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Production of methanol from biogas using methanotrophs for the application in a microbial fuel cell

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Abstract. Methane is available in abundance as natural and renewable gas. Methane-rich biogas has low power density so cannot be considered as a good substrate for the microbial fuel cell. The power density of methane-rich biogas can be increased by its conversion to methanol. Two-step strategy has been followed for the production of methanol from methane-rich biogas and its utilization in a microbial fuel cell. Methanol production using methanotrophs is an attractive way for the valorisation of waste-derived biogas. Besides this, methanotrophs have been used in converting the methane-rich biogas into a variety of bioproducts which include single cell protein, polyhydroxyalkanoate bioplastics, biodiesel, extracellular polysaccharides, propylene oxide, and human health supplements. This paper focusses on the biological conversion of methanol in which solid-state anaerobic digestate can be used for the isolation of methanotrophic bacterium. A fresh isolate possesses the characteristics which resembled to constrained methanotrophs from the methylocaldum genus. This freshly new isolated methanotroph evolved on purified methane or biogas and successful production of methanol is accomplished from the biogas. This study also deals with the effect of formate and various inhibitors of methanol dehydrogenase for the biological conversion of methanol and where the formate act as an electron donor. But isolate produced methanol without any electron donor with the help of phosphate. Moreover, isolate also produced methanol without methanol dehydrogenase inhibitor by the use of formate. The maximum quantity of methanol consists of an optimum quantity of formate and phosphate as a growth medium. This study reveals an efficient conversion of methanol from biogas using solid-state anaerobic digestate. The produced methanol will not go for the purification process and can be used directly in a microbial fuel cell for the power generation purpose.

Keywords: Methanotrophs; methylocaldum genus; methanol dehydrogenase; formate; phosphate; microbial fuel cell.

1. Introduction

Million tons of biomass wastes which include crop residues, food wastes, and animal manure are generated [1]. These generated biomass waste can be used for energy production and different types of valuable products. Anaerobic digestion technology can help in degradation of role in these biomass



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waste for the biogas production [2]. Biogas generally contains methane (CH_4), carbon dioxide (CO_2), and less quantity of contaminant gas which includes ammonia (NH_3) and hydrogen sulfide (H_2S). The major drawbacks of biogas are its low energy density and are costly as well difficult to store, distribute and transport. That's why biogas in small scale anaerobic digester (AD) is often gets flared and wasted [3]. Biogas can also be captured from landfills and can be used as renewable fuels. Biogas can be converted to compressed natural gas as well as liquid chemicals via thermochemical processes [4]. Biological conversions are generally more attractive than thermochemical conversions [5]. A diverse group of bacteria commonly known as methanotrophs uses monooxygenase (MMO) for the oxidation which converts methane to methanol. These bacteria consume two reducing equivalents for the oxidation of a single molecule of methane. Normally, formate dehydrogenase (FDH), methanol dehydrogenase (MDH), and formal dehydrogenase (FaldH) further result in oxidation of methanol to carbon dioxide (CO_2) which generates reducing equivalents of six per molecule of methanol. That's why, production of methanol with methanotrophs is accomplished by the use of MDH inhibitors such as phosphate and sodium chloride (NaCl), and usually provides external electron donors which are generally formatted to carry out the metabolic activity and survival of methanotrophs [5]. Biological upgradation of biogas using methanotrophs is an interesting approach to add the value to the waste-derived methane gas because methanotrophs grow at ambient pressure and moderate temperature, can utilize a low concentration of methane (CH_4) which is generally less than 20%, and capable of producing methanol at high efficiency [5-7].

Trickle bed bioreactors have a fascinating design for the cultivation of methanotrophs because of their low costs and limited power requirements as compared to that of membrane bioreactors [8, 9]. Trickle bed bioreactors (TBRs) are a type of cylindrical packed reactors which is packed with inert materials having high specific surface area [10]. A thin layer of liquid is provided on the packing surface by circulating the nutrient medium through the trickle bed bioreactor (TBR) while gases are blown up either co-current or counter-current to the liquid [8]. In this study, the two-step process has been discussed for the generation of power from methanol derived from biogas.

