Study on Methanation of CO_2 and CO from Blast Furnace Gas by Anaerobic Fermentation under Mesophilic conditions

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Abstract

Greenhouse gas emissions (GHG) and energy crisis forced humans to research for alternative energy development. Although sustainable energy has taken great leaps forward, many alternative energy sources have not been marketed due to high costs, inefficiencies, technical difficulties, etc. Blast furnace gas (BFG), as a kind of syngas, the main components are CO and CO_2 which can be used as substrates by microorganisms and to be converted into biofuels. Thus, BFG as a by-product of steelworks, could be used in situ for energy generation. It is currently being used primarily as fuel for steam boilers, dynamos, however, high concentrations of CO (toxic) and CO_2 (greenhouse gases) make it low calorific value.

Several methods are available for treating BFG from steel mills including absorption, cryogenic separation, membrane separation, chemical transformation and biological transformation. Compared with their advantages and disadvantages, biological transformation from BFG to CH_4 has been recognized as a favorable technology with the advantages of economic, environmental friendly and convenient. Furthermore, CH_4 produced from BFG can be further used as heating fuel or power generation for steel plant operations, which fulfills the sustainable development. Therefore, the aim of this research is to study biomethanation of CO_2 and CO in BFG to CH_4 for eliminating GHG and obtaining higher quality fuel. Two kinds of inoculums were used in this study for BFG conversion to CH_4 and the comprehensive comparison was carried out.

Firstly, methanogens acclimated by H_2/CO_2 conditions were developed to explore the feasibility of converting CO from BFG to methane. Even though a CH_4 production rate reached to 13.2 L/L_{liquid} d by acclimated methanogens under H_2/CO_2 conditions in a semi-continuous reactor when trace metal elements were added. When the gas substrate was CO, the inhibition of methane production by CO partial pressure (P_{CO}) started at 0.1 atm. While, the CO partial pressure in BFG ranged from 0.2 - 0.35 atm, in order to sustain $P_{CO} \leq$ 0.1 atm, BFG needed to be diluted with nitrogen requiring high cost. Therefore, the enriched methanogens were not suitable to use CO in industry exhaust (BFG) as sole energy and

carbon source for CH_4 synthesis.

Secondly, anaerobic granular sludge (AGS) was used to convert CO_2 and CO from blast furnace gas to methane via the addition of exogenous hydrogen in mixed culture. The hydrogenotrophic and acetoclastic methanogenic potentials (18.31 ± 1.2 mmol/g VSS d and 6.58 ± 0.38 mmol/g VSS d) were obvious. While, AGS also presented a promising carboxydotrophic methanogenic potential (1.19 ± 0.03 mmol/g VSS d and 5.56 ± 0.26 mmol/g VSS d) under CO/N_2 and $CO/CO_2/N_2$ conditions. When testing the effect of different CO partial pressure on methane production, the inhibition of methane production by P_{CO} started at 0.4 atm. $P_{CO} \geq 0.4$ atm presented an inhibitory effect on methane production by anaerobic granular sludge during the first 96h. After 96 hours' operation, due to that CO was gradually consumed by the microorganisms in the reactors, the partial CO pressure in the reactor gradually decreased and the inhibition was relieved to some extent. Therefore, the cumulative methane production under P_{CO} of 0.4 atm exceeded those from 0.2 atm and 0.1 atm reactors. In the presence of BES, the intermediate metabolites from CO to methane included acetate, propionate, H_2. And they accumulated at higher CO concentrations. It illustrated that CO was converted to methane via acetogenic CO-oxidizing pathway, however, the H_2/CO_2 pathway may be co-existing. After the introduction of H_2 and BFG to the bottles, although hydrogen partial pressure up to 1.54 atm led to maximum CH_4 yield (5.57 mmol/ g VSS,), only 4.70% higher than that of 0.88 atm condition, it was unsuitable for the stability of the whole system due to the accumulation of VFAs. The optimum hydrogen partial pressure on CH_4 production (5.32 mmol/ g VSS) was observed at 0.88 atm.

This thesis would provide useful information for applying acclimated methanogens into BFG treatment. Also, the results indicate a bright future for adopting AGS into converting BFG to CH_4, which would make this study more meaningful.

Keywords: Carbon monoxide; Biomethanation; Blast furnace gas; Exogenous hydrogen; Fermentation

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Chapter 1 Introduction

Industry, particularly iron and steel industry, occupied for approximatively 1/3 of global final energy use and around 40% of total energy-related CO_2 emissions [1]. The development of alternative sustainable energy sources and fuels has become the current attractive research point as the depletion of unsustainable fossil resources has been foreseen, and the process presented the negative impacts on environment [2]. Synthesis gas (syngas), formed by the process of gasification or reforming of natural gas, biomass, tar, digested sludge or coal, could be used as critical intermediates during energy sources or chemical fuels production [3]. The composition of syngas mainly consisted of nitrogen (N_2), carbon monoxide (CO), carbon dioxide (CO_2) and hydrogen (H_2) [4]. Here, waste-off gas and biomass-derived producer gas belong to syngas. Waste-off gas is considered to be a cheap gas with a high content of carbon sources and desired to be treated to prevent greenhouse gas emissions and energy loss.

In this chapter, blast furnace gas and treatment methods, biological fermentation of syngas to methane and methanogenic metabolism pathways were introduced as follows.

1.1 Blast furnace gas and treatment methods

During the process of steel production, four steps are involved: coking, iron making, steel making, and metal working [5]. Gas emissions are from three streams which formed during processes of steel making in a steel mill: coke oven gas (COG), blast furnace gas (BFG), and basic oxygen furnace gas (BOF). The compositions are summarized in Table 1-1. Among these three streams, BFG is responsible for about 69% of the emitted CO_2. The CO_2 and CO separation technology to study alternative uses for BFG is a method to reduce its emissions. The separation technologies usually include chemical absorption and physical absorption such as pressure swing adsorption (PSA), selective adsorbents for CO_2 and/ or CO, cryogenic separation and membrane separation. The main differences of separation technologies typically depend on the separation agent and the property of its interactions with the gas kinds to be separated.

For chemical adsorption, Farla et al. [6] studied a CO_2 capture system which based on methyldiethanolamine (MDEA) as chemical sorbent. It is suitable for gas streams containing low CO_2 concentrations (≤ partial pressure 5 bar). As referred above, the concentrations of CO_2 and CO in BFG are about 20 - 35% (Table 1-1), this could cause CO to be converted into CO_2 by using shift reaction, resulting in a doubling concentration of CO_2.

Compared with chemical adsorption, physical adsorption is more cost effective. A steel corporation from Japan developed PSA technology applying to the physical separation and recovery of CO_2 and CO from BFG [7]. The system includes two parts: CO_2 recovery from the pretreated BFG and the remained CO recovery. Zeolum F-9 was used to be as the adsorbent for CO_2 adsorption and desorption, higher recovery ratio was obtained at higher adsorption pressure. At last, 6.3 tons of CO_2 was recovered with the following conditions: cycle time of 225 seconds, initial CO_2 concentration 33%.

The cryogenic separation technology needs more power than the physical absorption process. Cryogenic separation is the separation of material at a temperature that is below the freezing point of the material, which is well known in industry. It is relatively easy to compress gaseous CO_2 from BFG to liquefied CO_2 by supercritical pressure, and the liquefied CO_2 can be used as a cooling agent due to low temperature. Lampert et al. [8] studied the possibility of integration of the Corex process, blast furnace, CO_2 removal installation and metallurgical combined heat and power (CHP) plant. As the Corex emission gas (after CO_2 removal) was proposed to be used as a reducing gas injected to the the blast-furnace process. In this system, physical absorption and cryogenic separation technology together applied to CO_2 separation.

The advantages of membrane seperation [9] are obvious, like low cost, flexible installation and application, good space efficiency, small footprint, minimal associated hardware, low environmental impact, reliability, and the ease of developments. The disadvantages include high feed cleanliness (particulates and in most cases entrained liquids must be removed) and high energy requirements for gas compression. Lie et al. [10] evaluated 3 kinds of membranes for CO_2 capture in the BFG: semi-commercial adsorption selective

carbon membranes, in-house tailored carbon molecular sieving membranes, and fixed site carrier (FSC) membranes with amine groups in the polymer backbone for active transport of CO_2. The FSC membrane presented the most excellent selectivity for CO_2 over the other co-existing gases (N_2, CO and H_2), also the highest CO_2 capture ability in the three types. Even though the good property in single gas experiment results, the treatment of simulated BFG with an FSC membrane showed a noticeable deterioration in selectivity. Fortunately, the problem was found and solved. Through economic evaluation, this technology is notable for the low energy cost.

For treatment of BFG, another promising method is to produce valuable products from CO_2 and CO, like industrial intermediates and fuels either by chemical or biological transformations.

Chemical transformation relies on passing syngas over metal catalysts and requires high temperatures and pressures, strict gas ratio and high energy consumption. There is no doubt that chemical conversion has high production efficiency. However, the catalysts used in the conversion processes are easy to be poisoned by low concentrations of co-existing contaminants such as BTEX (aromatics benzene, toluene, ethyl benzene, and xylene) and sulfur [11]. Compared to the chemical transformation process, biological transformations method requires less pretreatment and presents more flexibility regarding to gas composition. In addition, biocatalysis can tolerate the specific contaminants. A certain concentration of sulfur (hydrogen sulfide and carbonyl sulfide) is actually as a nutrient for the microbes in the fermentation process. Fermentation of syngas to biofuels including liquid products and gas products with biological method has been studied intensively in the past decades [12, 13]. Liquid products include acetate [14], ethanol [15] and 2,3-butanediol [16], gas products include methane [17] and hydrogen [18].

As mentioned above, each method has its own advantages and disadvantages. The most concerned topic is to find the proper material for each method. In this study, methane production from BFG by biological method would be discussed in detail.

1.2 Biological fermentation of syngas to methane

1.2.1 Previous studies

Methane production from syngas by pure cultures has been investigated elsewhere [19,20]. Anaerobic conversion of CO and H_2 /CO_2 respectively to CH_4 by mixed cultures is feasible.

Sipma et al. [21] investigated seven kinds of anaerobic sludges from wastewater treatment reactors to screen for their ability to convert CO at 30 ℃ and 55 ℃. The tested granular sludges were from paper mills, distillery, brewery, reactor treating kraft-pulping wastewater and anaerobic gas-lift reactor. At 30 ℃, the products included CH_4 and/or acetate. Inhibition experiments (using 2-bromoethanesulfonic acid and vancomycine) showed that the precursor was acetate from CO to methane, but not by H_2. At 55 ℃, sludges originally cultivated at 30 - 35 ℃ or 55 ℃ converted CO rapidly into hydrogen or methane. Inhibition experiments showed that hydrogen was the intermediate from CO to methane.

Some researchers combined anaerobic digestion with coke oven gas methanation [22], anaerobic fermentation could be selected to be a universal, technologically simple and effective method of producing biofuels from organic wastes [23]. Biogas mainly contains CH_4 (40 - 75%) and CO_2 (25 - 60%). The so-called in-situ biogas upgrading is to import the exogenous hydrogen directly into the real biogas fermentation system, and to convert the CO_2 in the biogas into CH_4 in situ to purify and upgrade the biogas. As an anaerobic reactor treating domestic and industrial organic wastes usually contains a wide variety of microorganisms, bioconversion of H_2 and CO simultaneously is possible. Here, the simulated coke oven gas including 92% H_2 and 8% CO was used to be as the exogenous hydrogen for the in-situ biogas upgrading. The maximum methane content in the reactor could reach to 98 - 99% and CO and H_2 are almost completely consumed during steady period. The investigation of microbial community structures showed the addition of coke oven gas in the reactor affected the dominance.

Guiot et al. [24] evaluated whether carboxydotrophic methanogenic potential exists in the industrial granular sludge and could be enriched. The results showed that no matter under mesophilic or thermophilic conditions, methanogenic potential from CO was existing even though the inoculum was not specially acclimated. Compared to mesophilic operation, thermophilic conditions provide the anaerobic granules with a significantly faster CO-bioconversion potential

Luo et al. [25] investigated ex-situ biogas upgrading by addition of hydrogen in an anaerobic reactor containing enriched hydrogenotrophic methanogenic culture. After upgrading, CH_4 content in the biogas was higher than 90%. The process completed increase of heating value and made the quality of biogas close to natural gas. And after enrichment under thermophilic conditions, CO_2 and H_2 bioconversion rate was more than 60% higher than that under mesophilic temperature. As revealed by PCR–DGGE, no matter under which temperature, the dominant species (the order *methanobacteriales*) was hydrogenotrophic methanogenesis.

Luo et al. [26] also researched the process of anaerobic digestion for simultaneous sewage sludge treatment and CO biomethanation. Batch experiments showed that under thermophilic conditions, CO partial pressure at 0.25 - 1 atm was inhibitory to methanogens. During anaerobic digestion of sewage sludge supplemented with CO, CO partial pressure up to 1 atm did not inhibit the hydrolysis and acidification process but strongly inhibited methanation process.

Alitalo et al. [27] designed two solid state bioreactors to produce CH_4 through the reaction of CO_2 and H_2 by recirculation system. Cheap vermiculite and granule perlite were used to obtain a structure and leave pores to provide microorganisms to habitat. Wood ash and some nutrient element were added to the reactor. The pH of the system was slightly acid because CO_2 dissolved of water and formed HCO_3 - buffer systems. The maximal methane productivity was achieved of 6.35 L/ $L_{reactor}$ d. The low reactor power, simple reactor design and waste material as nutrients for methanogens made this study significant and interesting.

1.2.2 Species of microorganisms and routes in syngas biomethanation

Methanogens are strictly anaerobic prokaryotes and are the only known organisms to metabolize methane, phylogenetically belonging to Euryarchaeota. Up to date, the known methanogens are divided into 7 orders: Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales, Methanopyrales, Methanocellales and Methanoplasmatales. Most of methanogens are able to utilize H_2/CO_2 to produce methane. In the past several decades, some known anaerobic microorganisms species have been able to grow with CO as substrates (Table. 1-2). Methane is produced by methanogenic bacteria from either acetate or H_2/CO_2.

