1. Introduction

Biogas, a promising renewable energy source, is generated during anaerobic digestion (AD) process where different microbial species degrade organic materials (OMs) in the absence of oxygen [1, 2]. A wide range of waste types can be used as feedstock for biogas production. For instance, sludge from water resource recovery facilities (WRRFs) [3], agricultural residues [4-7], paper waste [8, 9], food waste [10], and livestock manure [11, 12]. The yield and composition of biogas from AD depend largely upon the type of feedstock and operating conditions [13, 14]. The composition of biogas originating from AD process fed with different types of feedstocks is listed in Table 1 [15-32].

It can be seen from Table 1 that CH₄ and CO₂ (two largest contributing greenhouse gases) are the main constituents of biogas. Therefore, biogas release to atmosphere must be avoided due to the adverse environmental impact. Biogas generally contains hydrogen sulfide (H₂S) from a trace amount to a concentration as high as 80,000 ppmv depending on the feedstock's sulfur content [17]. When H₂S and water vapor co-exist, biochemical reactions by thiobacilli rapidly produce sulfuric acid [33, 34] that is highly corrosive and causes the metal equipment and concrete structure to wear down. The combustion of biogas containing H₂S leads to the formation of sulfur dioxide (SO₂). The product SO₂ can chemically react with moisture in the atmosphere to form sulfuric acid resulting in acid rain [35]. Therefore, prior to biogas utilization, an appropriate pre-treatment technique considering the economic and environmental benefits is required. Biogas desulfurization techniques - biological and physicochemical - have been widely reported in literatures [36-38]; they are beyond the scope of this review.

Biogas, after pre-treatment (cleaned mainly for the content of H₂S and water vapor), is nowadays used in internal combustion engines (ICEs) and boilers for electricity and thermal energy generation [39, 40]. In addition, it can also be utilized as a vehicular fuel [43, 44]. The incentives to utilize biogas for the aforementioned applications might weaken in the near future. Shale oil and gas are expected...
to be plentiful and cheap due to the advanced extraction technologies and fast expansion of technically recoverable shale reserves worldwide [45, 46]. In addition, the use of biogas for electricity generation faces competition with other renewable sources such as solar and wind. The unit cost of solar panels (called photovoltaic (PV) modules) and equipment has declined so dramatically over the last decade [47] that is now cheap enough and yet much more efficient to outcompete biogas for the purpose of electricity generation. According to Kost et al. [48], in Germany, the largest mature European market of biogas for electricity generation, the levelized cost of energy (LCOE) for biogas-based power generating plants (specific investments of 2,000-4,000 €/kW) ranges between 0.10 €/kWh (7,000 full load hours (FLH) per year) and 0.15 €/kWh (5,000 FLH per year), while the LCOE of large scale PV rooftop power plants with a global horizontal irradiation (GHI) of 950-1,300 kWh/m²a ranges between 0.05 €/kWh and 0.08 €/kWh. In meanwhile, the LCOE of onshore wind power plants (1,800-3,200 FLH per year) lies in the range of 0.04 €/kWh and 0.08 €/kWh. The LCOE is a measure of the average revenue per unit of electricity generated by a given generating plant over its lifetime. The International Renewable Energy Agency (IRENA) published a technical report in November 2019, which shows that the LCOE of solar PV plants has decreased significantly over years, 77% since 2010, reaching $0.08/kWh in 2018. Further drop in the LCOE of solar PV plants is expected over the next decades, reaching ($0.08-$0.02)/kWh and ($0.05-$0.01)/kWh by 2030 and 2050, respectively [47]. For these reasons, novel uses of biogas have drawn great attention in recent years. This review focuses on existing studies related to the utilization of biogas for producing single cell protein as animal feed supplement, and methanol as an important building block in the chemical industries. Yet, to the best of the authors’ knowledge, the lack of such a review paper is evident in literature. This review is organized into two sections. The first section deals with single-cell protein production, and the second is concerned with bio-methanol production. In each respective section, the main mechanism together with the microorganisms involved and the key process parameters are systematically presented, which could point to the research gaps where further studies should be directed.

### 2. Biogas Conversion to Single-cell Protein

The rapidly growing world population (9-10 billion people by 2050) [49, 50] will lead to an increase in demand on protein-rich foods, which rely mainly on animal proteins [51, 52]. The higher demands on animal-based food, the higher the production of agricultural crops (e.g., soybean meal) for animal feeds [53]. The crops production is often energy-intensive, has a high water/land footprint, and generates large quantities of residues [54]. In addition, in case where the application of fertilizers is essential for better crop yield, their excessive use contributes toward eutrophication, which causes...
detrimental impact on our ecosystem [55]. Furthermore, the crop yield depends on soil characteristics and the weather conditions [56] that limit the productivity and cultivatable plant species. In recent years, scientific community has focused on developing innovative approaches to supply animal feeds. In this context, microbial-based protein production processes have caught remarkable attention because they do not necessarily exhibit any of the above-mentioned drawbacks [54, 57, 58]. Microbial protein - popularly termed single cell protein (SCP) [59] - refers to protein derived from the dried cells of bacteria, fungi, algae, or yeasts [60]. In dealing with SCP production, different sources have been utilized as feedstock. For instance, edible sugars (QuornTM, Marlow Foods Ltd, UK), natural gas [60-62], and agro-industrial wastes [63-65]. However, there are some challenges associated with the use of these feedstocks for SCP production: i) edible sugars-based SCP production process may be economically less viable, ii) the direct utilization of agro-industrial wastes may lead to accumulation of heavy metals in the SCP, which might hinder the consumption of this bio-product [66], and iii) natural gas-based SCP production processes are becoming less attractive because of reliance on the unsustainable fossil resources.

