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# Methane Remediation Using Biocatalysts in Gas-Solid Reactor – Challenges and Prospects

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**ABSTRACT:** One of the engineering grand challenges of the 21st century is to develop carbon sequestration methods due to human activities. Carbon dioxide and methane are the two most abundant greenhouse gases with methane having a higher global warming potential (GWP) than carbon dioxide. Methanotrophs are a time of bacteria that consumes methane and can produce different kinds of organic acids. Wild methanotrophs produce a range of products and can be specifically selected and genetically engineered to produce a specific product. Improvement of bioreactor design for a solid-gas mass transfer is necessary for this technology to move forward. Poor solubility of methane requires high energy input for the conversion of methane. This paper reviews some relevant technology in bioreactor design of gas and liquid/solid interfaces and describes the scope of the project here at LLNL and the objectives it seeks to achieve in the geometric design of reactor of methanotroph.

## Introduction

Mitigating the human derived excess of greenhouse gases has been at the forefront of environmental scientists' minds in protecting the planet. Since the industrial revolution, GHG emissions have permanently damaged the atmosphere. In 2020, approximately 5,000 million metric tons of CO2 equivalents of greenhouse gases were released into the atmosphere[1]. Due to the COVID-19 pandemic halting commuting, this is approximately 10% less than what was released in 2019. Carbon dioxide ($CO_2$), methane ($CH_4$), nitrous oxide ($N_2O$), and fluorine-containing compounds (HFCs, PFCs, $SF_6$, and $NF_3$) are the four main contributors of greenhouse gases. Since the industrial revolution, concentration of methane has increased 167%[1]. The global warming potential (GWP) of methane is 25 times higher than that of carbon dioxide. The GWP is an empirical value that describes the amount of radiative force 1 kg of gas is able to accumulate over a specific time. This measurement is used to standardize these GHGs and compare the damage they are capable of. The capture and reduction of methane concentrations is a key part of mitigating our current climate crisis.

There are several different approaches in downstream processing and in design that seek to reduce and capture GHGs. While CO2 emissions are the highest, the lifespan and warming capacity of methane makes it much more impactful. Mitigation and control of methane and carbon dioxide is necessary for protection of the planet. The production of GHGs is not avoidable as it is the most abundant product of common manufacturing processes. Scientists have developed several different methods to reduce emissions of processes and harnessing the power of these products to create additional value-added materials, yet they still lack in industrial markets.

Methane is a natural byproduct of the life cycle decaying organic matter but the introduction of methane from human derived processes, has resulted in a dangerous concentration of methane in the atmosphere. The oil and gas, agricultural, and municipal waste disposal industries are the three largest contributors to excess methane production[2]. Methane release from these sources is responsible for approximately 70% of all methane emissions into the atmosphere[3]. Agricultural derived methane is primarily from raising cattle and rice cultivation. With the global population increase and demand for food, methane produced from agriculture has also increased. The second contributor is from the energy sector, specifically the oil and gas industry. Natural gas is 95% methane and is a byproduct of oil drilling and fracking. The United States and Russia are the largest contributors to methane release from the oil and gas industry[2]. The last largest source of methane is from municipal waste. Uncontrolled, anaerobic decomposition of waste produces methane as a byproduct. In robust design of aerobic wastewater treatment, methane emissions can be reduced greatly. Yet, wastewater treatment facilities in developing countries are often stagnant water treatment such as septic tanks or open sewers allowing for uncontrolled anaerobic decomposition producing methane[2].

There are currently two different approaches for conversion of methane into value added materials: chemical and biological processing. When using a chemical route to create different chemicals, it involved high capital costs and can also have toxic side reactions and byproducts. The benefit of biological conversion is that the microorganisms used are naturally occurring species that consume methane and produce value added products with little to no harmful side products. There are several different approaches to remediating the amount of methane such as using it for fuel of microbial fuel cells to generate electricity, control and produce methane from municipal waste as a source of energy, and create liquid fuels using complex chemical processing, the Fischer-Tropsch Process. In large manufacturing facilities, gas to liquid fuel processing plants can convert the methane into long hydrocarbon chains for fuel[4]. The Fischer-Tropsch process was developed in the 1920's during WWII by Germans, Franz Fischer and Hans Tropsch. The Fischer-Tropsch process converts solid and gas carbon sources into liquid fuel. High pressure and temperature conditions are required for the gasification of carbon monoxide and hydrogen gas for the reaction of long hydrocarbon chains. The volumetric flow rate of methane