Acetate may be produced by the following reactions:

$$4CO + 2H_2O \rightarrow CH_3COOH + 2CO_2,\ \Delta G^0 = -176\ kJ/mol \quad (1\text{-}1)$$

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + H_2O,\ \Delta G^0 = -104\ kJ/mol \quad (1\text{-}2)$$

They could be completed by several anaerobic bacteria, including *Peptostreptococcus productus, Acetobacterium woodii and Eubacterium limosum* [28]. Among these bacteria, *P. productus* strongly adapts to CO and utilizes CO rapidly doubling in 2 hours. Also, H_2 and CO_2 are known to be utilized by *Acetobacterium woodii* to produce acetate [29–31], which anaerobically oxidises H_2 and reduces CO_2.

Hydrogen may be produced by the water gas shift reaction:

$$CO + H_2O \rightarrow H_2 + CO_2,\ \Delta G^0 = -20\ kJ/mol \quad (1\text{-}3)$$

Rhodopseudomonas gelatinosa and *Rhodospirillum rubrum* [28], are able to perform the reaction to convert CO to hydrogen. As anaerobic bacteria, *R. R. gelatinosa* could exist via CO as the only carbon and energy source by the addition of trypticase simultaneously under shading conditions. While, different from *R. R. gelatinosa*, the growth *for R. rubrum* needs tungsten light and other carbon sources (sugars, yeast extract etc.) besides CO. According reports, *R. rubrum* could not only grow and utilize CO faster than *R. R. gelatinosa* but also be easier to be cultivated since it tolerates slight oxygen, sulfur compounds and CO partial pressures up to 2.0 atm.

CO_2 and H_2 may be used to produce methane by the reaction:

$CO_2 + H_2 \rightarrow CH_4 + H_2O$, $\Delta G^0 = -130.7$ kJ/mol (1-4)

Almost all methanogens, such as *Methanospirillum hungatii*, *Methanobacterium formicicum*, *Methanobrevibacter smithii* and *Methanosarcina barkeri* could finished the reaction. Methane may also be produced from acetate as following reaction:

$CH_3COOH \rightarrow CH_4 + CO_2$, $\Delta G^0 = -31$ kJ/mol (1-5)

The related methanogens include *Methanosarcina barkeri*, as well as *Methanothrix soehngenii* and so on.

From the above summary, we can realize that two - step will be contained during the production of methane from syngas: CO, H_2 and CO_2 formation of the precursors (H_2 or acetate) by bacteria; Biomethanation of the precursors by methanogens. Usually, methane production may be operated in separate reactors or in a co-culture reactor depending on the precursors [4]. It has been reported that the when the concentration of acetate is higher than 12 g/L, it would present inhibitory to methanogens. The inhibition usually results in low productivities and a large second stage reactor. For syngas, it is possible that R. rubrum firstly converts CO to hydrogen and then the whole hydrogen and CO_2 may be converted by M. formicicum to methane. The premise is to find the best co-cultivation conditions.

1.2.3 Methanogenic metabolism pathways

Methanogens are widely distributed, mainly in anaerobic ecosystems, such as (a) Lakes, marsh wetlands, paddy fields, sludge, etc.; (b) In vivo, such as rumen of ruminants and intestinal flora of humans, insects, etc. (c) Anaerobic granules in the soil, such as soil pellets in forests; (d) Artificial biodegradation facilities such as biogas digesters and bioreactors; (e) Some extreme environments such as hot springs, sulfur-containing hydrothermal ports, etc [32, 33]. Although the kinds of methanogens are diverse, only a limited number of substrates (methyl-group containing compounds, acetate, H_2/CO_2, CO) can be utilized to form methane. The methanogenic process of methanogens includes a series of steps involving a few enzymes (Fig 1-1), eventually reducing the methyl groups in the CO_2 or C1 compounds to CH_4.

The biogenic methanogenic process requires the participation of various enzymes and

coenzymes. These coenzymes can be divided into two categories according to their functions. One is carriers of C1, mainly including methanofuran (MF), tetrahydromethanopterin (H_4MPT), tetrahydrosarcinapterin (H_4SPT), and coenzymeM (HS-CoM). The others are electron carriers that mainly include ferredoxin (Fd), coenzyme B (HS-CoB), coenzyme F_{430}, cytochrome, FAD, methophenazine (MP), and coenzyme F_{420} [34–36].

Reaction (12) and (13) in Fig. 1-1 are common steps in the biomethane production process. The reaction (12) is the final step in the biological methanogenesis process which is catalyzed by the nickel-containing methyl-CoM reductase (Mcr), and the CH_3-S-CoM is reduced by the direct electron donor HS-CoB to generate methane and CoB-SS-CoM. Reaction (13) is also required for all biogenic methane production. CoB-S-S-CoM can be considered as the terminal electron acceptor of the methanogenesis process. It is reduced by heterogeneous disulphide reductase (Hdr) and re-releases HS-CoB and HS-CoM. The reaction equation is CoB-S-S-CoM + $2e^-$ + $2H^+$ →CoB-SH + CoM-SH [37].

In the view of methanogenic substrates, the methanogenic metabolism includes CO_2-reducing pathway, aceticlastic pathway and methylotrophic pathway.

(1) CO_2-reducing pathways

The CO_2-reducing pathway is widespread in nature. Except for a few methanogens, most methanogens can use this pathway to produce methane [35]. In the CO_2-reducing pathway, most of the methanogens use H_2 as an electron donor and transfer electrons to Fd_{ox} to reduce CO_2 under the action of hydrogenases. Therefore, these microorganisms are also called hydrogenotrophic methanogens. There are also a few methanogens that use CO as an electron donor. In this process, 3CO is first oxidized to $3CO_2$ to generate electrons, and CO_2 is reduced to methane [38]. While, methanogenic growth on CO as sole substrate seems not to be more efficient than H_2/CO_2 conditions. Generally, CO metabolizes under anaerobic conditions through carbon monoxide dehydrogenase (Codh). The structures of Codh isolated from three different carboxydotrophic organisms include iron-sulfur center [39] and nickel as a cofactor. The following is a detailed introduction to the biochemical process of hydrogenotrophic methanogens.

In Fig. 1-1, reactions (1) to (5) are processes related to CO_2 reduced by H_2. Reactions (1) to (4) are catalyzed by enzyme complexes located in the cytoplasm; Reaction (5) is catalyzed by a protein complex located on the cell membrane. For reaction (1), formaldehyde methanofuran dehydrogenase (Fdh) uses reduced form of ferredoxin (Fd_{red}) as an electron donor, and reduced CO_2 to CHO-MF and oxidized form of ferredoxin (Fd_{ox}), CHO-MF is formed by a formyl group covalently linked to amino group of methanofuran (MF) [40], Fd_{ox} then used H_2 as an electron donor to produce Fd_{red}, this process requires energy consumption or reverse electron transfer. Next, formyl on CHO-MF is transferred to the N^5 group of H_4MPT to form CHO-H_4MPT (reaction (2)). Subsequent the reaction (3) is catalyzed by formyl tetrahydromethane peptidyl cyclase and formyltetrahydromethane dehydrogenase. Reaction (4) was accomplished by the catalytic conversion of formyltetrahydromopterate reductase with the coenzyme $F_{420}H_2$ as an electron donor. Finally, in reaction (5), HS-CoM methyltransferase (Mtr) acts as a catalyst to transfer the methyl group to the thiol group of HS-CoM to form CH_3-S-CoM.

(2) Aceticlastic pathway

About two-thirds of the biogenic methane production in the nature comes from the aceticlastic pathway, whereas only *Methanosarcina* and *Methanosaeta* which has different mechanisms can use the aceticlastic pathway to produce methane [41]. *Methanosarcina* utilizes 1 molecule of ATP to catalyze the conversion of acetic acid to acetyl-CoA through acetate kinase (catalytic reaction (7)) and phosphotransacetylation (catalytic reaction (8)); *Methanosaeta* uses acetyl-CoA synthetase to catalyze the conversion of acetic acid to acetyl-CoA using 2 molecules of ATP [42]. Acetyl-CoA is then catalyzed by a carbon monoxide dehydrogenase/acetyl-CoA synthetase complex (Codh/Acs) with Fd_{ox} as an electron acceptor (reaction 9) to produce CO_2 and CH_3-H_4SPT, during this process excess CO_2 in the cytoplasm can be removed, increasing the efficiency of methanogenesis. Subsequently, CH_3-S-CoM is produced in reaction (11), a process similar to reaction (5), capable of producing a sodium ion gradient for synthesizing ATP.

(3) Methylotrophic pathway

In nature, only *Methanosphaera* in the Methanococcales and Methanosarcinales can use the methylotrophic pathway to produce methane. Methyl compounds are firstly treated with HS-CoM as electron donor under the action of a special coenzyme M methyltransferase system (Mt), and the methyl group is transferred to the sulfhydryl group of HS-CoM to generate CH_3-CoM (reaction (6). Subsequently, in the presence of H_2, CH_3-CoM uses H_2 as an electron donor to generate methane through reactions (12) and (13); in the absence of H_2, a portion of CH_3-CoM is oxidized to produce CO_2 through reaction (4) to reverse reaction of reaction, $F_{420}H_2$ and H_2, and the other part of CH_3-CoM produces CH_4 using $F_{420}H_2$ and H_2 as electron donors through reaction (12) and (13).

1.3 Inoculums

1.3.1 Acclimated methanogens

Alves et.al. [43] studied the effect of long-term exposure of a thermophilic anaerobic sludge to syngas and CO over 1 year. They successively transferred with syngas (16 transfers) or CO (9 transfers) from anaerobic sludge. The microbial diversity of the sludge rapid decreased after incubation with syngas and CO. CO seriously affected methanogenic activity of the sludge. Then methane production stopped when the anaerobic sludge was subcultured with CO alone. Also methane production ceased after 4 subsequent transfers under syngas condition for the reason that methanogens can not tolerate CO [20] and acetate was the main product from CO. It seems that acetogenic and hydrogenogenic bacteria could be more tolerant to CO.

As referred in section 1.2, methane may be produced by most methanogenic bacteria from H_2/CO_2. While, only a few species of methanogens can use CO as a substrate and energy source. Many studies were reported that methane was efficiently produced by methanogens enriched with H_2/CO_2 [44, 45], however, there are few reports of further use such methanogens to convert CO to methane.

1.3.2 Anaerobic granular sludge

Anaerobic granular sludge(AGS) was first reported in the upflow anaerobic sludge blanket (UASB) reactor which was developed in the late 1970s. AGS in UASB or EGSB (expanded granule sludge blanket) reactors presents excellent performance in treating high-strength organic wastewaters, requires low energy consumption and can produce valuable byproduct (biogas). For the structure, acidifying bacteria and hydrolytic bacteria growing on lactate or propionate are dominant species in the outer layer of AGS, while, in the inner layer archaea like methanogens are predominant [46, 47]. Extracellular polymer substances (EPS) consisting of polysaccharides, proteins, lipids, phenols, and nucleic acids play very important roles in forming and maintaining anaerobic granules. The process of anaerobic sludge granulation is complex and even though some hypotheses have been proposed, the process is still not explored very clearly [48, 49]. It is efficiently applied to treat brewery, beverage, distillery, food leachate, dairy, textile, and paper wastewaters [50].

It is well known that CO is toxic to microorganisms, the special structure of AGS may make it resistant to CO toxicity [24,51].

1.4 Objective and structure of the dissertation

The former part of introduction has concluded that sustainable production of chemicals and fuels have been one of the greatest challenges for society in the present, methane is a very promising alternative energy source. Microbes are able to grow on C1 compounds (CO or CO_2) derived from syngas including waste-off gas or gasification of biomass. However, fermentation of syngas to methane still involves practical challenges, such as the toxicity of CO on methanogens, the low methane production efficiency, etc. In this study, BFG as industrial waste-off gas was used to be converted to methane. The specific objectives are listed as follows:

The whole research aimed at biomathanation of CO and CO_2 from BFG, and the contents were divided into two parts.

The first part focused on cultivation of methanogens as dominant species under H_2/CO_2 and evaluating the ability of methane production in semi-continuous reactor. Besides, due to the existence of CO in BFG, the effect of CO partial pressure on methane production from CO by acclimated methanogens was also conducted. Moreover, feasible research on the removal of CO from BFG by acclimated methanogens was explored (Chapter 2).

In the second part, to efficiently resist the toxicity of CO, anaerobic granular sludge (AGS) was chosen to be as the inoculum to convert CO and CO_2 from BFG to methane (Chapter 3). The methanogenic potential of AGS, the effect of CO partial pressures and exogenous H_2 partial pressures on the methane production by AGS were investigated. Based on the experimental results, the optimum H_2 partial pressure was finally discussed and carbon balance was studied.

Table 1-1 Composition of gas streams from steel making process.

Gas streams	CO (%)	H_2 (%)	CO_2 (%)	N_2 (%)	Other (%)
Coke oven gas (COG)	5 - 10	55	3 - 5	10	25
Blast furnace gas (BFG)	20 - 35	2 - 4	20 - 30	50 - 60	-
Basic oxygen furnace (BOF)	50 - 70	1 - 2	10 - 20	15 - 30	-

Table 1-2 Anaerobic carboxydotrophic microorganisms [52].