In order to eliminate the risk of accumulation of toxic substances in the SCP and to ensure the sustainability and economic viability of SCP production process, biogas could be a promising and attractive feedstock. This section aims to represent the proof-of-concept for biogas conversion to SCP.

2.1. Choice of Microorganisms and Culture Mediums

Various microorganisms possess the capability of utilizing CH₄ and/or CO₂ in biogas as carbon source while assimilating nitrogen from a cultivation medium leading to SCP production. These microorganisms include:

i) Aerobic methane-oxidizing bacteria (MOB), so-called methanotrophs, which utilize CH₄ as both carbon source and energy source. Methanotrophic bacteria rely on O₂ to oxidize CH₄ into CO₂ while building up their new cellular material [67]. Examples of the most popular methanotrophic genera (and species, if applicable) for biogas-based SCP synthesis are Methylocystis [57], Methylophorum [57, 68, 69], Methylophilus [68, 69], Buriramicrobium [68], Comamonadaceae [69], Methylophilales [70], Methylococcales [70], Methylococcus capsulatus [53, 71], Methylobacterium butyrate [72], Methylophilus parvus [73], Methylophorus marinus [74], Methylophilus alcalophilus 20Z(R) [75, 76], and Methylophilus acidiphilus DSM 13967 [77]. It is worth noting that several methanotrophic genera have been reported to be capable of CO₂ fixation, which make them suitable candidates to grow on biogas; for instance, Methylococcus, Methylophilus, and Methyloferula [67].

ii) Autotrophic hydrogen-oxidizing bacteria (HOB), sometimes called Knallgas bacteria [58], which utilize O₂ as an electron acceptor and H₂ as an electron donor, and fix CO₂ to biomass while assimilating ammonia nitrogen [78, 79]. According to the literatures, the following HOB genera (and species, if applicable) demonstrated to be capable of producing SCP. These include Hydrogenomonas [80], Aquaspirillum [81], Alcaligenes eutrophus Z1 and Ralstonia eutropha B5786 [82], Pseudomonas [83], Pseudomonas denitrificans Y5, and Pseudomonas versutus D6 [78]. However, HOB can hardly be a suitable candidate for SCP production from biogas because hydrogen generated as an intermediate in acidogenesis stage during AD is quickly converted to methane by methanogenic archaea. It can exist only if methanogenesis stage is intentionally inhibited for bio-hydrogen production [84], which is not commercially practiced today.

iii) Algae, which use CO₂ in the presence of sunlight (or artificial light) as energy source leading to O₂ and biomass production [85]. Several potent algal species recently used for SCP synthesis include Chlorella sorokiniana [53, 71, 75], Synechococcus PCC 7002 [75, 76], Scenedesmus [73], and Arthrospira platensis [72].

The most frequently used mediums to cultivate the SCP producers (methanotrophs, HOB, algae) are synthetic nitrogen-rich sources such as ammonium mineral salts (AMS) [66, 68, 71], nitrate mineral salts (NMS) [77], Zarrouk’s medium [72], A-plus medium and P-medium [75, 76]. However, such culture mediums are expensive [59]. Hence, in the recent years, application of waste streams as nitrogen source for microorganisms’ growth has shown a good promise with zero or negative cost when adopted to replace high-energy intensive process such as activated sludge. These waste streams include supernatant of the anaerobically digested sludge/bio-waste (ADs) [68, 70, 71], and food industry wastewater (FIWW), for instance, the effluent of a potato processing plant [53]. The ADs/FIWW can be considered as a promising alternative to the synthetic mediums for SCP production making the process economically more viable.

A challenging issue associated with the use of ADs as a culture medium for SCP production is that they contain high ammonium concentration [86, 87], e.g., 1,000-3,000 mg NH₄⁺/L [85], which should be diluted to meet the desired ammonium concentration (below 150 mg NH₄⁺/L) [66, 71]. This implies that a large volume of freshwater for the dilution process is required while going to produce SCP at a large scale. This is not realistic because freshwater is becoming a scarce resource worldwide. In order to address this limitation, the secondary clarifier water (SCW) from the WRRFs could be used instead without affecting the microbial growth [71].

