cultivation medium using distillation attaining a maximum purity of 94.74%.

Eventually, different blends of methanol (0-20% v/v) with diesel were evaluated for their suitability as alternate transportation fuel. Physicochemical properties of diesel-methanol blends either improved or exhibited equivalence relative to pure diesel, as control. The blends were investigated for their effect on emission and performance characteristics of four-stroke internal combustion engine under varying engine loads (25, 50, 75 and 100%). Emissions of carbon monoxide, hydrocarbon and SO_x, and opacity of smoke decreased with increasing methanol content in the blend, attaining maximum decrements of 38.8-48.4%, 39.8-61.6%, 79.6-91.0%, and 21.0-27.5%, respectively, with 20% methanol-containing blend (M20) under varying load conditions. Superior performance parameters *e.g.*, brake specific fuel consumption (0.29 kg kW^{-1} h^{-1}) and brake thermal efficiency (28%) were achieved using M20, while brake power and mechanical efficiency exhibited equivalence with pure diesel. To the best knowledge of the author, this is the first study unveiling the potential of methanotroph-derived bio-methanol as a renewable alternative transportation fuel.