Species	Temperature (℃)	pH	Products	References
Acetobacterium woodii	30	6.8	Acetate	[53]
Clostridium autoethanogenum	37	5.8-6.0	Acetate, ethanol	[15]
Peptostreptococcus productus	37	7.0	Acetate	[54]
Eubacterium limosum	38-39	7.0-7.2	Acetate	[53,55]
Moorella thermoacetica	55	6.5-6.8	Acetate	[56]
Citrobacter sp Y19	30-40	5.5-7.5	Hydrogen	[57]
Rubrivivax gelatinosus	34	6.7-6.9	Hydrogen	[58]
Carboxydothermus hydrogenoformans	70-72	6.8-7.0	Hydrogen	[59]
Thermincola carboxydiphila	55	8.0	Hydrogen	[60]
Methanosarcina barkeri	37	7.4	methane	[19]
Methanosarcina acetivorans strain C2A	37	7.0	Acetate, formate, methane	[20]
Methanothermobacter thermoautotrophicus	65	7.4	methane	[61]

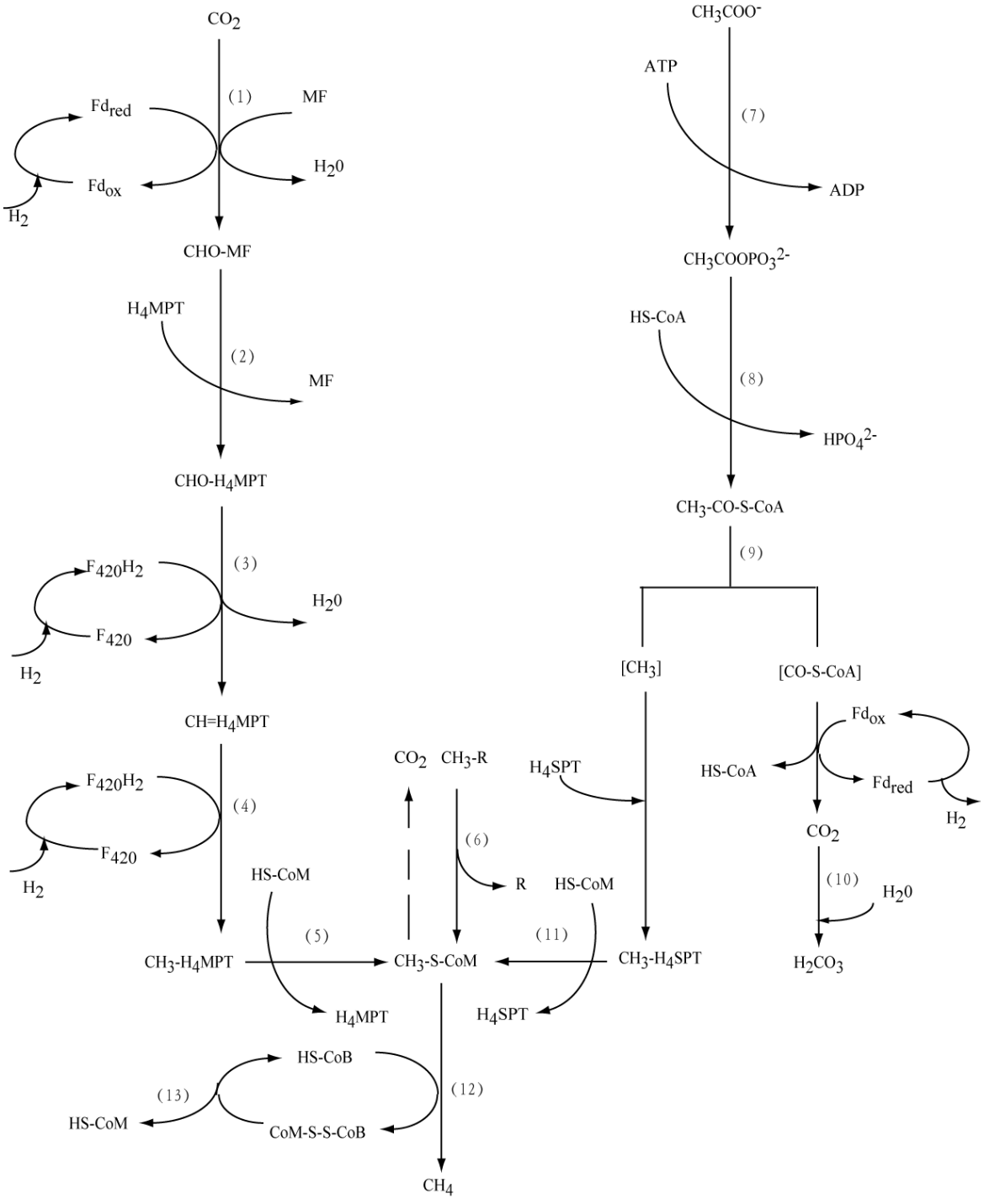


Fig. 1-1 Biochemical pathways of methanogenesis [41].

Reaction (12) and (13) are essential for all the methanogens; reaction (1) to (5) exists in CO_2-reducing pathway of hydrogenotrophic methanogen; reaction (6) exists in methylotrophic pathway; reaction (7) to (11) exists in aceticlastic pathway. Fd: ferredoxin; MF: methanofuran; H_4MPT: tetrahydromethanopterin; H_4SPT: tetrahydrosarcinapterin; F_{420}: oxidized form coenzyme F_{420}; $F_{420}H_2$: reduced form coenzyme F_{420}; HS-CoM: coenzyme M; HS-CoA: coenzyme A; HS-CoB: coenzyme B; CoM-S-S-CoB: heterodisulfide of CoM and CoB.

Chapter 2 Feasibility study on methanation of blast furnace gas using acclimated methanogens

2.1 Introduction

The drastic climate change, greenhouse effect (GHG), depletion of fossil fuel reserves and conflictive increased energy consumption, aroused many attention on the research for sustainable, alternative energy sources and innovative fuels [62]. In order to ease the impact on the environment and respond to future energy crisis, a readily available, higher heating value and low-cost energy source is required. Methane (CH_4) seems to be a good candidate due to its easy storage and high calorific value advantages, and there are ready-made pipe networks for CH_4 transportation and distribution.

Waste-off gas from steel mills contains high content of carbon monoxide (CO) and carbon dioxide (CO_2), which could be used as the intermediates for methane production. Capturing and recovering carbon from waste-off gases can contribute to the depletion of current fossil fuels, and form a virtuous circle. For waste-off gas from steel mills (coke oven gas, blast furnace gas and basic oxygen furnace), blast furnace gas (BFG) contains 20 - 35% CO_2, 20 - 30% CO, 2 - 4% H_2 and 50 - 60% N_2 [63]. In principle, BFG is suitable feedstock for biological fermentation. Compared with metal catalysts used in the chemical industry, the biocatalysts usually present ability to be more tolerant to intrinsic gas contaminants.

Methanogenic bacteria can reduce CO_2 to CH_4 at mesophilic temperature with H_2 as electron donor. However, methanogens usually grow more slowly than aerobic microbes, and need long lag time for the substrate utilization. CO_2 can be utilized as the carbon source only in the presence of CO or H_2 [63]. And a variety of methods for biological conversion of CO_2 to CH_4 have been referred over the years [64]. However, CO is a less studied substrate of methanogenesis which was first reported by Fischer et al in 1931. Some researchers found that only *Methanosarcina acetivorans*, *Methanothermobacter thermoautotrophicus* and *Methanosarcina barkeri* have been studied to utilize CO as sole energy source and a reductant for methanogenesis from CO_2 [61]. As shown in the following reaction:

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2,\ \Delta G_0 = -53\ kJ/mol \quad (2\text{-}1)$$

The growth of microorganisms with CO is not so effective, resulting in the doubling time of more than 200 hours for M. thermoautotrophicus and 65 hours for M. barkeri.

In this study, a semi-continuous reactor was used to cultivate methanogens sampled from a nearby pond sediment under H_2/CO_2 conditions. Hope to investigate the tolerance of the enriched methanogens to CO partial pressure and determine whether it is suitable for converting BFG to methane.

2.2 Materials and methods

2.2.1 Enrichment cultures

Microbial inoculum collected from the sediment of Matsumi pond and was acclimated to the H_2/CO_2 gas mixture (80/20, % v/v) under anaerobic condition for about 6 months at 37℃ as previous studies [44]. The basal medium for the microorganisms consisted of the following components (per liter of distilled water): KH_2PO_4, 3.4 g; K_2HPO_4, 3.4 g; NH_4Cl, 2.13 g; Na_2CO_3, 2.54g; $Na_2S\ 9H_2O$, 1mM; Resazurine, 0.001g; Yeast extraction, 2 g and 10 ml trace metals given [65] (Table. 2-1). Because these two gases were used as the sole carbon and energy sources, therefore, methanogens became more dominant to efficiently convert CO_2 and H_2 to methane in the mixed culture.

2.2.2 Experimental set-up

(1) Semi-continuous reactor

Methane production ability of methanogens under H_2 /CO_2 was determined in semi-continuous reactor. The semi-continuously operated reactor (Fig. 2-1) was a 2.32 L bottle with working volume of 1 L. The inlet gas (80/20, % v/v) was injected at 54.72 L/d to the bottom of the reactor, and 143 ml of mixture was daily replaced with culture medium.

(2) Batch experiment

To investigate the effect of CO partial pressure (P_{CO}) on methane production by using acclimated stains as inoculum, batch experiment was conducted in cylindrical pressure bottles (4.4 cm in diameter, 7 cm in height) with a volume of 110 ml. 45 ml of the basic medium and 5 ml of seed culture was used for inoculation of the bottled medium. The pH of the mixture was then adjusted to 7.2 with 2 M NaOH. In order to create anaerobic conditions, the bottles were flushed with pure N_2 gas for 3 min after being capped and sealed with butyl rubber stoppers. A certain volume of N_2 was removed from the bottle and replaced by an equivalent volume of CO using a gas tight syringe to obtain the required CO partial pressure (0.025 - 0.4 atm) in the headspace. Then the bottles were incubated in a thermostatic water bath oscillator at 37 ± 2 °C and 100 rpm. All the tests were performed in triplicate.

2.2.3 Analytical methods

For observing the surface morphology of samples, the samples were washed with PBS and fixed with 2.5% glutaraldehyde (pH 7.2 - 7.4) overnight at 4℃, and then washed with PBS for 2 h. Samples were fixed for 1.5 h by 1% osmic acid, and then washed with double distilled water for 2 h. Then samples were dehydrated by successively passaging through 25, 50, 75, 80, 90, 95, and 100% ethanol followed by drying with a critical drier. The samples were then used for SEM by an field emission scanning electron microscope (FE-SEM, S-4800; Hitachi Hitec Corp, Japan) at 5 kV accelerating voltage after Pt-Pd being coated with an ion sputter coater (E-1030; Hitachi Ltd., Japan).Two gas chromatographs (Shimadzu GC-8A, Japan) equipped with TCD were used to detect the concentrations of gaseous components. For H_2, CH_4, and CO_2, the temperatures for detector and injector were both 60 ℃, and the column temperature was 80 ℃ with nitrogen being used as the carrier gas. For CO analysis, the detector and injector temperatures were both 170 ℃, and the column temperature was 80 ℃ with helium as the carrier gas. Volatile fatty acids (VFAs) concentrations were analyzed by a Shimadzu GC-14B/FID, and the column and the injector temperatures were set at 150 ℃ and 190 ℃, respectively with nitrogen being the carrier gas. In this study, the concentrations of

VFAs were expressed as equivalent carbon values calculated from the theoretical formula of each VFA component.

2.3 Results and discussion

2.3.1 Biomethanation potentials from H_2 and CO_2

The morphology of acclimated microorganisms was shown Fig. 2-2. As seen, most of the microorganisms are slender and crooked with rod shape and blunt round ends. After cultivation of 2 months, the situation of the enrichment reactor was evaluated using 2 L H_2/CO_2 mixed gas. In Fig. 2-3, as the higher solubility, CO_2 decreased sharply, and it was calculated that around 80 mL CO_2 dissolved in the liquid phase in the first 30 min. Within 8 hours, CO_2 and H_2 are almost completely consumed to produce methane, but there is still a small amount of CO_2 remaining even if the ratio of H_2 to CO_2 is 4:1. It might be caused by the bicarbonate in the inoculum, contributing to residue of CO_2. Regarding solubility, H_2 is almost insolubl**e** which is different from CO_2, while the decrease of H_2 presented linear tendency suggested no gas–liquid mass-transfer limitation in the experiment. Fig. 2-4 showed the CH_4 production rate. In the first four hours, the average value was 74.5 ml/h, and then with the consumption of CO_2 and H_2, CH_4 production rate reduced and the yield tended to be stable.

In the semi-continuously operated reactor, CH_4 content changed in the outlet during the first 24 hours (Fig. 2-5). As shown, the content of CH_4 increased rapidly and reached 52%. After keeping at this content for 10 hours, it decreased to 38% at 52 hour and then a steady-state was achieved with CH_4 production rate of 9.56 L/L_{liquid} d after 5ml trace metal elements (TME) being added. In order to test the effect of TME, in the followed-up hours 5ml TME was not added and then added, the similar phenomenon occurred. Results showed that the trace metal elements are crucial to keep the steady efficiency of CH_4 production. Then, the effects of NH_4Cl and S were also tested, which seemed to have no obvious influence on CH_4 production (Fig.2-6). As for the addition amount of TME, 5 ml is enough to keep its steady operation at inlet of 54.72 L/d. From 333h on, an inlet flow rate of 89.28 L/d was tested, and

CH_4 production rate increased to 13.2 L/L_{liquid} d since the supply of H_2 and CO_2 were about 2 times that at 54.72 L/d. Various methanogens (*methanosarcina barkeri; methanospirillum hungatii; methanocorpusculum parvum* and so on) require trace metal element especially Fe, Co, Ni, Mo, Se. Trace metals play an important role in sustaining the stable and efficient conversion of waste crop, livestock manure, industrial wastewaters [66] or gas [65] to methane. Metal elements are essential for enzymes to participate in methane production and biochemistry [67].

Some specific operation parameters and results are summarized in Table. 2-2. The acclimated methanogens reflected obvious hydrogenotrophic methanogenic activity, as H_2 in the inlet gas was efficiently consumed even during the startup period of the reactor. Acetate was also detected as the major metabolite in the liquid phase with the concentration about 10 mM. During the operation of reactor, pH (around 7.1, Fig.2-4) was relatively stable possibly due to the buffer capacity of the culture medium. In addition, the increase in dry cell weight during the operation between 333 h to 434 h could be ascribed to the increase in gas injection rate [45].