The gravitationally settled ADs, diluted with SCW, can be directly used as a culture medium without requiring pre-treatment, e.g., filtration or autoclaving, which present an inhibitive cost to this application. An example is the study of Roberts et al. [71], who investigated biogas conversion into microbial biomass using a co-culture of Chlorella sorokiniana and Methylococcus capsulatus grown separately on the gravitationally settled ADs or the sterilized AMS medium (as the control). According to their findings, the growth profile of the co-culture grown in ADs and the sterilized co-culture medium (as the control) was comparable to those obtained growing separately on the gravitationally settled ADs, pre-treated ADs or the sterilized AMS medium (as the control). According to their findings, the growth profile of the co-culture grown in ADs and the sterilized AMS medium (as the control) was comparable to those obtained using either the pre-treated ADs or the sterilized AMS medium.

However, prior to the use of FIWW as a culture medium, it should be subjected to centrifugation and autoclaving (to ensure neither solids nor indigenous bacteria are present) that make full scale SCP production infeasible [53]. In this context, ultrafiltration could be instead applied. According to the study of Podlevin et al. [88], an ultrafiltered IWW stream was successfully utilized as a culture medium for algal growth in a full-scale reactor.

2.2. Production Process

The production of SCP from biogas takes place in enclosed bio-
reactors where the selected microorganisms, in the presence of biogas, are grown in a sterile medium containing the essential nutrient for their growth. The generated biomass is then processed for the protein extraction. Fig. 1(a) illustrates biogas-based SCP production process driven by methanotrophs, which metabolize \( \text{CH}_4 \) in the biogas. Such a process is less attractive in converting biogas into SCP because \( \text{CO}_2 \) in the biogas remain unused; in other words, \( \text{CO}_2 \) emission to air persists. This is a problematic issue because \( \text{CO}_2 \) is a major contributor to global warming. In addition, since biogas and \( \text{O}_2 \) are directly bubbled in the bioreactor culture medium, an explosive mixture in the bioreactor headspace may occur [53, 57].

In order to enable both \( \text{CH}_4 \) and \( \text{CO}_2 \) contribution to the production of SCP, a possible solution is the use of methanotrophs co-cultivated with autotrophic HOB in a single stage system [66] (Fig. 1(b)). This process relies on \( \text{O}_2 \) and \( \text{H}_2 \) gases, which are expensive resources [54] as they are usually generated by electrolysis of water that is an energy-intensive method [58, 89]. In addition, the risk of an explosion still appears to be a challenge for the safety of such a process [58].

A highly promising alternative process to the above mentioned methanotroph- and (methanotroph-HOB)-based SCP production processes is the use of a consortium of algae and methanotrophs in a single stage phototrophic reactor (Fig. 1(c)). In this process,
Table 2. Recent Studies of Biogas-based SCP Produced Using Various Microbial Cultures

| Microbial culture              | Culture medium                                                                 | Biogas Composition (% v/v) | Biomass SCP (%) w w<sup>-1</sup> DCW | Reference |
|-------------------------------|--------------------------------------------------------------------------------|---------------------------|--------------------------------------|-----------|
| M.capsulatus                  | IWW<sup>1</sup>, 19 Synth.                                                    | (60% CH<sub>4</sub>; 10% CO<sub>2</sub>; 30% O<sub>2</sub>)<sup>2</sup> | 53                                   | [53]      |
| HOB-MOB                      | AMS<sup>3</sup>, 131 Real<sup>3</sup>                                          | (52% CH<sub>4</sub>; 44% CO<sub>2</sub>; 3% N<sub>2</sub>, 0.8% O<sub>2</sub>)<sup>5</sup> | 61 ± 8                              | [66]      |
| Mixed MOB<sup>6</sup>         | dAMS<sup>7</sup>, 28 Real<sup>7</sup>                                         | n.r.                      | 46 ± 2                               | [68]      |
| Mixed MOB<sup>8</sup>         | ADs<sup>9</sup>, 28 Real<sup>7</sup>                                         | n.r.                      | 41 ± 2                               |           |
| C.sorokiniana-M.capsulatus    | ADs<sup>10</sup>, 120 Synth.                                                 | 70% CH<sub>4</sub>; 30% CO<sub>2</sub> | 43 ± 4                               | [71]      |

<sup>1</sup>centrifuged-autoclaved

<sup>2</sup>gas composition at the end of C.sorokiniana cultivation test using a synthetic biogas (60% CH<sub>4</sub> and 40% CO<sub>2</sub>)

<sup>3</sup>supplemented with vitamin solution of DSMZ medium 81

<sup>4</sup>originated from a mesophilic AD fed with agricultural wastes (pumpkin and pig manure)

<sup>5</sup>averaged of 30 measurements on a daily basis

<sup>6</sup>dominated by 34.80% Methylphilus sp.1, 12.64% Rurimicrobium sp., and 10.50% Methylomonas sp.1

<sup>7</sup>a thermophilic AD fed with a mixture of primary and secondary sludge

<sup>8</sup>dominated by 56.26% Methylomonas sp.1, and 24.60% Methylphilus sp.1

<sup>9</sup>centrifuged-filtered-pasteurized-diluted supernatant of a thermophilic AD fed with a mixture of primary and secondary sludge

<sup>10</sup>gravitationally settled-diluted supernatant of a mesophilic sludge-based AD

The methanotrophs use the in situ generated O<sub>2</sub> by algae to convert CH<sub>4</sub> into biomass while generating CO<sub>2</sub> that is subsequently fixed by the algae. Such a process offers several advantages over the methanotrophs and methanotrophs-HOB processes: i) it does not require an external O<sub>2</sub> supply, ii) CH<sub>4</sub> and CO<sub>2</sub> in the biogas can be simultaneously converted to biomass, and iii) the risk of an explosion is eliminated [53, 71, 72].