that is a byproduct of these processes is too low for chemical plants of conversion, rather this methane is just released into the air or burned for fuel, using a high value chemical as a low value fuel. Alternative to chemical treatments is the use of a biological catalyst. Utilizing microorganisms that naturally consume the undesirable toxin is an underexplored technique. Advantages of Fischer-Tropsch synthesis is that this process is not specific to GHGs so research doesn't have to come specifically from methane consumption; in comparison with the use of a biocatalyst is that it has to be highly specified for a single feedstock to produce the desired products. The use of a biocatalyst microorganism specificity and reactor design will be the focus of this paper. It will describe current literature regarding methods of using biocatalyst in methane consumption and the limitations and areas for additional research.

**Biocatalyst - Methanotrophs**

Methanotrophs are anaerobic and aerobic type of bacteria that oxidize methane into products such as methanol and formaldehyde in the presence of oxygen. Type I methanotrophs are a γ-proteobacteria and utilize the ribulose monophosphate (RuMP) pathway; Type II methanotrophs are α-proteobacteria that use the serine pathway to consume methane as the source of carbon[5]. The RuMP pathway utilizes formaldehyde to convert these larger carbon intermediates[6]. Methanotrophs' carbon source is methane gas and is able to convert gas into liquid products such as: methanol, lactic acid, carboxylic acids, and 2,3-butanediol[7]. Aerobic methanotrophs oxidize methane using two different kinds of enzymes, particle and soluble methane monooxygenases (pMMO and sMMO, respectively) in the first steps if methane oxidation[3]. Figure 1 shows an overview of the different metabolic cycles methanotrophs utilize for methane consumption. There has been much research done in screening wild strains to see if there is potential for it to be used as a biocatalyst. Products of methane consumption, growth conditions, consumption rate, and robustness against infection are a just a few parameters that are of interest in the design and use of biological catalysts.

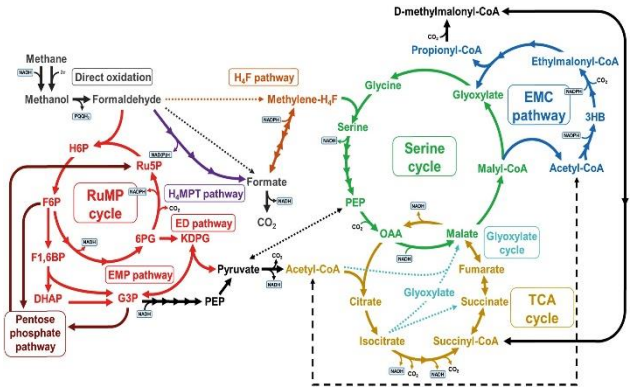


Figure 1. Overview of RuMP and Serine cycle, reprinted from Lee et. al[8].

There is high value in using a biological catalyst for gas-to-liquid conversion. Methanotrophs do not require culture conditions that are high in energy and can be cultured at ambient temperature and pressure. Succinic acid is a building block for bio-based plastics[8]. *M. capsulatus (BATH)* have been developed and isolated for the production of succinate using genetic engineering for over-expression and down-regulation of specific genes. Poly-lactic acid (PLA) is a bioplastic that has become significantly more popular in the biodegradable/compostable plastic world and the demand is growing. There are several different methods of producing PLA and methanotrophs would be contributing to the production [7,8]. Figure 2 shows the variety of chemicals that are products of methanotrophs[9].

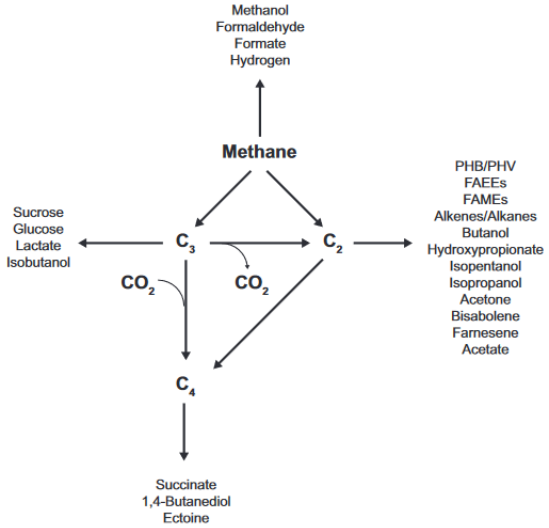


Figure 2. Reprinted from Kalyuzhnaya et. Al describing possible intermediate carbon intermediates from methanotrophic conversion[9].