2.3.2 Effect of CO partial pressure on methane production by acclimated methanogens

It is well known that CO is an inhibitor of methanogenesis, the effect of CO partial pressure varying from 0.025 atm to 0.4 atm on methane production was tested to achieve maximum CO and CH_4 specific activities. The result was provided in Table 2-3. During the fermentation of 30 days, CO at 0.025 atm and 0.05 atm were completely consumed, however, P_{CO} higher than 0.1 atm were consumed more than 60%. It illustrated that the inoculums acclimated under H_2/CO_2 were not adapted to CO as energy source. The highest CH_4 specific activity was achieved at P_{CO} 0.05atm and it decreased with the increasing of P_{CO}, suggesting that CO presented inhibitory to inoculum for methane production started from P_{CO} of 0.1 atm. Here, methane produced from CO through indirect or direct conversion, even though the direct conversion is uncommon [21, 68]. The CO specific activity presented positive correlation to P_{CO} and the optimal CO specific activity was observed at P_{CO} of 0.4 atm in this

study. Table 2-4 summarized the final products, with the increase of P_{CO}, methanogenesis started to decrease, the methane precursors (acetate) accumulated.

In BFG, the CO content is 20 - 35% corresponding to 0.2 - 0.35 atm at atmospheric conditions. It is necessary to dilute the P_{CO} below 0.1atm for conversion of CO to CH_4 by the acclimated methanogens. Nitrogen is in general an inert gas and does not relate to a toxicity issue. Here, N_2 can be used to lower the CO partial pressure in the gaseous stream, however, it can be assumed to influence the gas transfer rate of CO and H_2 into the solution according to Henry's law, which may limit the rate of fermentation. Therefore, it seems that methanogens enriched under H_2/CO_2 is not efficient to convert CO to methane at mesophilic conditions.

2.4 Summary

A semi-continuous bioreactor was developed for using acclimated methanogens in the H_2/CO_2 fermentation. Among the different factors, besides basic medium, trace metal element can maintain the performance of the system for methane production. This research also provided insight into that enriched methanogens under H_2/CO_2 was not suitable for using CO from BFG as sole energy and carbon source for CH_4 synthesis.

Table 2-1 Composition of trace metal solution

Components	Concentration (mg /L)
$MgCl_2 \cdot 6H_2O$	820
$MnCl_2 \cdot 4H_2O$	200
$FeCl_2 \cdot 4H_2O$	200
$NiCl_2 \cdot 6H_2O$	15
$ZnSO_4 \cdot 7H_2O$	30
$CoCl_2 \cdot 2H_2O$	10
$CaCl_2 \cdot 6H_2O$	160
Na_2SeO_3	12
$Na_2MoO_4 \cdot 2H_2O$	3
$CuSO_4 \cdot 5H_2O$	8
$AlK(SO_4)_2$	1
H_3BO_3	2
$NaWO_4 \cdot 2H_2O$	10

Table 2-2 Performance of the mesophilic reactor under different operation conditions.

Parameters (hour)	Period		
	0 - 142[a]	142 - 333(I)	333 - 434(II)
Gas injection rate (L/(L_{liquid} day))	54.72	54.72	89.28
Gas retention time (h)	0.44	0.44	0.27
Biogas composition			
CH_4 (%)	57.18 ± 0.83	58.73 ± 1.64	36.07 ± 0.04
H_2 (%)	38.08 ± 1.58	36.98 ± 1.72	9.37 ± 0.43
CO_2 (%)	4.73 ± 0.75	4.29 ± 1.13	54.56 ± 0.48
CH_4 production rate (L/(L_{liquid} day))	9.52 ± 0.04	9.59 ± 0.08	13.18 ± 0.06
Acetate concentration (mM)	9.98 ± 1.02	10.07 ± 1.17	9.75 ± 0.06
Cell dry weight (g/L)	1.4 ± 0.21	1.49 ± 0.04	1.67 ± 0.03

[a]The operation was not in steady state during this period

Table 2-3 CO consumption ratio, carboxydotrophic and methanogenic activities during CO fermentation by acclimated methanogens at 37 ℃.

Parameters	CO partial pressure (atm)					
	0.025	0.05	0.1	0.15	0.2	0.4
CH_4 specific activity (mmol/L d)	0.79	1.38	0.95	0.90	0.84	0.70
CO specific activity (mmol/L d)	0.31	0.32	1.03	1.43	1.44	3.19
Initial CO (mmol/L)	1.10	2.44	5.72	8.95	11.66	24.14
CO consumption ratio (%)	100.00	100.00	81.59	65.56	62.24	64.30

Table 2-4 Final products during CO fermentation by acclimated methanogens at 37℃

Products formed after 30d incubation	CO partial pressure (atm)					
	0.025	0.05	0.1	0.15	0.2	0.4
Acetate (mmol/L)	0.0047	0.005	0.0063	0.0067	0.0071	0.0083
CH_4 (mmol/L)	1.01	2.43	1.5	1.14	1.04	0.86

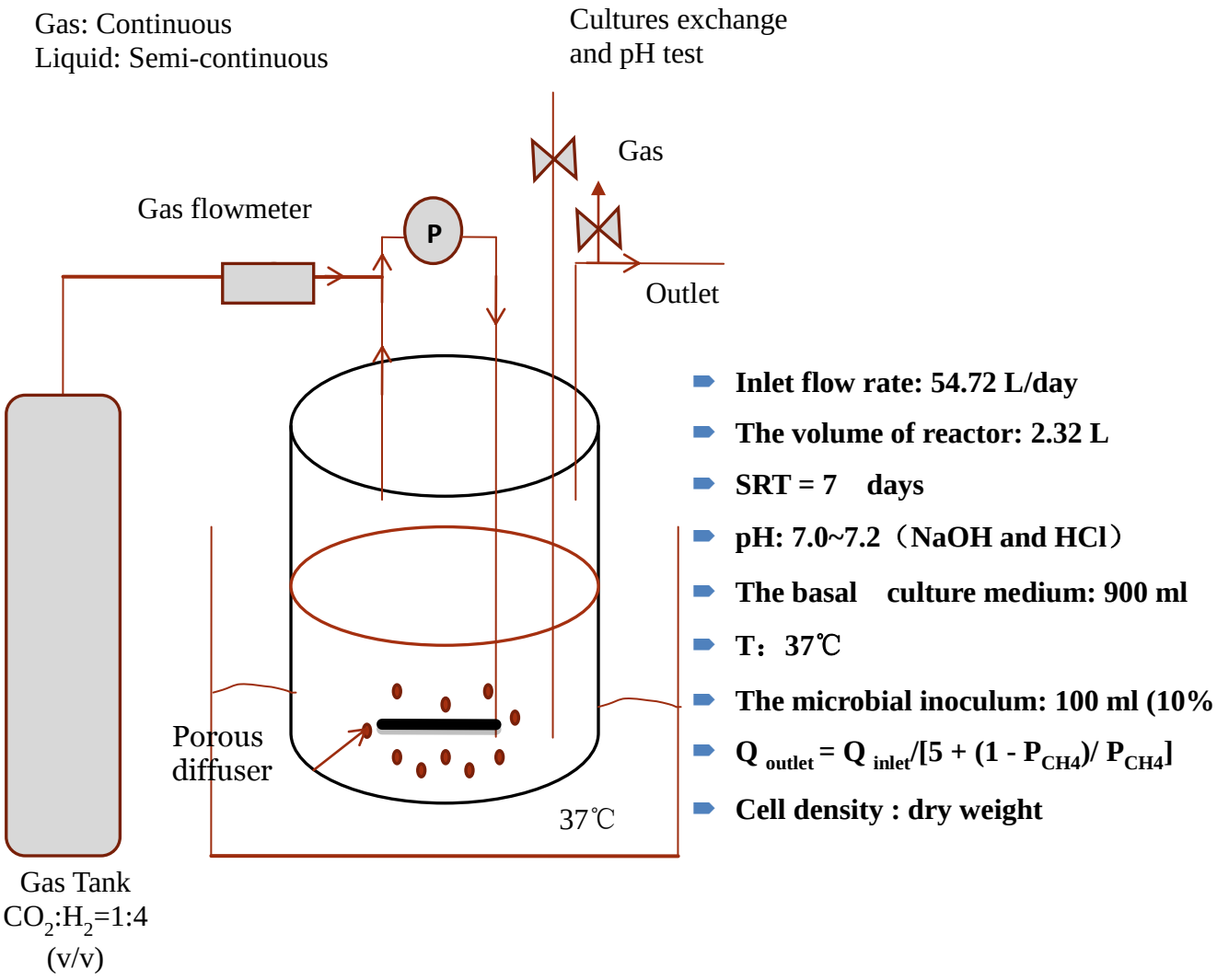


Fig. 2-1 Schematic diagram of the semi-continuous methane fermentation system.

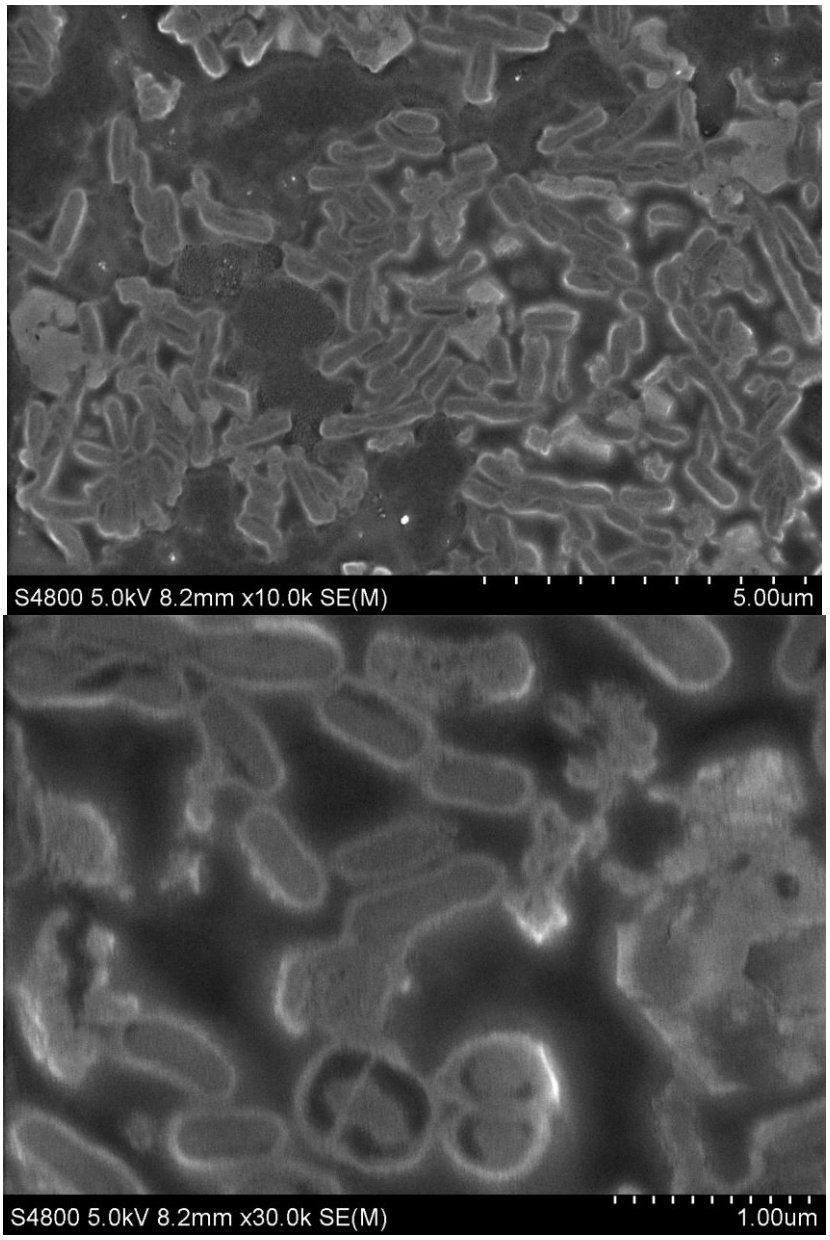


Fig. 2-2 SEM of acclimated methanogens after 2 months enrichment.

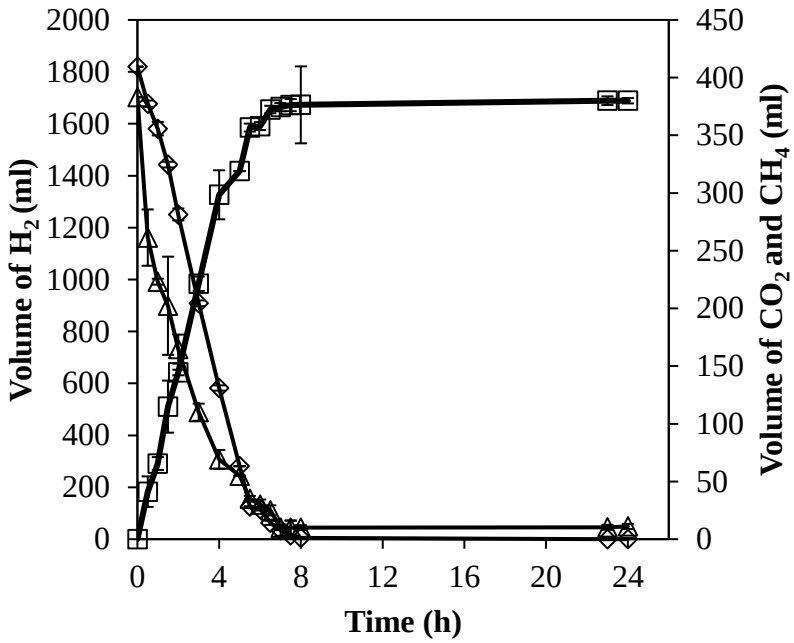


Fig. 2-3 H_2 and CO_2 consumption and CH_4 production profiles. H_2 (○), CH_4 (□), CO_2 (Δ).