Table 2 summarizes the results of the existing studies related to the biogas-based SCP production by means of methanotrophs, autotrophic HOB-methanotroph consortia, and algae-methanotroph consortia.

As seen in Table 2, the protein content of the biomass is within the range of 41-61% on a dry cell weight (DCW) basis, which is superior to soybean meal (35-44%) [54, 58, 90] that is the most common vegetable-based protein for livestock feed, and comparable with fishmeal (55-65%) [90, 91]. Therefore, SCP products can replace such costly conventional protein sources for aquaculture/animal feed supplement. A number of researchers successfully tested the SCP products in feed trials with various fish species and mono-gastric animal such as pigs, broiler chicken, mink and fox [61, 92, 93]. However, as of today, there exists a number of issues making the SCP products unsuitable for the use in human diets: i) in the case of obtaining SCPs from bacterial-based biomass, they may contain nucleic acid, notably ribonucleic acid (RNA), as high as 8-16% [82, 94, 95], which is too high for human consumption. The maximum safe limit of RNA for a person is 2 g/d. Intaking RNA more than 2 g/d may pose serious health problems such as gout and kidney-stone [58, 62, 96]. This is because purine (C<sub>5</sub>H<sub>4</sub>N<sub>4</sub>) compounds - generated from breaking down of RNA - are degraded to uric acid, which are then accumulated in the body due to the lack of uricase enzyme. It is worth noting that RNA-rich SCP products as feed for ruminants and short-lived mono-gastric animals does not cause serious problem [60, 97], ii) algal-based SCP products, although having relatively low nucleic acid content (3-8%) [60], their rigid cellulosic cell wall (about 10% of DCW) is indigestible by humans due to the lack of cellulose degrading enzymes [95], iii) SCP may carry heavy metals - when waste streams are used as the cultivation medium - that can cause detrimental effects on human health, and iv) SCP may contain toxin compounds if the microbial culture and the process conditions are not properly controlled. Rudravaram et al. [74] reported that a methane-oxidizing bacterium Methylomonas methanica, which is capable of storing high levels of protein, produces endotoxins at certain environmental conditions.

Taking into account the above-mentioned issues, if SCP products are going to be marketed for direct consumption as human food, it will have to go thru a long journey of the regulatory approval process. First, they must undergo a number of proper treatments to disrupt the cellulosic cellular wall and reduce nucleic acid content to a level that meets the guidelines for the use as food or food supplements, without affecting the quantity and quality of the amino acids. Second, a series of costly and time-consuming toxicological tests are required to ensure the SCP products are truly safe for human consumption. These tests involve short-term studies for acute toxicity and long-term chronic toxicity, where laboratory/farm animals are fed with normal diets incorporated with different amounts of SCP. In this context, animal species such as mice, rats, rabbits, and guinea pigs can be served as animal models. The duration of the toxicological tests ranges from few weeks to few years (equal to the lifespan of the animal under study). After that period, hematological, biochemical and pathological examinations are performed to check possible adverse effects of SCP on the tested animal organs. The more specific toxicity tests include carcinogenicity, multiple generation, mutagenicity, teratogenicity, and sensitization [98]. Shacklady [99] has reported an extensive range of toxicological test results on "Toprina", which is a product of Candida lipolytica. Useful information concerning the safety evaluation of SCP products is available in [100-104].
For instance, allergenic responses may not be detectable through animal trials. Therefore, once SCP products are demonstrated to be free of toxic compounds, and not to leave unpleasant residues in the tested animal tissues, they have to be subjected to clinical tolerance trials [104-106]. The toxicity tests for Quorn™ Myco-protein (Marlow Foods Ltd, UK) lasted 16 years, with many more years needed to obtain approval for sale outside the UK [107]. It is worth mentioning that, in order to perform toxicological tests on SCP, a considerable amount of SCP must be produced. As an example, Imperial Chemical Industries (ICI) “Pruteen” factory in Billingham, England, utilized approximately 500 tons of Pruteen in feeding 200,000 animals (7 different species) at a cost of approximately $6 million to carry out toxicological studies on Pruteen [98]; Pruteen was derived from the methanol-assimilating bacterium *Methylocapsa methylotrophus*.