**Bioreactor design**

The biggest barrier of design of a gas to liquid bioreactor is the mass transfer limitation of methane. The solubility of methane is low in water, ~22 mg/L at 20C and 1 atm[10]. Many reactor designs contain the products and reactants in the liquid phase. While this is not feasible for a methanotrophic bioreactor, methods for increasing mass transfer between gaseous methane and microorganisms is important. The combined parameter, $k_La$, is a measurement of the convective mass transfer coefficient of a gas bubble moving through a liquid. Where $k_L$ is the convective mass transfer coefficient of the liquid and the bubble surface area, $a$ where the combined units of $k_La$ are $h^{-1}$. The mass transfer coefficient of methane, $k_La$, through polymers and other complex materials is often not calculate but otherwise experimentally determined.

The application of using methanotrophs to produce value-added materials requires disruption of the metabolic cycles allowing for the over production of desirable compounds. For example, inhibition of enzyme methanol dehydrogenase (MDH) prevents conversion of methanol to a desirable liquid product, formaldehyde[11]. Alternative methods of disrupting these metabolic cycles can include selectively editing genes for over production of a certain product or inhibiting enzymatic activity of metabolism cycles.

Two common reactor designs for two phase reactions is to use a bubble column or a stirred tank reactor, STR. The most common method of increasing the active area for mass transport, $a$, is to have a continuously stirred reactor (CSTR) with a gas sparger to produce additional bubbles. CSTRs with gas sparging are high energy input with low catalyst density and conversion rates. The volume required for these reactors is also something to consider. Process controls of CSTRs require precise control over parameters such as temperature and mixing rate to maintain high conversion and yield. Alternative reactor designs are of interest and alternatives will be described with parallel or similar applications. Increasing the impeller rpm in a STR can move the reaction forward but the shear of the liquid on methanotrophs decrease their viability.

Bubble columns are a type of reactor that is tall column with a sparger at the bottom. The sparger produces gas bubbles and gas/liquid mass transfer occurs throughout the length of the column. The mass transfer coefficient of a bubble column was experimentally measured in a bubble column to be 102.9 $h^{-1}$ with a gas flowrate of 3L/min and 300 rpm mixer[10]. A limitation of using a bubble column is that the reaction rate decreases as the number of bubbles decrease as you are further away from the sparger.

Traditional methods of increasing mass transfer include operating at high pressure, decreasing gas bubble diameter, and increasing quantity of bubbling. For example, R. Tschentscher et. Al describes increasing the gas-liquid mass transfer using rotating foam reactors. By encapsulating catalyst within the pores of the foam, it reduces required downstream processing and limits damage of the catalyst. The rotating blades creates turbulent environment creating the bubbles, reducing the need for a sparger. R. Tschentscher et al. compared two different geometries, foam paddles and donut-shaped cone with a traditional Rushton stirrer. This experiment measured the mass transfer of oxygen to water. When using a Rushton stirrer achieved a $k_{GL}a_{GL}$ of $0.1s^{-1}$ at rmp of 500 while the foam block stirrers had a 25% increase of $k_{GL}a_{GL}$ at a lower RPM[12].

Reactor designs include immobilization of biomass to increase the density of proteins and overall productivity. 3D culture technique is useful for long-term experiments where culture on flat surfaces is not representative of the system. Bioreactors that are packed with gel beads, made from alginate, silica, or other hydrogels are used in continuous flow reactor design. Immobilizing methanotrophs in sodium alginate beads shows prospect of using them in a continuous-flow reactor. Taylor et. al[11] measured the methane uptake of different seeding densities of *Methylosinus trichosporium* OB3b, a strain of methanotroph, in sodium alginate beads in batch and semi-steady state reactors. They inhibited the enzyme methanol dehydrogenase (MDH) which converts methanol to formaldehyde using cyclopropane to allow accumulation of methanol. An interesting result from this report showed that the higher seeding density did not improve the methanol production. There was not significant differences in the uptake between the free, suspended biomass compared to the packed alginate beads[11]. Scaling effects were observed where increasing volume of biomass did not increase the methanol production showing that the solubility of methane was a limiting factor.