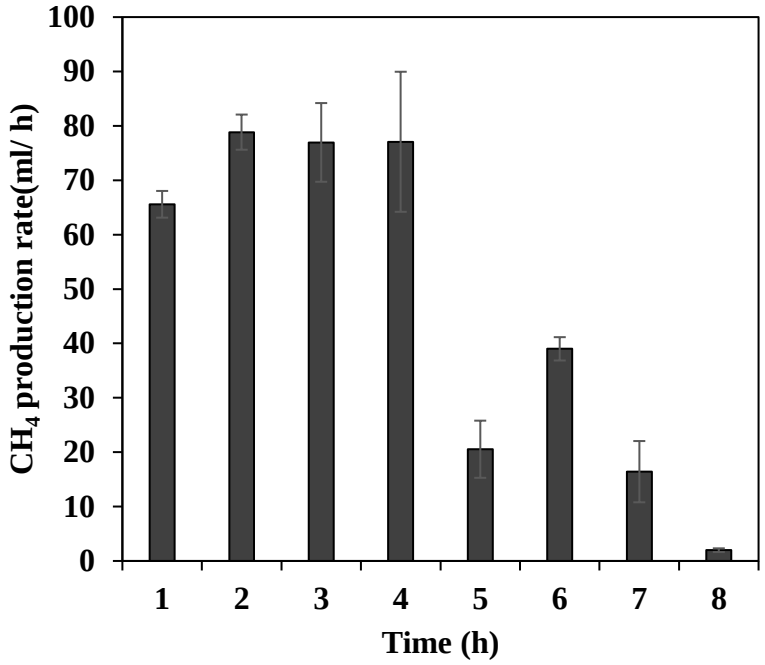


Fig. 2-4 The CH_4 production rate per hour with time.

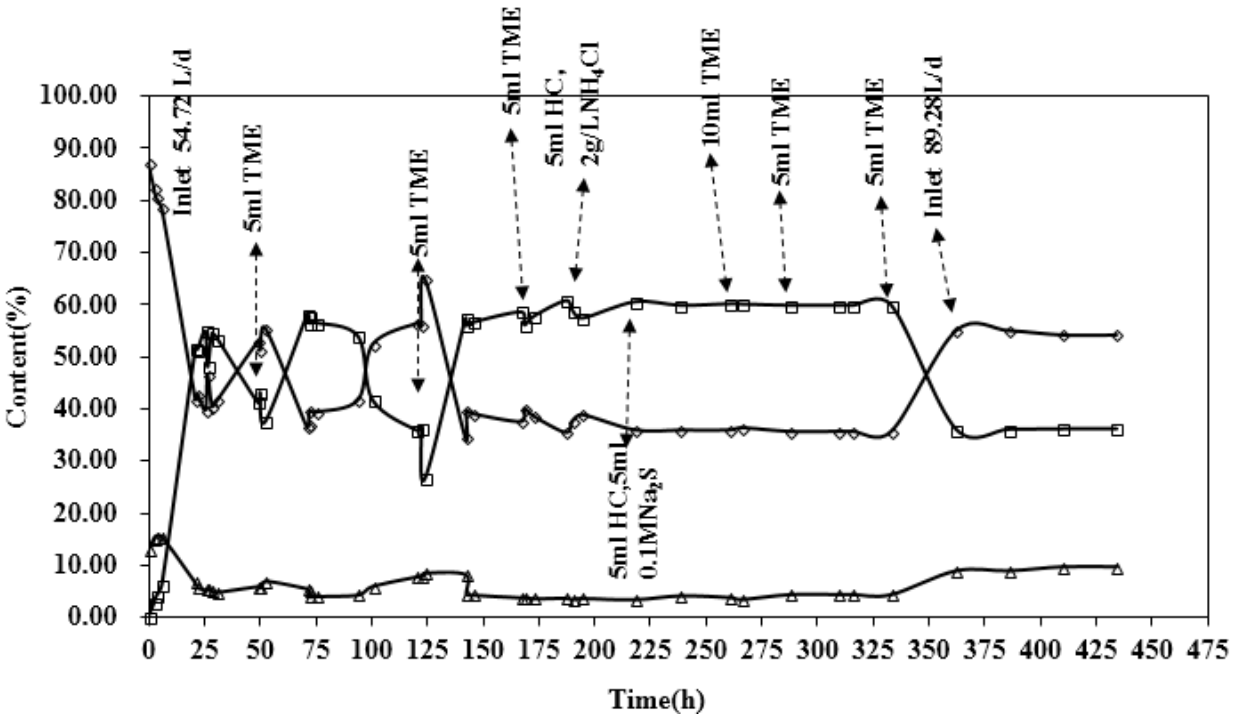


Fig. 2-5 Changes in outlet CH_4 content from the semi continous reactor. H_2 (◇), CH_4 (□), CO_2 (Δ).

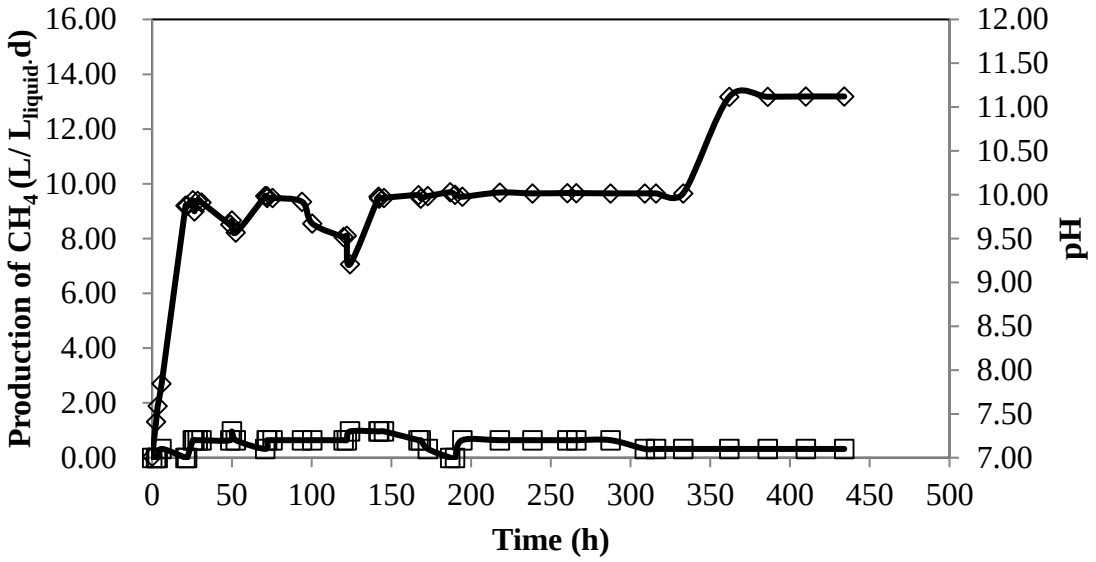


Fig. 2-6 CH_4 production rate (L/L_{liquid} d) (○) and pH (□) changes in the semi-continuous reactor.

Chapter 3 Biomethanation of blast furnace gas using anaerobic granular sludge via addition of hydrogen

3.1 Introduction

In 2014, Japan's total carbon dioxide (CO_2) discharge was approximately 1.2 billion tons, accounting for 92.8% of total greenhouse gas (GHG) emissions. The steel industry (14%) is the second largest source of CO_2 emission after the power industry (39%) according to a recent survey [69]. In other words, the steel industry should take an important role in reducing GHG emissions [70]. Blast furnace gas (BFG) is a byproduct gas produced during the production of hot metal (liquid iron) in a blast furnace from iron and steel industry. BFG is almost a colorless gas and has a stable chemical stability under normal storage and handling conditions. Although BFG could be directly used as fuel for steam boilers, dynamos, and as the supplement of traditional fossil fuels in thermal units [71], the calorific value is low for the reasons that carbon monoxide (CO) has low energy density and CO_2 is non-flammable [12].

It is well known that the high affinity to metal-containing enzyme makes CO be toxic to many microorganisms [72]. Therefore, the high concentration of CO in BFG makes it to be hazardous.

For treating BFG, absorbents could be developed for CO_2 capture from BFG to recover the carbon source for integrated steelworks [70]. Besides, hydrogen could be produced from CO in BFG through water-gas shift reaction (the following reaction 3) regardless of CO_2 is captured or separated [73], however, strict reaction conditions need to be controlled such as the specific catalyst development and high temperature. The mixtures of CO, CO_2, and H_2 can also be used as sources for biofuels production by microorganisms, like ethanol [74], volatile fatty acids [75] and 2,3-butanediol [76].

Compared with H_2, although it is also a good source of clean energy, the storage cost of CH_4 is only about 1/3 of H_2 [77]. Higher quality of fuel will be obtained through biomethanation of CO_2 and CO from BFG, which could be further utilized as heating fuel or

power generation for steel industry. Therefore, this form of carbon recycling not only saves costs but also helps reduce GHG. In addition, there are ready-made pipe networks for CH_4 transportation and distribution. For biological method, the moderate temperature and pressure, low energy consumption are the advantages. Besides, the high specificity of the enzyme lead to higher product yields and fewer by-products. Biological conversion of CO_2 [27, 78] and/or CO [26] to CH_4 by anaerobic microorganisms have been studied and confirmed previously, such as Bugante, E.C et al. [79] used a column bioreactor to convert BFG to methane under thermophilic conditions. Two steps are generally involved in this process: the conversion from CO to CO_2 and methane, and then extra H_2 is added to the reactor for accomplish the conversion of CO_2 to methane. The related reaction processes of carboxydotrophic microorganisms and hydrogenotrophic methanogens are shown in the following reactions.

$$4\ CO + 2\ H_2O \rightarrow CH_4 + 3\ CO_2,\ \Delta G^0 = -53\ kJ/mol \quad (3\text{-}1)$$

$$CO + 3\ H_2 \rightarrow CH_4 + H_2O,\ \Delta G^0 = -151 kJ/mol \quad (3\text{-}2)$$

$$CO + H_2O \rightarrow H_2 + CO_2,\ \Delta G^0 = -20\ kJ/mol \quad (3\text{-}3)$$

$$4\ CO + 2\ H_2O \rightarrow CH_3COOH + 2\ CO_2,\ \Delta G^0 = -176\ kJ/mol \quad (3\text{-}4)$$

$$2\ CO + 2\ H_2 \rightarrow CH_3COOH,\ \Delta G^0 = -67\ kJ/mol \quad (3\text{-}5)$$

$$CH_3COOH \rightarrow CH_4 + CO_2,\ \Delta G^0 = -31\ kJ/mol \quad (3\text{-}6)$$

$$4\ H_2 + CO_2 \rightarrow CH_4 + 2\ H_2O,\ \Delta G^0 = -130.7\ kJ/mol \quad (3\text{-}7)$$

The above reactions about methane production include direct reaction (reaction 3-1, 3-2, 3-7) and indirect reaction (reaction 3-3, 3-4, 3-5, 3-6). For indirect methane production, two steps will be contained: CO, H_2 or CO_2 formation of the precursors (H_2 or acetate) by bacteria and biomethanation of the precursors by methanogens. It can be seen that BFG could not be completely converted to methane without exogenous H_2 addition, due to the co-existence of high concentration of CO_2 in the BFG. Up to now, little information could be found on methane production from the mixture of BFG and H_2 gases under mesophilic conditions. Here, exogenous H_2 can be obtained from hydrogen containing industrial exhaust gases, such as coke oven gas (COG) [80], the byproduct from the coke making process, contains around 54 – 59% of H_2. Most of the COG is directly discharged into the air, resulting in seriously

environmental pollutions. Therefore, the conversion of H_2 by microorganisms to methane would be more sustainable. For the hydrogen from electrolysis of fluoride-contaminated wastewater [81], in order to obtain only hydrogen, electrocoagulation technology can be applied to treat hydrofluoric acid wastewater by using renewable electricity without oxygen production. Exogenous H_2 can also be obtained from in-situ anaerobic corrosion of metallic iron [82], under anaerobic conditions, and the hydrogen produced by iron corrosion could serve as electron-donor for hydrogen-consuming microorganisms.

In the biological methods for treating CO or CO_2, pure culture is sensitive to changes of environment and strict sterilization conditions [83–85]. However, it is well known that mixed culture presents rich functions, such as non-sterile conditions, high ability to adapt to different components of syngas [86], existence of rich variety of microorganisms and low cost than pure culture [74], these advantages make it more suitable for application in industry. Anaerobic granular sludge (AGS) with excellent settling property, the retention of a high biomass concentration and ability to treat high concentrations of organic wastewater is a promising technology that has attracted more and more attention [87]. It is usually used in upflow anaerobic sludge blanket (UASB), expanded granular sludge bed (EGSB) and internal circulation (IC) reactors to be the bio-catalyst treating the wastewater of high organic loading rates. The structure of AGS is favorable to resist CO inhibition that the outer layer is dominated by heterogeneous population and bacteria, the inner layer consists of large numbers of archaea like methanogens [88]. AGS has been used in the mixed culture to convert CO to hydrogen [18] or methane [24]. However, until now, there is no documentation on the biomethanation of CO and CO_2 from BFG by AGS via addition of exogenous H_2 in mixed cultures at mesophilic conditions. Since anaerobic digestion of wastewater by AGS in above mentioned reactors involving multiple steps requires the participation of various microorganisms, it is possible for the microorganisms in the anaerobic reactor to convert CO and CO_2 from the BFG to methane, while addition of exogenous hydrogen was assumed to promote the biomethanation of BFG more thoroughly.

Based on the above considerations, the present study aimed to investigate the feasibility

to achieve biomethanation of BFG by the addition of exogenous H_2. The potential of AGS to convert CO and CO_2 to CH_4 will be tested. And it is important to understand the possible effect of CO which is toxic on the activity and ecology of the microorganisms and the possible CO methanation routes. Since H_2 is a possible inhibitor to the anaerobic process, it is challenging to add both BFG and hydrogen in the reactors at the same time. In this study, the mechanisms of enhanced CH_4 production by AGS via exogenous H_2 addition were explored using batch tests.

3.2 Materials and methods

3.2.1 Sludge source

The sludge used in this study was obtained from a mesophilic Expanded Granular Sludge Blanket (EGSB) reactor treating brewery wastewater (Asahi, Ibaraki, Japan) and was stored at 4 ℃. The major physicochemical characteristics of the AGS were as follows: Total suspended solids (TSS) 11.7 (± 0.5) g/L, volatile suspended solids (VSS) 9.9 (± 0.3) g/L, and pH 6.8 (± 0.1) with extracellular proteins of 128.1 (± 2.9) mg/g VSS, extracellular polysaccharide of 9.4 (± 0.3) mg/g VSS, and total organic carbon (TOC) of 679.9 (± 0.8) mg/g VSS, respectively.