Methanotrophs isolation can improve the feasibility of methanol production from the biogas. Isolation of methanotrophs from various sources such as waste treatment facilities, natural gas fields, and soils [5]. Few studies also focus on the production of methanol by methanotrophs isolation from hydrogen-rich anaerobic digester using biogas as a source [11]. Methanotrophs can last long without oxygen and can use biogas as their main source of carbon as well as carbon. This paper focusses at the role of formate and inhibitors of MDH for the biological conversion of methanol, Isolation and characterization of methanotrophs from the solid state anaerobic digester, comparison of purified methane (CH_4) and biogas as a substrate for isolation of methanotrophs. Finally, the produced methanol using methanotrophic isolation can be utilized in a microbial fuel cell (MFC). This paper also includes the details regarding the construction associated with a MFC.

2. Materials and methods

2.1. *Methanotrophs isolation*

Eight samples of digestate can be collected from solid-state AD reactors. The quantity of each sample should be 5g [12]. The switchgrass and anaerobically digested wastewater sludge in the ratio of 3:1 can be fed into one liter solid-state AD reactors. Total solids (TS) required for controlling the solid-state reactor is either 20% or 30%, and incubation period under mesophilic conditions (36 ± 1) is 70 days. The variation in methane yield of solid-state AD reactors is varied from 88 l/kg VS_{add} (TS of 30%) to 113 l/kg VS_{add} (TS of 20%) [12]. The procedure of isolation is the modified version of protocols explained in Dedys and Bowmen [13, 14].

The incubation of solid-state AD samples can be performed in 250 ml flasks which consisted of nitrate mineral salts medium (45 ml per flask). The nitrate mineral salts medium consisted of 0.2% (v/v) of chelated iron (Fe) solution along with 0.284 g/l of sodium diphosphate (Na_2HPO_4), 1.0 g/l of magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), 0.134 g/l of calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$),

0.272 g/l of Monopotassium phosphate (KH_2PO_4), 1.0 g/l of potassium nitrate (KNO_3), and 0.05% (v/v) of a trace element solution. The chelated iron (Fe) solution consisted of 2 g/l of Ethylenediaminetetraacetic acid (EDTA), 1 g/l of ferric (III) ammonium citrate, and 0.3% (v/v) of concentrated hydrochloric acid in deionized water. The composition of trace element in solution includes 200 mg/l of Iron(II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$), 30 mg/l of Boric acid (H_3BO_3), 10 mg/l of Zinc sulfate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), 500 mg/l of EDTA, 20 mg/l of Cobalt(II) Chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$), 3.0 mg/l of Sodium molybdate dihydrate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$), 1.0 mg/l of calcium chloride dihydrate ($\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$), 3.0 mg/l of Manganese(II) chloride tetrahydrate ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$), and 2.0 mg/l of Nickel(II) chloride hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) in deionized water. The initial pH of nitrate mineral salts should lie in the range of 6-7. The rubber stopper can be used for sealing the flasks and 0.2 μm of a filtered mixture of pure methane (99% purity) should be filled in the headspace of the flasks and 1:4 (v/v) of a CH_4 : air ratio can be selected. The incubation temperature of 37 °C and continuous shaking with 200 rpm can be followed. After the completion of three days, an enriched culture of 5–10 ml should be transferred into new flasks having 40–45 mL of freshly nitrate mineral salts (NMS) medium and similar ratio of methane to air can be followed. This process has to be repeated every three days. After the completion of 25 days, the enriched mixture has to be spread on the nitrate mineral salt agar plate. These plates will be incubated in an anaerobic jar at a temperature of 37 °C for 1-2 weeks having headspace of air and methane (CH_4) in the ratio of 1:4 (v/v). The freshly nitrate mineral salts plates could have been selected for streaking of selected individual colonies until single colonies based on size, shape, and color are visible. The obtained isolates should be shifted to 1 ml of nitrate mineral salts (NMS) medium in a sealed vial of 15 ml capacity with the reactor headspace containing 0.2 μm of filtered methane and air in the ratio of 1:4. The incubation temperature of 37 °C can be followed by shaking at 300rpm until the visible increase of turbidity in culture. Light microscopy can be used for determining cell morphology. If the culture doesn't grow in nitrate mineral salts (NMS) improved with glucose (0.05% (w/v)) [13, 14].

The analysis and classification of 16S rRNA gene sequences can be followed by the BLAST and RDP Classifier against the NCBI RefSeq database [15]. The *Methylocaldum* sp. 14B is one the fastest growing isolates that's why it can be selected for further experiment.