Biotrickling reactor, or a trickle bed reactor (TBR), have been used with ceramic beads or polyurethane foams where the beads have grown a biofilm on the surface, or pores of foam infiltrated with biomass[13,14]. Trickle bed reactors are advantageous to alternate reactor designs such as STR and bubble columns because they do not require energy requirements as the liquid component primarily uses gravity to determine the volumetric flowrate. These are packed columns, with encapsulated beads or ceramic beads

containing biofilm, increase the surface area, which is key for gas to liquid/solid mass transport. Limitations of this bioreactor is long term bioaccumulation. The biogas:air ratio[13] and the resonance time[14] were two main factors in increasing mass transfer and maximizing methane consumption. Limitations of trickle bed reactors is the accumulation of biomass results in clogging of the reactor. This increases the pressure drop across the inlet and outlet so the operating conditions cannot be assumed to be steadystate[15]. Long term operation of a reactor of this design would require regular maintenance which is not ideal for industrial use.

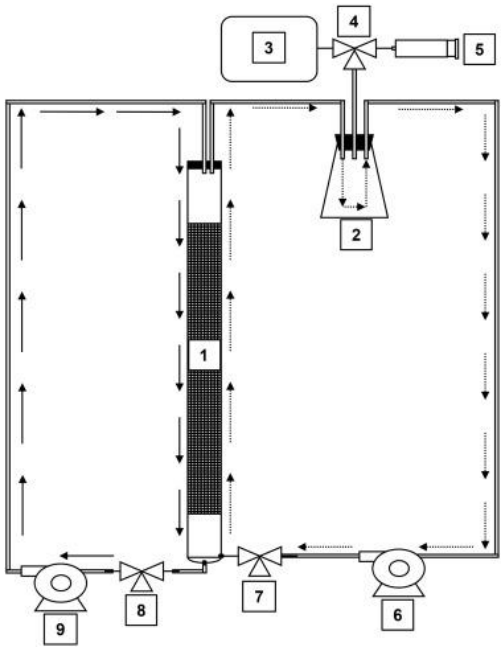


Figure 2. Overview of trickle bed reactor from Sheets et. Al.[13] This is an example of a counter current flow of liquid and gas in reactor. (1) the trickle bed reactor (2) gas feed and sample port (3) gas bag (4) gas sampling and feeding port (5) syringe for vacuum (6) circulation pump (7) gas circulation valve (8) sampling valve (9) liquid circulation pump. [13]

In contrast to a trickle bed reactor or a packed bed reactor discussed above, a fluidized bed reactor involves the passing of a fluid through bed of solid material at the minimum fluidization velocity, $U_{mf}$, that suspends the particles in the liquid. Rather than the fluid moving through a packed bed in the void space depending on particle size, a fluidized bed has uniform mass transfer throughout[16]. Fluidized bioreactor beds have been used in wastewater treatment where the packed bed contains micro-organism coated particles that are suspended in the liquid and allow for the entire surface area of the particle as a biocatalyst. Fluidized bed reactors are often used with solid-liquid phases, but fluidized bioreactors using the gas-liquid-solid phases has also been shown to successful in culture of methanotrophs by Pfuger et. Al[5]. This reactor setup was a non-sterile environment containing aggregates of Type I and II methanotroph biomass that were fluidized with W1 media and dissolved $CH_4$ and $O_2$. This fluidized bed bioreactor experiment lasted 255 days where the first 33 days had higher substrate consumption, up to 286 mg $CH_4$ mg/hr, to 51 mg/hr after more than 255 days of culture[5].

There are several different geometries of reactor vessels, stirred tank reactors, bubble columns, trickle bed reactors, and fluidized beds, that have been used for methane conversion or could be easily adapted for biocatalysts in theory. Advantages of these designs is that there has been much research done in optimization and modeling of reactors. Disadvantages of these methods are the high capital costs due to the process control of parameters such as temperature, pressure, and volumetric flowrate. A bioreactor for methanotroph culture at ambient temperature and pressure, addressing the disadvantages of common reactor design, is where Lawrence Livermore National Lab seeks to design.