3.2.2 Experimental set-up

(1) Methanogenic Potential of AGS

As CO and CO_2 in BFG were designed to be converted to methane, batch experiment 1 was firstly conducted to investigate the methanogenic potential of the AGS sampled from the EGSB reactor. In this part, acetoclastic, carboxydotrophic and hydrogenotrophic methanogenic activities of the AGS in anaerobic cultures were tested in cylindrical pressure bottles (4.4 cm in diameter, 7 cm in height) with a volume of 110 ml. 50 ml of the basic medium [65] and granular sludge (washed with phosphate buffer) were loaded into the bottles to reach a final VSS concentration of 2 g/L. The pH of the mixture was then adjusted to 7.2 with 2 M NaOH. In order to create anaerobic conditions, the bottles were flushed with pure N_2 gas for 3 min after being capped and sealed with butyl rubber stoppers. A certain volume

of N_2 was removed from the bottle and replaced by an equivalent volume of CO (CO_2, or H_2) using a gas tight syringe to obtain the required partial pressure in the headspace. Different substrates were filled [89] in the headspace with volume of 60 ml or in the liquid including R1-sodium acetate (30 mM) for acetoclastic methanogenic activity, R2-H_2/CO_2 (80/20, 2.5 atm) for hydrogenotrophic methanogenic activitiy, R3-CO/N_2 (20/80,1 atm) for carboxydotrophic-a methanogenic activity, R4-$CO/H_2/N_2$ (20/64/16, 1 atm), or R5-$CO/CO_2/H_2$ (20/16/64, 1 atm) for carboxydotrophic-b methanogenic activity. R4 was designed to investigate the effect of H_2 on CO fermentation under mesophilic conditions. The bottles with granular sludge and medium only (without gas substance) were used as the control (R0). These bottles were incubated in a thermostatic water bath oscillator at 37±2 °C and 100 rpm. All the tests were performed in triplicate.

(2) Effect of CO partial pressures on methane production by AGS

To investigate the effect of CO partial pressure (P_{CO}) on methane production from CO and the possible pathway involved, the experiments were divided into two parts, with the scales and procedures being similar as the above except for that the VSS of AGS was 4g/L. The first part (batch experiment 2) was conducted for 6 days to observe the effect of CO partial pressure (P_{CO}) (0, 0.1, 0.2, 0.4, 0.8atm) on methane production. In this experiment the headspaces of the bottles were purged with the mixture of CO and N_2 at different ratios to obtain the required initial partial pressures of CO. The second batch experiment (batch experiment 3) was performed for 7 days to explore the CO conversion route under different P_{CO} (0, 0.2, 0.4, 0.6, 0.8, 1atm) with the methanogens inhibitor, 25mM 2-bromoethanesulfonic acid (BES) [21] being added. Fig. 3-1 demonstrates the impact of BES on the pathways of CO conversion to CH_4.

(3) Effect of exogenous H_2 partial pressures on methane production from blast furnace gas

In this experiment, the scales and procedures were similar with the batch experiment 1, which investigated the effect of exogenous H_2 partial pressures on methane production from BFG by using AGS. The compositions of simulated BFG (TOMOE SHOKAI Co., LTD,

Japan) consist of CO, CO_2, H_2, and N_2 at a volume ratio of $CO/CO_2/H_2/N_2$ = 22/22/4/52. Besides BFG (1 atm, P_{CO} was 0.22 atm) in the headspace of the bottles, exogenous H_2 was also added into each bottle up to a final hydrogen partial pressure (P_{H_2}) of 0.04 atm, 0.88 atm, and 1.54atm, respectively (with a total pressure being adjusted to 2.6 atm with N_2). In this study, soluble total organic carbon (STOC) was measured and used in the carbon balance analysis.

3.2.3 Analysis and chemicals

Total suspended solids and Volatile suspended solids (TSS and VSS) were tested according to the standard methods [90]. Two gas chromatographs (Shimadzu GC-8A, Japan) equipped with TCD were used to detect the concentrations of gaseous components. For H_2, CH_4, and CO_2, the temperatures for the detector and injector were both 60 ℃, and the column temperature was 80 ℃ with nitrogen being as the carrier gas. For CO analysis, the detector and injector temperatures were both 170 ℃, and the column temperature was 80 ℃ with helium as the carrier gas. Volatile fatty acids (VFAs) concentrations were analyzed by a Shimadzu GC-14B/FID, and the column and the injector temperatures were set at 150 ℃ and 190 ℃, respectively with nitrogen being the carrier gas. In this study, the concentrations of VFAs were expressed as the equivalent carbon values calculated from the theoretical formula of each VFA component. And the carbon content of VSS was calculated using $C_5H_7O_2N$ [91]. The concentration of total carbohydrate was measured by phenol-sulfuric method and the concentration of total protein was determined by Lowry-Folin method.

3.3 Results and discussion

3.3.1 Methanogenic Potential of AGS

During the anaerobic biomethanation process of gases with different compositions, it was observed that AGS obviously possessed hydrogenotrophic and acetoclastic methanogenic potentials (18.31 ± 1.2 mmol/g VSS·d and 6.58 ± 0.38 mmol/g VSS·d, respectively, Fig. 3-2), which are similar with previous researches [24, 89]. In order to investigate the

carboxydotrophic potential of the AGS used in this study, two kinds of gases were prepared and tested. As seen, AGS also exhibited a promising carboxydotrophic potential (1.19 ± 0.03 mmol/g VSS·d and 5.56 ± 0.26 mmol/g VSS·d) even though the microorganisms from AGS might not adapt to CO as energy source in comparison to H_2. For all runs, no lag phase was detected during CH_4 production (Fig. 3-3). It was worth mentioning that during the conversion of CO/N_2, CO was the only substrate, while a very small amount of H_2 and VFAs (data not shown) were detected, possibly due to that they were the intermediates for CH_4 production

The CO metabolism by microorganisms from AGS was somehow influenced by the presence of CO_2 and/or H_2 [74, 92], although it is thermodynamically feasible (reaction 3-2) for methane production from CO and H_2. In the process of CO fermentation under mesophilic conditions, the effect of H_2 has been rarely reported [92]. Compared with R3, the presence of hydrogen (R4) led to a slower trend of CO consumption which presented negatively influence (Fig.3-4). Probably CO was in part consumed during the production of the intermediates (e.g., H_2 and acetate) for methanogenesis (reactions 3-3 and 3-4). If H_2 is an intermediate product, the presence of extra H_2 in R4 is not conducive to the smooth progress of reaction 3-3. Assuming acetate is the intermediate, it may be formed from extra H_2 and CO through reaction 3-5, and the consumption of CO in reaction 3-5 is slower than that of reaction 3-3 since 2 moles and 4 moles of CO are required to participate in the formation of 1 mole of acetate, respectively. In addition, by measuring VFAs concentrations, it accumulated at 109.5 h in R4 but not in R3 and R5 (Fig. 3-6). A similar phenomenon was noticed by Heiskanen et al. who applied *Butyribacterium methylotrophicum* to convert different gas substrates to biofuels (mainly acetate) [93], and found that butyric acid production was increased after the supplementation of hydrogen into CO. However, the CO consumption of R5 was not affected by the supplementation of CO_2 and H_2 probably attributable to that CO_2 reacted with H_2 first (reaction 3-7), eliminating the negative impact of CO on methane production to some extent. Compared with R3, R4 seemed to have increased methane production that might be caused by the addition of hydrogen, stimulating the production of CO_2 from the organics contained in

the AGS [94], and then the formation of CH_4. According to a previous study [92], the addition of hydrogen would not promote the direct methanogenic CO conversion in reaction 2-2. Fig. 3-5 shows that the microbial population presented in AGS could rapidly convert CO_2 into methane within 15.5 h in R5; however, the extra CO_2 production detected from 37.5 h on presented a similar increasing trend as that in R3. This extra CO_2 might be from CO and AGS itself. In R3, if calculated according to the reaction 3-1 which assumes that CO can be completely converted to CH_4 and other intermediates, the produced methane (0.56 ± 0.09 mmol/g VSS) was lower than the theoretical value (1.34 ± 0.04 mmol/g VSS). This observation is attributable to that many intermediates were simultaneously generated from CO during the methanation process. At 109.5 h, the intermediates have not been completely utilized.

From the above results, it can be concluded that AGS possessed a great potential for the conversion of CO and CO_2 to methane. And supplementation of H_2 to CO as substrate might lead to the accumulation of VFAs.

3.3.2 Effect of CO partial pressures on the methane production by AGS

Fig. 3-7 shows the relationship between P_{CO} and methane production from CO. In general, more methane was accumulated when P_{CO} was lower than 0.2 atm at the initial stage (within 96 h). Being similar with other reports [51, 68], the methane production rate was found to be obviously inhibited when P_{CO} was higher than 0.4 atm. When the pressure was 0.4 atm, the varying trend could be divided into two phases. During the 96 h after starting this test, the cumulative methane production from different reactors followed a descending order as reactors at 0.2 atm > 0.1 atm > 0.4 atm > 0.8 atm, illustrating that $P_{CO} \geq 0.4$ atm presented an inhibitory effect on methane production by anaerobic granular sludge. After 96 hours' operation, due to that CO was gradually consumed by the microorganisms in the reactors, the partial CO pressure in the reactor gradually decreased and the inhibition was relieved to some extent. Therefore, the cumulative methane production under P_{CO} of 0.4 atm exceeded those from 0.2 atm and 0.1 atm reactors. However, it was different from the flocculant digested

sludge in which the methanogens were inhibited at a P_{CO} of 0.25 atm [26]. This observation reflects that the special dense structure of AGS is favorable to resist CO inhibition. Along with the operation, the methane production at P_{CO} > 0.4 atm gradually increased and exceeded the control. This observation is possibly due to that CO was gradually consumed by the microorganisms in the reactor, and the partial CO pressure in the reactor gradually decreased and the inhibition was relieved to some extent, then CO could be used by the microorganisms and converted to methane. It has been speculated that CO in the anaerobic reactor can be first converted by bacteria into some intermediates and then produce CH_4 and CO_2 by methanogens [24]. The CO consumptions at different partial pressures are shown in Fig. 3-8.

Oelgeschläger and Rother [72] investigated the influence of CO on metabolite formation in Methanosarcina acetivorans when methanogenesis is inhibited and methane production showed the decreasing phenomenon, CO went to acetate, propionate and H_2 accumulation. Methanosarcina acetivorans conserves energy via acetogenic and formigenic process [20].

To explore the pathways from CO to methane and elucidate the effect of CO partial pressure on the intermediates produced, the gaseous components and VFAs were measured and recorded respectively. Seen from Fig. 3-9, the concentrations of VFAs (129–247 mg C/L) on day 7 in all the bottles containing CO were obviously higher than that of the control (106 mg C/L), demonstrating an increasing trend with the increase in P_{CO}. This observation indicates that VFAs could be produced from CO by bacteria when methanogens were inhibited by BES. The VFAs species and proportion of each individual VFA on day 7 were also examined. As seen from Fig. 3-10, no matter under which P_{CO} condition, the dominant VFA was acetate ranging from 47% to 68%. From the control to 1 atm of CO, the second largest amount of individual VFA changed from isovaleric acid (18% in the control and 10% at 0.2 atm of P_{CO}) to propionic acid (16% at 0.4 atm, 33% at 0.6 atm, 36% at 0.8 atm, 35% at 1 atm of P_{CO}). This phenomenon can be explained from the following two aspects. (1) In the presence of BES, the fermentation products of organic matter contained in AGS (control) were VFAs, especially acetate and isovaleric acid in this study. (2) With the increase of substrate (CO) addition to the reactor, acetate and propionate were the main intermediates of

the CO conversion, which is partially in agreement with the findings by Navarro et al. [68,89] who identified an acetate-producing bacterium [95] and a propionate producing bacterium [96] after a long-time exposure to high CO concentrations. In addition, hydrogen was detected in the gas phase. CO has been claimed to be converted to methane via acetate (acetogenic CO-oxidizing pathway) as the precursor under mesophilic conditions, while via hydrogen (hydrogenotrophic CO-oxidizing pathway) under thermophilic conditions [21]. In this study, as shown in Fig. 3-11 H_2 was produced from all P_{CO} conditions, and it might be the intermediate when P_{CO} was higher than 0.2 atm in the headspace as a similar amount of H_2 was detected in the control and the P_{CO} at 0.2 atm reactors. Table 3-1 summarizes the related contents of acetate, propionate and H_2. In this study, H_2 was produced from all the P_{CO} conditions, which might be the intermediate when P_{CO} was higher than 0.2 atm in the headspace as a similar amount of H_2 was detected in the control and the P_{CO} at 0.2 atm reactors. It has been pointed out that the carboxydotrophic activity was insignificant when both inhibitors (BES and vancomycin) were present at the same time; Still, H_2 could be detected, illustrating that hydrogen-producing bacteria were not inhibited in the presence of the above two inhibitors [89]. Among the three intermediates, acetate (reactions 3-4 and 3-5) was considered as the major one due to its highest content; however, the H_2/CO_2 pathway (reactions 3-3 and 3-6) may be co-existing.

The above results showed that when $P_{CO} < 0.2$ atm methane production from CO by AGS was not obviously affected. Conversely, $P_{CO} > 0.4$ atm started to inhibit the activity of methanogens. The conversion of CO to methane was mainly via acetate as intermediate probably accompanied by the H_2/CO_2 pathway.