2.3. Effect of H₂S in Raw Biogas on SCP Production

Raw biogas usually contains 500-1,000 ppmv H₂S [77], sometimes as high as 10,000-26,000 ppmv depending on the feedstocks used for biogas production [108, 109], which can adversely affect biogas conversion to SCP. Tsapekos et al. [69] operated a lab-scale aerobic fermenter fed with either raw biogas or pure CH₄. The fermenter was enriched with a mixed methanotrophic culture grown in the liquid digestate from a municipal biowaste digester. They observed that biomass production using raw biogas (CH₄ 61%, CO₂ 39% and H₂S 913 ppmv) was 38% lower than that of obtained using pure CH₄. Xu et al. [77] studied SCP production from H₂S-rich synthetic biogas using acid-tolerant *Methylocapsa acidiphila* DSM 13967, belonging to type II *methanotrophs Alphaproteobacteria*, grown in NMS medium. The focus of their study was to investigate how H₂S would influence the SCP productivity. Their results demonstrated that the presence of H₂S in the biogas could significantly affect the biomass quality. Protein content in the biomass of *Methylocapsa acidiphila* grown on H₂S-free biogas sample was approximately 59% DCW. It decreased to about 40% and 28% when the biogas supplied with 165 ppmv and 725 ppmv H₂S, respectively. Based on the results of Tsapekos et al. [69], and Xu et al. [77], it is of high importance to desulfurize raw biogas prior to use for SCP production. More updated and comprehensive information on biogas desulfurization can be found in the studies of Khoshnevisan et al. [37], and Okoro and Sun [110].

2.4. Future Research Needs

Current developments and improvement of the biogas-based SCP production processes are noteworthy. However, despite large amount of research devoted to such processes, there remains two major aspects, given below, which need to be taken into account in future research.

1) In biogas-based SCP production processes, CO₂ is dissolved in the medium (and dissociated into bicarbonate), which results in lowering the medium pH into the acidic zone [70]. Hence, caustic addition would be required to neutralize the medium pH that leads to an increase in chemical costs. Therefore, exploring acid-tolerant methanotrophic strains for converting biogas to SCP should come into focus as a future research effort. This could make the SCP production process economically more attractive because pH neutralization could no longer be essential. In addition, acidic wastewater streams could be used as growth medium or nitrogen source without an external alkaline addition.

2) Utilization of genetically modified microorganisms (GMM) in SCP production from biogas has not been previously reported. The GMM technique could improve the safety of SCP products because the genes contributing to toxin production can be removed or inactivated. It could also enhance the quality of SCP products in terms of amino acids and vitamins. Therefore, future study might look into biogas conversion to SCP by means of GMMs, specifically when the SCP products are intended for food applications.

3. Biogas Bioconversion to Methanol

Methanol, known as methyl alcohol, is the simplest alcohol with chemical formula CH₃OH that is often abbreviated as MeOH. It has a wide range of applications in chemical industries. For instance, it is used in the synthesis of organic compounds such as acetic acid (HAc), dimethyl ether (DME), methyl tert-butyl ether (MTBE), mono-methylamines (MMA), dimethyl terephthalate (DMT), olefins, etc. [111-113]. In addition, MeOH is considered as a cleaner fuel with a lower risk of flammability and higher octane number (a quantitative measure of the knock resistance of a fuel) compared to gasoline [114]. Moreover, it is a theoretical carbon source for biological denitrification (DN) process [115], and hence, MeOH synthesis from biogas generated in anaerobic digesters of WRRFs can result in a reduction in operational cost of the wastewater treatment processes.

Today, the most developed technologies for the production of MeOH rely on the catalytic thermochemical reactions. However, such technologies incur a high operating cost because of the need for high pressure (up to 150 atm) and high temperature (200°C-1,000°C) [116]. A cost-effective alternative route to the thermochemical pathways for MeOH production is the biological processes. The bio-MeOH production process driven by the action of microorganisms can be operated at ambient conditions [117]. In this direction, the utilization of biogas as the feedstock draws a great attention. The biological production of biogas-based MeOH offers an environmental advantage because it can replace the fossil-based MeOH, and thus fight global warming. Besides, it requires much less energy.

3.1. Insight into the Mechanism

Biological conversion of biogas into MeOH is carried out by aerobic methanotrophs via sequential reactions (Fig. 2). The reactions are catalyzed by different enzymes, including methane mono-oxgenases (MMOs), methanol dehydrogenase (MDH), formaldehyde dehydrogenases (F₅₆DH), and formate dehydrogenases (F₅₇DH) [118, 119, 121].

There are two forms of MMO: i) particulate MMO (pMMO) that is a membrane-bound enzyme, and ii) soluble MMO (sMMO), which is a cytoplasmic enzyme. The expression of MMOs is largely dependent on copper concentration in the growth medium. pMMO contains copper active sites, and has higher affinity for CH₃OH compared to sMMO. The pMMO is active when copper concentration exceeds
one μmol per one gram dry weight of cells, whereas sMMO activity is only present under limited-copper conditions [122-124].

The primary reaction involved in the conversion of biogas to MeOH is CH$_4$ oxidation driven by the action of MMOs. The MMOs utilize reducing equivalents such as cytochrome complex (CytC) or NADH$_2$ (reduced form of NAD; nicotinamide adenine dinucleotide; H stands for hydrogen), depending on whether pMMO or sMMO is used to cleavage the O-O bonds in molecular oxygen (O$_2$) into monovalent oxygen atoms. One of the oxygen atoms is reduced to produce H$_2$O, and the other is incorporated into CH$_4$ leading to MeOH formation. The product MeOH is then oxidized to formaldehyde (CH$_2$O) by MDH, a pyrroloquinoline quinone (PQQ)-dependent enzyme. Formaldehyde is further oxidized to formate (CH$_2$O$_2$) and then to CO$_2$ through the action of F$_{ald}$DH and F$_{ate}$DH, respectively. The oxidation of MeOH to CO$_2$ generates reducing equivalents (six reducing equivalents per molecule of MeOH), which are utilized by MMOs to sustain CH$_4$ conversion to MeOH [118, 125, 126]. The CO$_2$ can be reduced to MeOH via the reverse oxidation of MeOH if sufficient amount of reducing equivalent is supplied [121].