**Novelty of project**

There is not one clear solution to limiting the amount of greenhouse gases released into the atmosphere. Lawrence Livermore National Lab (LLNL) seeks to create a bioreactor system utilizing methanotrophs ability of methane oxidation to create a bioreactor which can be used in waste streams, such as water treatment plants and other industrious processing that produces greenhouse gases, to convert methane into value added products. Methanotrophs ability to be used in gas to liquid of fuels is a great advantage to the biofuel industry when the storage of natural gas has low energy density. Engineering of the bioreactor system and biological strain are two parts of the project that have been conducted with help from the National Renewable energy Laboratory, NREL. The desired biological characteristics of a methanotroph strain that can be used in the application of methane conversion are: to be productive in presence of containments, such as carbon dioxide and hydrogen sulfide and have high yield of liquid product, such as methanol, lactic acid, or succinate. The objectives of the reactor system is to have low operating energy requirements and cost; and be scalable for a range of volumetric flow rates.

Collaborators at National Renewable Energy Laboratory, NREL, work on the isolation and optimization of bacterial strains of methanotrophs. There is an abundance of different strains of methanotrophs: aerobic, anaerobic,

gram-negative/positive, ect. One example of a bacterial strain that shows promis is *Methylomicrobium alaliphilum 20*$^{R}$. It was isolated as a strain that is able to feed off of a mixed feed stream, representative of commercial biogas containing methane, carbon dioxide, and hydrogensulfide[7]. They also concluded that $CO_2$ does not effect methanotroph growth and that biogas of various compositions can be used in culture.

Two key design parameters of this project, 1) that it has low operating costs and 2) is scalable. Designing a system that is scalable allows for application of mid to low volume waste producers. Chemical purification and separation plants require large volumes of reactants to be profitable and for certain separation processes to occur. These additional waste remediation systems also come with high capital costs with low profitability. The advantage of a biological approach, processes can be scalable by the number of units or concentration of bacteria used. This is important for waste streams where chemical plants are not feasible.

The use and design of a bioreactor utilizing gas-solid mass transfer of methane gas through a solid hydrogel is a novel technique. Traditional reactors with reactants that are poorly soluble in water increase the mass transfer area, $k_La$, parameter by using a sparger in a bubble column. This requires higher energy input and much lower cell density, and increases overall cost and space required.

The project at LLNL utilizes immobilization of methanotrophs in a hydrogel. This hydrogel is supported by a cylindrical scaffold to aid in structural integrity and to increase mass transfer between the gas and solid, immobilized methanotrophs. Poly(ethylene glycol) tetraacylate (PEGTA) is that is initiated using Lithium phenyl-2,4,6,-trimethylbenxoylphosphinate, LAP. The capillary forces between the pores of the scaffold hold the uncured hydrogel until it is UV cured. A UV cured hydrogel has been used because of the methods of curing of other common hydrogels used for cell encapsulation, such as alginate and agar. This UV-click chemistry only requires a short exposure to UV light to cure without damaging the cell viability.

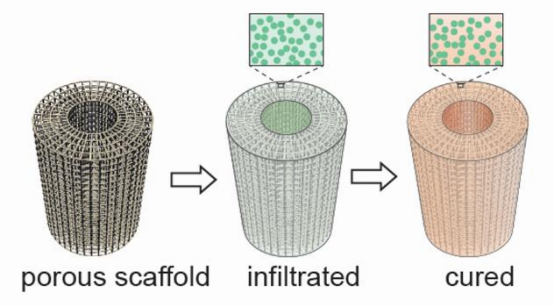


Figure 3. Overview of proposed design. Porous scaffold is 3D printed from commercially available polymer. It is then infiltrated with a mixture of hydrogel and methanotroph bacteria (green) that is UV cured. The cured scaffold is approximately 10mm in height and an inner diameter of 3mm with 250 um wall thickness.

There has been much research done to convert carbon dioxide and methane into value-added chemicals. Several improvements have been made on the manufacturing side where chemical plants are able to be built onsite of oil and gas refineries, yet a solution for mid to small scale waste streams still need to be addressed. Biological conversion of methane to methanol or other organics can be used as a catalyst for the fine chemical industry where methane is a side product. Improving the gas/solid mass transfer using physical or chemical methods would be beneficial for applications other than growth of methanotrophs. The applications and design of bioreactors for environmental remediations is a field that has been researched but is yet to be adapted for industry.

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