3.3.3 Effect of exogenous H_2 partial pressures on the methane production from blast furnace gas

High P_{CO} presented inhibition on methane production, thus CO should be controlled at a low concentration in the anaerobic system. In this study, the CO partial pressure in BFG is about 0.22 atm, slightly higher than 0.2 atm which has been demonstrated to be a partial

pressure without obviously inhibitory effect on methanogens. Due to the slight difference between 0.22 atm and 0.2 atm, the CO in BFG was not diluted in the experiments and its initial partial pressure was controlled at 0.22 atm in this test. The effect of exogenous H_2 partial pressures on methane production from BFG by AGS is shown in Fig. 3-12. For the control, there was almost no lag phase. Obviously, the lag period for methane production at P_{H_2} = 0.88 atm and 1.54 atm was about 42 h, suggesting that the anaerobic microbes need some adaptation time to accommodate to the BFG and H_2 environment. For BFG at P_{H_2} of 0.04 atm, this lag period lasted 71 h, ending up with slightly more methane than the control. The methane production was 2.01 mmol/ g VSS, 5.32 mmol/ g VSS and 5.57 mmol/ g VSS at P_{H_2} of 0.04 atm, 0.88 atm and 1.54 atm, respectively. During the entire process, the highest methane production from BFG was obtained at P_{H_2} of 1.54 atm, therefore addition of exogenous H_2 favors methane generation. In addition, under the P_{H_2} of 1.54 atm condition, only 4.70% higher methane production was obtained than that under P_{H_2} of 0.88 atm condition, probably due to the formation of other intermediate products. The maximum H_2, CO consumption and methane production rates are summarized in Table 3-2. The maximum CH_4 production rate of 4.08 $\pm$ 0.71 mmol/g VSS d and 4.19 $\pm$ 0.54 mmol/g VSS d were both achieved under P_{H_2} of 0.88 atm or 1.54 atm on day 3. The different inoculum or other conditions might result in the different time for achieving the maximum methane production rate. For example, in a continuous CO-converting reactor at 35 ℃ [87], the seeded anaerobic granules from a UASB plant treating fruit processing wastewater produced CH_4 ranging from 0.49 $\pm$ 0.1 mmol/g VSS d to 4.77 $\pm$ 1.21 mmol/g VSS d during 100 days' operation, when operated at P_{CO} in the gas feeding (CO: 30 - 60%) of (0.42 - 0.96) $\pm$ 0.6 atm and gas recirculation flow of 0 - 69 L/h. As for the disaggregated sludge during 45 days' continuous CO injection to the headspace, its CH_4 specific activity was 0.7 $\pm$ 1.3 mmol/g VSS d on day 30 and 5.5 $\pm$ 1.2 mmol/g VSS d on day 45, respectively [68]. Even though the values obtained in this study are relatively lower than the above-mentioned 4.77 $\pm$ 1.21 mmol/g VSS d and 5.5 $\pm$ 1.2 mmol/g VSS d, it took only 3 days to reach the maximum methane production rate, far shorter than the 97 days and 45 days reported in the previous works.

The H_2 consumption rates under all tested groups increased with the increase in H_2 partial pressure at a shaking speed of 100 rpm which may control the hydrogen gas-liquid mass transfer during the methanogenesis [97]. Hydrogen gas-liquid mass transfer limitations has been commonly observed in anaerobic reactors [98]. In this study hydrogen gas-liquid mass transfer was obviously the limiting factor, as the hydrogen consumption rate was proportional to initial P_{H_2}. As for the CO consumption rate, there was no significant difference between P_{H_2} of 0.88 atm and 1.54 atm conditions, possibly due to its low concentration in BFG. In order to make the reactions 1-6 thermodynamically feasible, additional hydrogen which could react with CO_2 is favorable for the CO consumption no matter in direct or indirect methane production from CO. CO_2 in BFG was fully utilized at 71 h and 42 h when P_{H_2} was at 0.88 atm and 1.54 atm, respectively (Fig. 3-13). CO_2 was consumed by 84% at 42h when the initial P_{H_2} was 0.04 atm, which later slightly increased and was not fully utilized, most probably due to the fact that CO_2 was one of the intermediates during the methanogenesis in the reactions 3-1, 3-3, 3-4, 3-6. This further suggests the necessity of exogenous hydrogen addition since it is beneficial for the consumption of CO and CO_2 in BFG.

Esquivel‐Elizondo et.al [74] compared the effects of CO_2 and H_2 on CO metabolism using pure and mixed cultures (anaerobic sludge was acclimated to CO), and claimed that the main products were acetate and ethanol. In pure cultures, H_2 was not consumed along with CO since hydrogenase activity in microorganisms may be inhibited by CO [99,100]. In the pure cultures the additional H_2 did not promote acetate production which was opposite to the phenomenon in the mixed culture. A possible explanation is that, CO dehydrogenase (CODH), the enzyme that catalyzes the reversible reduction of CO to CO_2, possesses hydrogenase activity. Thus, the activity of hydrogenases in the pure culture of carboxidotrophs could be redundant. Previous studies with pure cultures also reported that H_2 was not consumed along with CO [97,98]. In this study, the target product was methane by using AGS (mixed culture). From the changes in VFAs species under different conditions (Fig. 3-14), the co-existence of CO may influence the balance between VFAs production and consumption to some extent. At initial P_{H_2} of 1.54 atm, not only some increase in methane production (Fig. 3-12) but also

VFAs accumulation were detected. This phenomenon may explain why only a slight increase (4.70%) in methane production was achieved at P_{H_2} of 1.54 atm in comparison to P_{H_2} of 0.88 atm condition, possibly due to the accumulation of VFAs. Another 2 reactions (3-8 and 3-9) can also be used to explain the changes in VFAs under different P_{H_2}. In order to make the reactions 3-7 and 3-8 thermodynamically feasible, low hydrogen concentration needs to be maintained during the degradation of propionate and butyrate [101].

$CH_3CH_2CH_2COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$, $\Delta G^0 = 45.4$ KJ/ mol (3-8)

$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + 3H_2 + CO_2$, $\Delta G^0 = 72.7$ KJ/ mol (3-9)

In this study, VFAs degradation seemed to be inhibited at P_{H_2} up to 1.54 atm, which was mainly composed of acetic acid, butyric acid and valeric acid. Meanwhile, the remaining VFAs species and their concentrations under P_{H_2} = 0.88 atm were almost similar with those of the control. This observation suggests that proper exogenous hydrogen can improve the biomethanation of BFG by using AGS.

3.3.4 Carbon balance

To monitor the anaerobic process and compare the participation of the main substances related to BFG fermentation, the carbon balance was also analyzed in this work (Fig. 3-15). During the biomethanation of BFG using AGS, carbon from VSS of AGS and BFG (CO and CO_2) may be converted to products including methane, carbon dioxide, VFAs and other substances. As seen from Fig 3-15, all CO under different tested conditions were consumed within 7 days, producing methane and VFAs as the major intermediate carbon products under higher P_{H_2} conditions (0.88 and 1.54 am), and the percentage of methane-C was found to increase with the increase of P_{H_2} (around 28%, 49% and 52% at 0.04 atm, 0.88 atm and 1.54 atm). Noticeably, CO_2 was not fully utilized at P_{H_2} of 0.04 atm, most probably due to insufficient hydrogen. Restated, a slight increase in methane production was detected under P_{H_2} of 1.54 atm in comparison to 0.88 atm, due to its higher accumulation of VFAs (19%) in the fermentation systems.

3.4 Summary

This work indicates that AGS possesses high potential for anaerobic conversion of CO from BFG to methane via VFAs (especially acetate) or H_2 as intermediates under mesophilic conditions. By using the simulated BFG, the batch tests demonstrated that either CO or CO_2 from BFG could be effectively converted by supplying exogenous hydrogen under an appropriate P_{H_2}(0.88 atm in this study). Although P_{H_2} higher than 1.54 atm could rapidly convert carbon source in BFG to methane, the accumulation of VFAs implies that additional design and operation should be considered for the whole BFG fermentation system. This work confirmed the possibility of biomethanation of BFG with exogenous H_2, reflecting the recovery potential of H_2-containing waste gas via biological treatment.

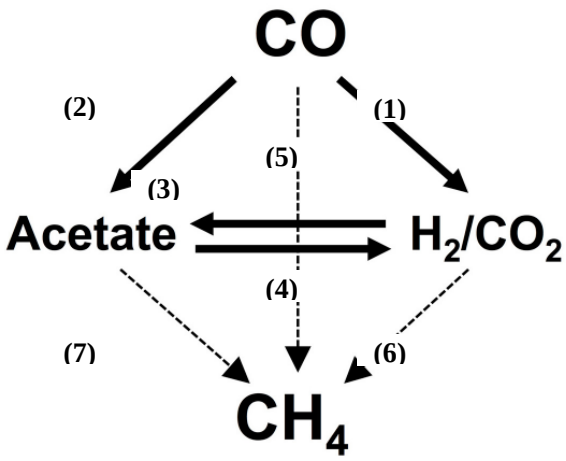
By comparing the effects of two inoculums, acclimated methanogens and AGS could adapt to CO partial pressure values up to 0.1 atm and 0.4 atm, respectively. Microbial populations in AGS can be more tolerant to the toxicity of CO, and be more in line with the actual demand. In addition, the results of this study would provide useful information for waste-off gas treatment, especially AGS as inoculum. Compression, transportation, and storage of CH_4 are technically feasible and mature, and it could be injected into the existing natural gas grid. The possible application of the idea depends on the necessity of BFG treatment, availability of economic and environmental hydrogen and equipment investment costs.

Table 3-1 Main products detected in the reactors under different CO partial pressures in the presence of BES

Main products	P_{CO} (atm)					
	Control	0.2	0.4	0.6	0.8	1
HAc (mmol/g VSS)	1.07 ± 0.04	1.84 ± 0.03	2.07 ± 0.01	2.04 ± 0.06	2.34 ± 0.01	2.49 ± 0.03
HPr (mmol/g VSS)	0.11 ± 0.02	0.10 ± 0.01	0.59 ± 0.02	1.44 ± 0.03	1.80 ± 0.02	1.82 ± 0.05
H_2 (mmol/g VSS)	0.02 ± 0.01	0.03 ± 0.01	0.08 ± 0.02	0.55 ± 0.04	0.75 ± 0.07	0.98 ± 0.03

Table 3-2 The maximum hydrogen and carbon monoxide consumptions, and methane production rates under different H_2 partial pressures

P_{H_2}	CO consumption rate (mmol CO/g VSS·d)	H_2 consumption rate (mmol H_2/g VSS·d)	CH_4 production rate (mmol CH_4/g VSS·d)
Control	-	-	0.53 ± 0.01
0.04 atm	1.67 ± 0.24	0.82 ± 0.05	0.70 ± 0.02
0.88 atm	1.94 ± 0.12	16.34 ± 1.38	4.08 ± 0.71
1.54 atm	1.90 ± 0.41	20.57 ± 0.96	4.19 ± 0.54



(The solid arrows: available pathways

the dotted arrows: blocked pathways)

Fig. 3-1 Possible catabolic routes from CO to methane in presence of 2-bromoethanesulfonate (BES) [68] (1) carboxydotrophic hydrogenogenesis, (2) carboxydotrophic acetogenesis, (3) homoacetogenesis, (4) syntrophic acetate oxidation, (5) carboxydotrophic methanogenesis, (6) hydrogenotrophic methanogenesis, (7) acetoclastic methanogenesis.

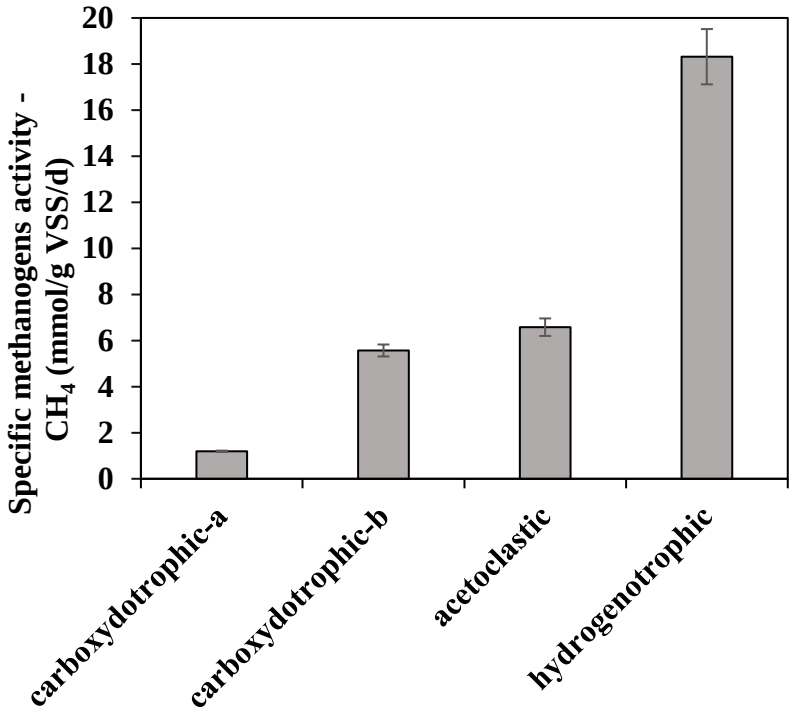


Fig. 3-2 Anaerobic biomethanation potential of AGS used in this study at 37°C. The substrates for carboxydotrophic-a, carboxydotrophic-b, acetoclastic, and hydrogenotrophic activities were CO/N_2, $CO/CO_2/H_2$, sodium acetate, and H_2/CO_2, respectively.