The formaldehyde, formate and CO$_2$ can be assimilated into microbial biomass depending upon the phylogeny and physiology of methanotrophs. Group I methanotrophs (Gammaproteobacteria) employ ribulose monophosphate (RuMP) cycle to assimilate carbon, whereas Group II methanotrophs (Alphaproteobacteria) and Group III methanotrophs (Verrucomicrobia) utilize serine (Ser) and Calvin-Benson-Bassham (CBB) cycles, respectively [122, 123, 127]. The detailed information on RuMP, Ser and CBB cycles can be found in the studies of Fei et al. [118], and Khmelenina et al. [120].

3.2. The Use of MDH Activity Inhibitors

A major issue associated with biogas bioconversion to MeOH is further oxidation of MeOH, which leads to lower extracellular MeOH accumulation [128]. In order not to allow MeOH to be oxidized, MDH metabolic activity must be suppressed. However, this approach creates a problem because NADH needed to sustain MMOs activity is depleted and cannot be regenerated. In other words, CH$_4$ will no longer be oxidized. To overcome this limitation, external electron donors, generally formate, are required [126, 129, 130].

Kim et al. [115] have shown the feasibility of using NaCl as an MDH activity inhibitor for improving MeOH production from raw biogas (composed of 68.9% CH$_4$) by means of mixed methanotroph species grown in NMS medium (30°C, pH 6.8, incubation period 48 h; biogas-to-air ratio 3:4:1 v v$^{-1}$). Their results indicated that supplementation of the medium with 60 mM NaCl and 40 mM sodium formate yielded 6.4 mM MeOH, which was 83% higher than that of obtained from the control (without NaCl and sodium formate). In the study of Patel et al. [128], MeOH accumulation using methanotrophs Methyloferula stellata (DSM 22108) and Methyloannes methanica (DSM 25384) was found to be 0.09 mM and 0.13 mM, respectively, when 100 mM phosphate buffer was supplied as an individual MDH inhibitor. Using 100 mM phosphate buffer together with 50 mM MgCl$_2$ as a combined MDH inhibitor, MeOH production increased to 0.29 mM and 0.48 mM, respectively. The addition of 100 mM formate significantly improved MeOH production by 9.1- and 8.0-fold, to 2.64 mM and 3.86 mM, by Methyloferula stellata, and Methyloannes methanica, respectively. Note that CH$_4$ (30%) was utilized as a feed in this study under 30°C, pH 7.0, incubation period 24 h, and inoculum conc. 3.000 mg DCW L$^{-1}$.

3.3. Key Parameters Affecting MeOH Production

3.3.1. Temperature and pH

Biogas conversion to MeOH, driven by methanotrophs, is a temperature and pH dependent process because the physiological behavior and growth of methanotrophs are greatly influenced by the medium temperature and pH [118, 131, 132]. Methanotrophic species, e.g., Methylocales sp. 14B [133], Methylosinus sporium DSMZ 17706 [134], Methyloannes methanica DSM 25384 and Methylocella tundra DSMZ 15673 [128, 135], Methylocystis bryophila DSM 21852 [136], Methylomonas album ATCC 33003 and Methyloferula
stellite DSM 22108 [137], Methylosinus trichosporium IMV 3011 [119], which have been used for MeOH production, are mesophilic with an optimum temperature range of 30-37°C.

Zhang et al. [125] reported that no significant growth of Methylocaldum gracile SAD2 was observed at temperatures 20°C, 25°C or 45°C. However, Su et al. [138], for the first time, reported MeOH production at relatively higher temperatures using a consortium of methanotrophs isolated from corn stover-based AD digestate. The consortium (dominated by 87.2% Methylocaldum and 7.0% Agrobacterium), grew effectively on raw biogas (72% CH₄, 26% CO₂, 1.7% N₂, and 0.3% O₂) within a temperature range of 30-55°C. A maximum MeOH production of 10.3 mM was obtained at 47°C (pH 6.8, biogas-to-air ratio 1:1 v v⁻¹, inoculum conc. 2,300 mg DCW L⁻¹, incubation period 144h). They also evaluated the influence of pH on CH₄ oxidation by the consortium at 47°C. According to their results, at pH levels from 5.5 to 7.5, the rate of CH₄ oxidation was found to be high and consistent (91%). The consortium grew more stably at pH value of 6.8 with a maximum cell yield of 0.31 g cells/g CH₄. When the pH was 5.0, only 7% of the CH₄ content was oxidized. Yoo et al. [139] reported that the oxidation of CH₄ using Methylosinus sporium was about 26% at pH value of 5.0, which was 48% lower than that of obtained at pH value of 7.0 (35°C, biogas-to-air ratio 2.3 v v⁻¹ where biogas composed of 50% CH₄ and 50% CO₂). These imply that proton activity in the medium has significant impact to MMOs activity of the consortium, which should be kept in near neutral environment.