2.2. Various physical and chemical inputs involved in cultivation.

The strain 14B ability can be tested for the utilization in different sources of carbon by growing aerobically in 1 ml quantity of liquid nitrate mineral salts (NMS) medium which is added with 0.1% (w/v) of citrate, methanol, acetate, glucose, formate, or xylose. Cultivation can be performed in vials of 5 ml with shaking at 200 rpm and a temperature of 37 °C. The growth can be observed by the variation in optical density and light microscopy can be used for examining the cell morphology. A fixed quantity (20 ml) of nitrate mineral salts medium in the flask of 125 mL can be selected for culturing the strain 14B with air and methane (CH_4) ratio of 4:1 (v/v) in headspace in order to determine the effectiveness of sodium chloride (NaCl), copper (Cu^{2+}), temperature, nitrogen source, and pH on growth of cell using methane (CH_4) as the only source of carbon. The copper should be selected for testing as it had shown influence on the growth of the cell specifically for the methanotrophs which acquire particulate methane monooxygenase [16].

The assessed concentrations of Cu^{2+} in a fixed quantity of CuCl_2 that needs to be supplemented in nitrate mineral salts (NMS). The liquid nitrate mineral salts (NMS) containing a fixed concentration of NaCl can be used to find out the effect of various concentration of salts. The growth which will take place can be assessed by the replacement of potassium nitrate (KNO_3) in nitrate mineral salts (NMS) medium with ammonium sulfate ($(\text{NH}_4)_2\text{SO}_4$). The cultures should be incubated at a temperature of 37 °C with continuous shaking of 200 rpm in order to determine the Cu^{2+} , pH, NaCl , and nitrogen source. The strain 14B should be cultured in nitrate mineral salts medium and incubation can be carried out at different temperatures with shaking at 200 rpm in order to determine the effect of temperature.

2.3. Formation of methanol

For the effective screening of methanol dehydrogenase (MDH) inhibitors, early growth phase should be selected for strain 14B cells which can be harvested by centrifugation process at 10000 rpm for 15 minutes, and resuspension can be done in 4.1 mL of nitrate mineral salts (NMS) medium containing a fixed quantity of sodium formate along with different concentration of methanol dehydrogenase inhibitors [17]. The most potent MDH inhibitors such as phosphate, magnesium chloride (MgCl_2), Ethylenediaminetetraacetic acid (EDTA), and sodium chloride (NaCl) provides a great impact on methanol accumulation. Before undergoing incubation, a fixed volume of cell suspension sample should go for removal and filtration process. Thus obtained filtrate can be analyzed using gas chromatography (GC) for determining the primal methanol presence. The leftover volume of the cell suspension should be shifted to glass vials which are closed with the help of rubber stopper. The purified methane (CH_4) having 99% purity should be added to vials to achieve a ratio of 5.15:1 for air: CH_4 in the headspace. The vials will be subjected to incubation at a temperature of 37 °C with continuous shaking at 200 rpm for 6 hours. After the completion of 6 hours, the cell suspension will be filtered and obtained filtrate will be analyzed using gas chromatography (GC) for the concentration of methanol.

Two replicates can be done for the vials. The volume of 6 ml of methane (CH_4) can be selected to provide an identical quantity of methane (0.25mmol) and 0.27mmol of oxygen (O_2) to the headspace of the reactor. Moreover, formate (80 mM) should be used to supply a quantity of formate (0.25 mmol) concordant to the levels of methane and oxygen in the reactor headspace. The impact of phosphate and formate on the production of methanol from biogas can be tested using 4 levels of sodium formate and 4 levels of phosphate (Na_2HPO_4 -Disodium phosphate or KH_2PO_4 - Monopotassium phosphate).

The reactors can be set up with above-described data and biogas have been considered as methane source (air: biogas of 3.1:1) in the headspace of the reactor with an optical density at 600nm. The time course of production of methanol can be tested by cultivating strain 14B at a temperature of 37 °C and shaking can be carried out at 200 rpm in nitrate mineral salts medium. This nitrate mineral salts medium will have phosphate with various sodium formate levels. The Tedlar gas bag will be connected to each of the flasks.