### 3.3.2. Biogas-to-air ratio

Before delivering biogas to methanotrophs, it should be mixed with air (or pure O₂) because methanotrophs need molecular oxygen to oxidize CH₄ [67]. Patel et al. [117] studied MeOH production using Methylosinus sporium DSMZ 17706 grown in NMS medium under an atmospheric pressure of raw biogas (62% CH₄), which was diluted with air to maintain CH₄ concentration in the range of 10-50% v v⁻¹. According to their findings, CH₄ concentration of 20% (corresponded to a biogas-to-air ratio of 2:1 v v⁻¹) resulted in the highest MeOH production of 4.63 mM (30°C, pH 7.0, inoculum conc. 3,000 mg DCW L⁻¹, incubation period 144h). Su et al. [138] conducted a series of experiments to optimize biogas-to-air ratio for MeOH production using a mixed culture of methanotrophs dominated by Methylocaldum (87.2%). Different biogas-to-air ratios (1:6, 1:4, 1:2, 1:1, and 1:2 v v⁻¹) were tested with raw biogas (72.0% CH₄, 26.0% CO₂, 1.7% N₂, and 0.3% O₂) originated from a food waste digester. They found that the biogas-to-air ratio of 1:1 v v⁻¹ resulted in the highest level of CH₄ to MeOH conversion (0.47 mol mol⁻¹) at a temperature and pH of 47°C and 6.8, respectively (after 168h incubation). In a recent report, Henard et al. [140] determined the growth of three methanotrophs strains Methylimonium alcaliphilum 20ZR, Methylosinus trichosporium OB3b, and Methylococcus capsulatus Bath with a simulated biogas (60% CH₄, 40% CO₂) at different biogas-to-air ratios (1:17, 1:7, 1:3, and 1:1). The growth of all strains positively correlated to the biogas-to-air ratio with an optimal growth at biogas-to-air ratio of 1:3-1:1 v v⁻¹. This could be due to higher CH₄ concentration in the headspace, leading to an increase in CH₄ availability in the culture medium.

### 3.3.3. H₂S Presence in biogas

One of the main challenges associated with the processes dealing with MeOH production from biogas is the inhibition of methanotrophs by H₂S [117, 126]. H₂S is inevitably present in biogas as mentioned earlier. In the study of Eun-Hee et al. [141], CH₄ oxidation capacity of methanotroph Methylomycistis sp. was inhibited approximately 48% in the presence of 200 ppmv H₂S. Caceres et al. [142] showed that the growth of Methylobacterium album (ATCC 33003) and Methylomycistis sp. (ATCC 49242) grown on a mixture of CH₄ and air, in the presence of 500 ppmv H₂S, was inhibited by 39% and 47%, respectively. It appears from these results that the need to desulfurize biogas is definitely required prior to its utilization for cell cultivation and MeOH production. To keep costs low, biogas desulfurization should be avoided. Thus, robust methanotrophic strains that can tolerate relatively high concentration of H₂S are desirable. Zhang et al. [125], for the first time, isolated a high H₂S-tolerant methanotrophic strain (Methylocaldum gracile SAD2) from digestate of a mesophilic digester of expired dog food to evaluate its performance in producing MeOH using raw biogas. The strain SAD2 had the capability of producing 8.6 mM MeOH when the raw biogas-to-air mixture (1:2 v v⁻¹) contained about 520 ppmv H₂S under 37°C, pH 6.8-7.5, and 48h incubation in NMS medium. In addition, the growth of this strain was inhibited by only 17%. These results imply that a more H₂S tolerant strain (other than SAD2) could be identified that would become effective for MeOH production from H₂S-rich biogas without requiring biogas desulfurization.

### 3.4. Enhancement of MeOH Production Using Cell Immobilization Technique

Cell immobilization can be performed by different methods, including i) adsorption that is driven by electrostatic forces on a solid support, ii) covalent linkage between the support and the cell membrane, and iii) encapsulation where the cells are entrapped inside a porous material. The main advantage of immobilized cell systems is that the loss of cells to the surrounding medium and ultimately off the reactor can be avoided or minimized, while diffusing of nutrient and metabolites are allowed into the densely populated immobilized matrix [143, 144].