3. Construction of microbial fuel cell and its operation

Microbial fuel cell test can be performed in triplicate which consists of single-chambered, cubic shaped air-cathode, and an anode chamber of cylindrical shape (4 cm in length and 6 cm in diameter) [18]. The anode of MFC consists of fiber brush (both length and diameter are 2.5 cm) made up of graphite and is subjected to heat treatment at 450 °C in the presence of air for 30 minutes before the application and placement can be done horizontally in the middle of microbial fuel cell (MFC) chambers. The preparation of cathode can be accomplished using a hot-pressing method [19]. The layer of catalyst can be prepared by mixing 60% of polytetrafluoroethylene emulsion (PTFE) with activated carbon (AC) at a mass ratio of 1:6. A stainless steel mesh (say 42×42) can be selected as a cathode current collector. The selected membrane for microbial fuel cell should be hydrophobic in nature so that it can be used in the diffusion layer in order to minimize loss of water. That's why polyvinylidene fluoride membrane (0.45 μm) is one the best choice for the microbial fuel cell. The pressure of 3×10⁷ Pa at a temperature 60 °C for 15 s of should be selected for the pressing of the current collector, PTFE: AC and diffusion layer until the surface of the membrane becomes dry [19, 20]. The cathode which has been pressed can be taken out for drying so that it can be used later on.

The methanol produced from solid state anaerobic digestate (using methanotrophs isolation) can be filled directly in the microbial fuel cell. The potential of the anode can be obtained by placing a silver or silver chloride reference electrode in the central part of microbial fuel cell (MFC) chamber, while the cathode potential can be determined by using whole cell voltage and anode potential. Coulombic

efficiency of the cell can be defined as the ratio of total charge displaced to the anode surface to the maximum theoretical charge extractable.

4. Results and discussion

4.1. Investigation of MDH inhibitors for production of methanol

It was interesting to note that without the addition of MDH inhibitors methanol gets accumulated with 80 Mm (Table 1). Few of the cases show less amount of methanol accumulated upon addition of MDH inhibitors (Table 1).

Table 1. Impact of MDH inhibitors for methanol accumulation [17].

| MDH Inhibitors | Concentration of MDH inhibitors (mM) | Formate (Mm) | Productivity of methanol ($\text{g l}^{-1}\text{d}^{-1}$) |
|---|--------------------------------------|--------------|---|
| Phosphate | 100 | 80 | 1.17 ± 0.04 |
| | 200 | 0 | 0.28 ± 0.00 |
| | 200 | 80 | 0.95 ± 0.13 |
| Magenesium chloride (MgCl_2) | 20 | 80 | 0.98 ± 0.13 |
| | 40 | 0 | Not determined |
| | 40 | 80 | 1.04 ± 0.01 |
| Ethylenediaminetetraacetic acid (EDTA) | 0.5 | 80 | 1.12 ± 0.03 |
| | 5.0 | 0 | Not determined |
| | 5.0 | 80 | 1.04 ± 0.14 |
| sodium chloride (NaCl) | 100 | 80 | 1.00 ± 0.04 |
| | 200 | 0 | 0.83 |
| | 200 | 80 | 0.85 ± 0.09 |
| Without any addition | 0 | 80 | 0.98 ± 0.05 |

4.2. Influence of phosphate and formate on the productivity of methanol

Production of methanol gets increased upon addition of phosphate in the absence of formate but maximum content of methanol was found out to be low which lies in between 0.04-0.05 g/l at the phosphate of 200 mM [17]. Moreover, interesting results were found which shows that higher productivity of methanol was obtained on addition of formate without phosphate which lies in between 0.5-1.0 g/l/d [17]. This results in hints that formate dehydrogenase provides limitation in methanol accumulation by strain 14B.

4.3. Methanol-powered microbial fuel cell

The methanol along with buffer solution of phosphate have been used for the operation of the microbial fuel cell. The maximum voltage and power density were found out to be 0.5 V and $426 \pm 17 \text{ mW/m}^2$ after the completion of the acclimation period of 45 days [21]. During the operation of the microbial fuel cell, fast consumption of methanol takes place and results in $51 \pm 4 \text{ mg/L}$ as final concentration after completion of 24 hrs of operation [21]. The acetate concentration during the operation reaches a

maximum in 12 hrs and after completion of 24 hrs, acetate concentration reduces. The coulombic efficiency of the microbial in a cycle was found out to be $22 \pm 3\%$ [21].

5. Conclusion

The two-step process can be followed to generate power from biogas obtained from the solid-state anaerobic digestion process. The methanotrophic isolate plays a crucial role in the production of methanol from biogas. Production of methanol can also be achieved with the addition of phosphate only in the cultivation medium but production can be further improved upon the addition of formate. Lastly, produced methanol from the biogas can be directly used in a microbial fuel cell without any purification process.

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