Patel et al. [117] investigated MeOH production from raw biogas composed of 20% CH₄ (diluted with air) by the methanotrophs Methylosinus sporium DSMZ 17706 immobilized on different types of supports such as Amberlite and Duolite A7 ion-exchange resins, and Chitosan. Based on their findings, MeOH productivity was significantly improved by the immobilized Methylosinus sporium compared to their suspended culture. After incubation for 120 h at 30°C and a pH value of 7.0, a maximum yield of 5.22 mM MeOH was observed, which was approximately 72% higher than that obtained from the free suspended culture. Patel et al. [136] later evaluated MeOH production using covalently immobilized Methylomycistis bryophila (DSM 21852) with a synthetic gas feed (30% CH₄, 15% CO₂, and 55% air) under repeated batch condition (eight reuse cycles of 24 h incubations). Their results demonstrated that the immobilized cells yielded a remarkably higher cumulative MeOH concentration (25.8 mM) than that of the free cells (15.50 mM). Table 3 summarizes the results of recent studies directed
towards the use of immobilized cell techniques for MeOH production from simulated or raw biogas by various strains of methanotrophs. These studies clearly suggest that cell immobilized processes are superior to the suspended process for biogas conversion to MeOH, noting that the efficiency can be further optimized if the operating parameters and environmental conditions are properly controlled.

### 4. Conclusions

Overview of the current state of bioconversion studies of biogas to single cell protein (SCP) and methanol (MeOH) are summarized. These are by far the most feasible lucrative means to up-value the biogas which is today in some areas are in excess or of low value. Biogas can be converted to SCP by methanotrophic bacteria alone, or in combination with autotrophic hydrogen oxidizing bacteria (HOB) or algae in a single-stage process. Among which, the process driven by the algae-methanotroph coculture is more economically viable. The product from algal-bacterial SCP is rich in protein, up to 60\% on dry basis, and has a great potential as a substitute for the costly traditional protein sources for animal and human. Another approach to valorize biogas is bioconversion to MeOH. It relies on the activity of methane monooxygenase (MMO) in methanotrophs. Most of the methanotrophic species used are mesophilic with optimum growth at neutral pH. To obtain MeOH from biogas by methanotrophs, it is necessary to inhibit methanol dehydrogenase (MDH), and supply an external electron donor, mainly formate, to sustain MMO metabolic activity. The bioconversion rate of biogas to MeOH is primarily affected by types of inhibitors, biogas-to-air ratio, and H2S in biogas. Cell immobilization technique and H2S tolerant strains are of major research interest in improving MeOH production efficiency.

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**Table 3. Results of Recent Studies Related to the Use of Immobilized Cell Techniques for MeOH Production from Simulated/raw Biogas by Various Strains of Methanotrophs**

| Strains | Feed (% v:v⁻¹) | Technique | Support | Incubation period (h) | pH/T (°C) | MeOH prod. (mM) | Reference |
|---------|----------------|-----------|---------|-----------------------|-----------|-----------------|-----------|
| M. album ATCC 33003 | 30% CH₄, 15% CO₂ | Free cells | n/a | 72 | 7.0/30 | 3.8 | [137] |
| Encapsulation | Alginate | 4.1 |
| | PVA | 4.6 |
| | Covalent | cmChitosan | 5.0 |
| Free cell | n/a | 7.0/30 | 3.9 | [145] |
| Covalent | cmChitosan | 24 | 12.5 | |
| Free cell | n/a | 72 | 4.4 | |
| Covalent | cmChitosan | 6.7 |
| (30% CH₄, 16% CO₂, 46 ppm H₂S) | Free cell | n/a | 24 | 19.5 | |
| Covalent | cmChitosan | 6.7 |
| M. stellate DSM 22108 | 30% CH₄, 15% CO₂ | Free cells | n/a | 72 | 4.9 | [137] |
| Encapsulation | Alginate | 5.0 |
| | PVA | 5.0 |
| | Covalent | Chitosan | 5.3 |
| M. bryophila DSM 21852 | | Free cells | n/a | 72 | 4.9 | [128] |
| Encapsulation | Alginate | 5.0 |
| | Silica gel | 5.9 |
| Free cells | n/a | 24 | 19.5 | |
| Encapsulation | Alginate | 25.7 | |
| | Silica gel | 32.0 | |
| (32% CH₄, 18% CO₂, 650 ppm H₂S) | Free cells | n/a | 48 | 4.6 | |
| Encapsulation | Alginate | 4.7 |
| | Silica gel | 5.7 |
| M. sporium DSMZ 17706 | 30% CH₄, 15% CO₂, 5% H₂ | Free cells | n/a | 6.8/30 | 4.9 | [146] |
| Covalent | Chitosan | 6.1 |

**Notes:** a\(^3\)000 mg DCW L⁻¹; b\(^3\)o-culture of M. tundrae DSMZ 15673 and M.methanica DSM 25384; c\(^3\)rice straw-based biogas (65.4% CH₄, 34.6% CO₂ and 100 ppm H₂S), which was diluted in a 1.18:1.00 (v:v⁻¹) ratio with air; d\(^3\)municipal waste-based biogas (63.4% CH₄, 35.6% CO₂ and 1,300 ppm H₂S), which was diluted in a 1:1 (v:v⁻¹) ratio with air; e\(^3\)chemically modified Chitosan with APTES followed by GLA; f\(^3\)repeated batch test (8 cycles of reuse; each cycle was incubated for 24 h); g\(^3\)cumulative MeOH production (mM).
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Author Contributions

R.S. (Ph.D. Independent Researcher) wrote the original draft, and did visualization. S.C. (Professor) reviewed and edited the article.

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