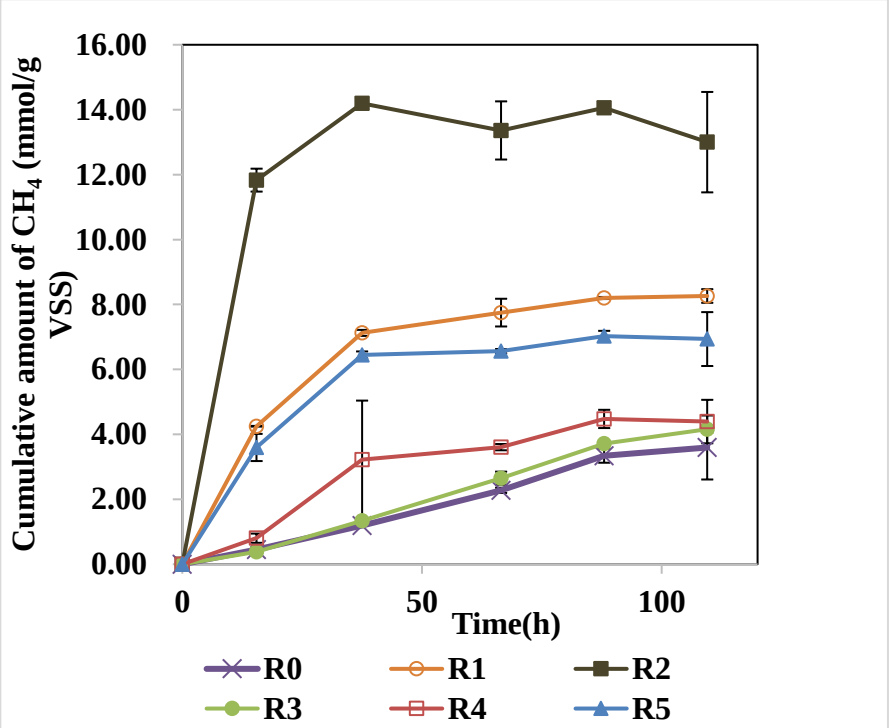


Fig. 3-3 Anaerobic biomethanation potential of AGS at 37°C: CH_4 production（R0-control, R1-sodium acetate, R2-H_2/CO_2, R3-CO/N_2, R4-CO/H_2; R5-$CO/CO_2/H_2$）

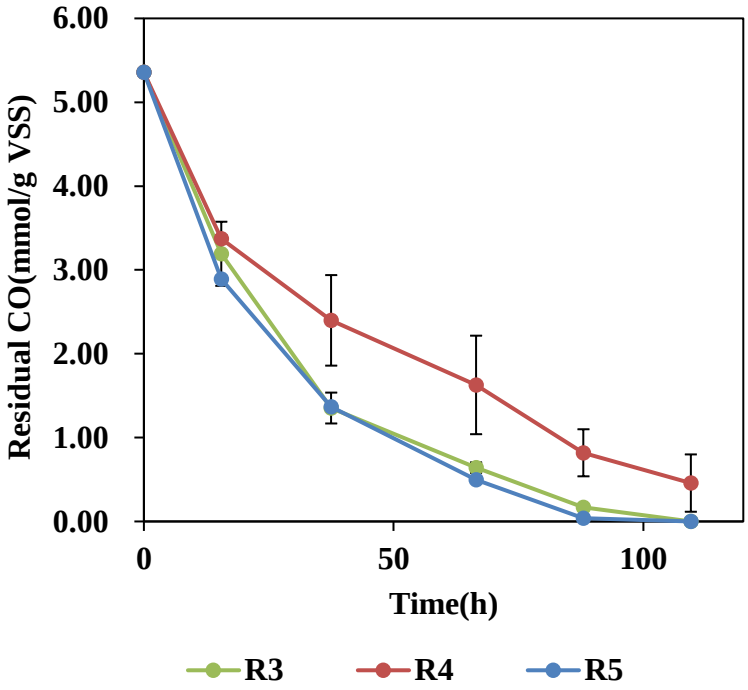


Fig. 3-4 Anaerobic biomethanation potential of AGS at 37°C: CO consumption（R3-CO/N_2, R4-CO/H_2; R5-CO/CO_2/H_2）

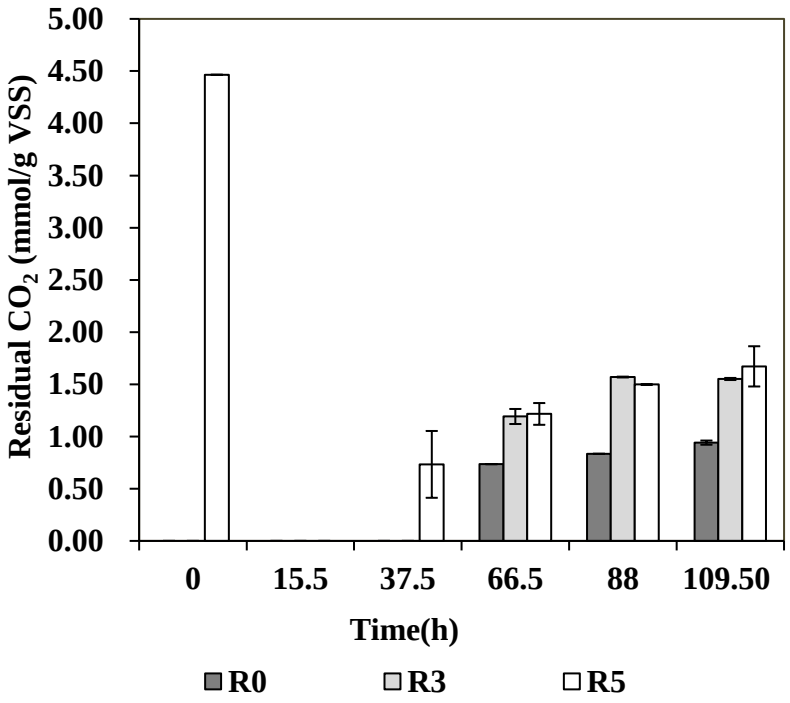


Fig. 3-5 Anaerobic biomethanation potential of AGS at 37°C: CO_2 consumption and production. （R0-control, R3-CO/N_2, R5-CO/CO_2/H_2）

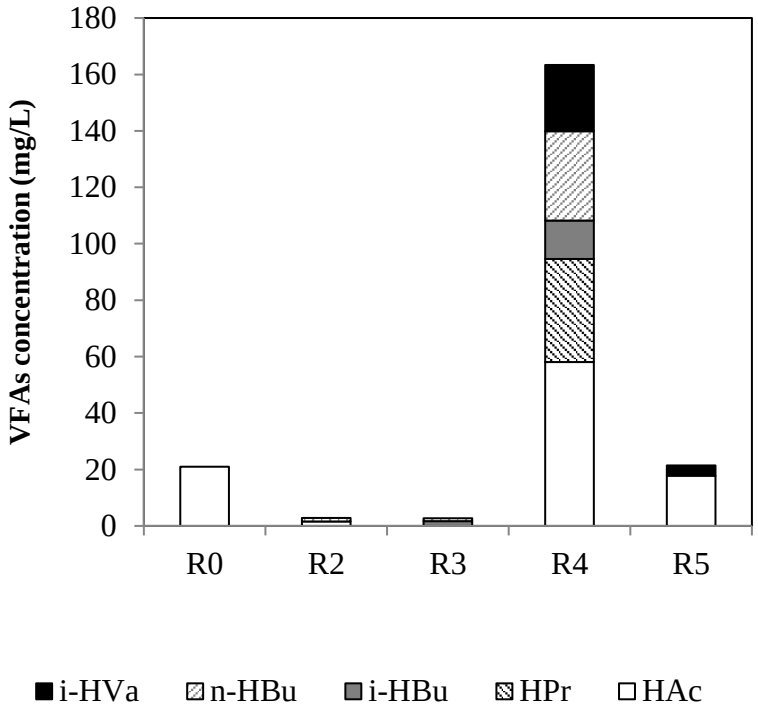


Fig. 3-6 Anaerobic biomethanation potential of AGS at 37°C: VFAs production（R0-control, R1-sodium acetate, R2-H_2/CO_2, R3-CO/N_2, R4-CO/H_2; R5-$CO/CO_2/H_2$）

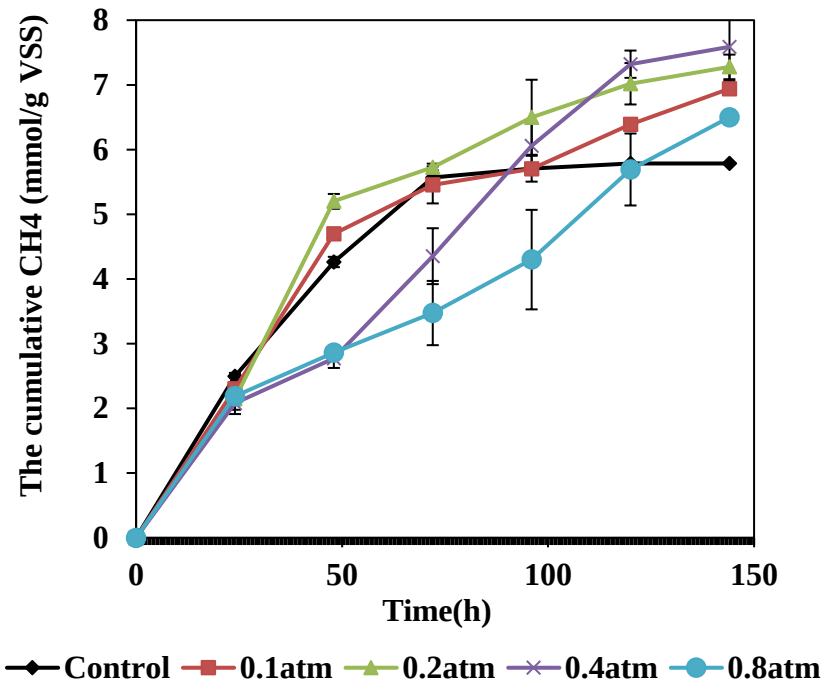


Fig. 3-7 Effect of CO partial pressures (atm) on methane production from CO by AGS.

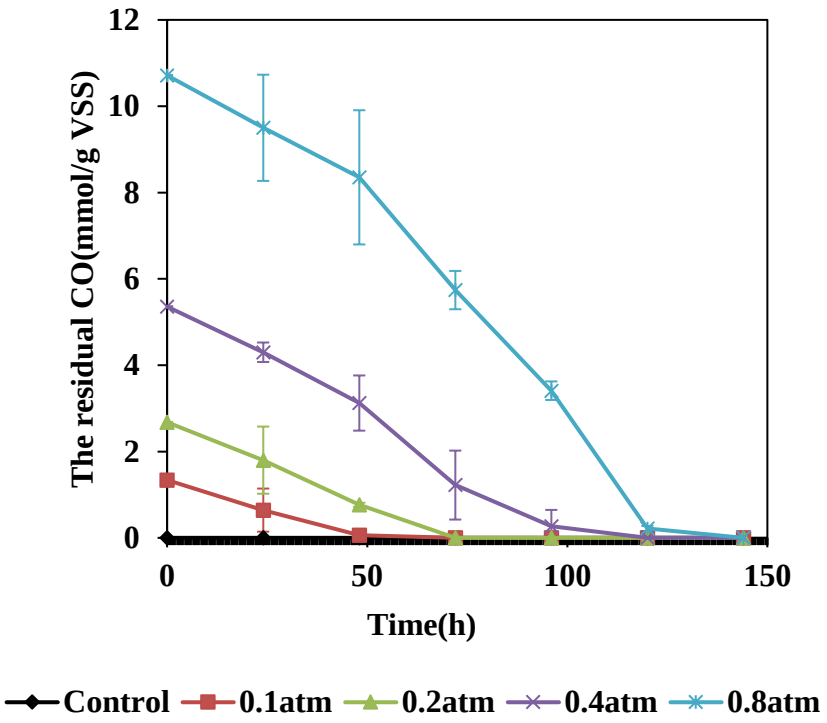


Fig. 3-8 Effect of CO partial pressures (atm) on CO consumption by AGS.

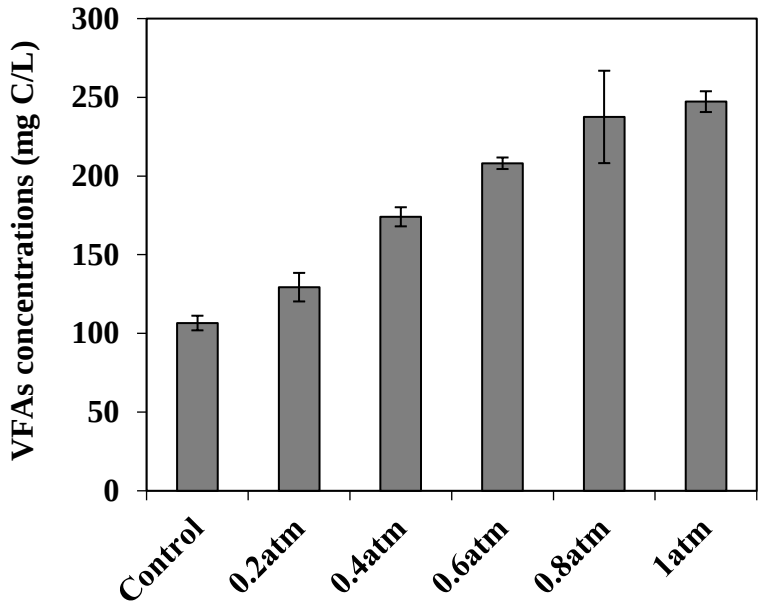


Fig. 3-9 Effect of CO partial pressure (atm) on intermediates formation from CO in the presence of BES at 37°C on day 7.

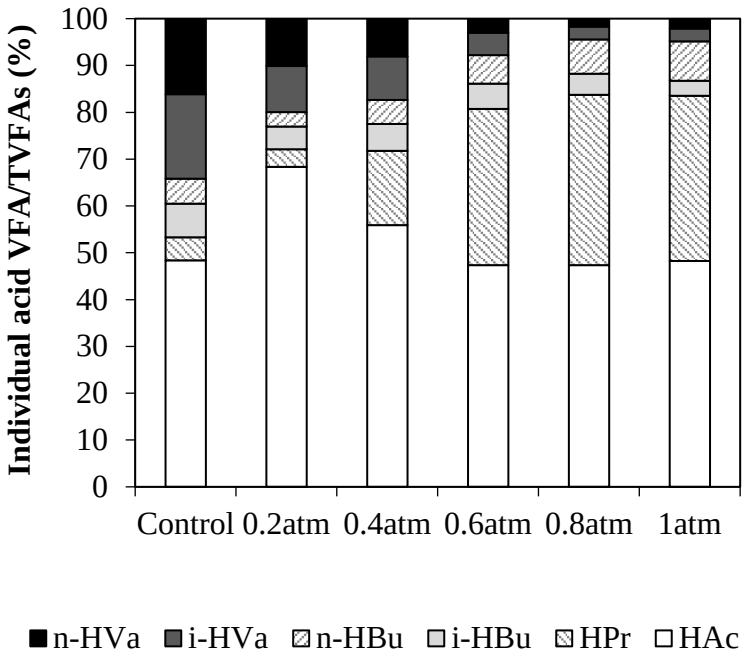


Fig. 3-10 Effect of CO partial pressures (atm) on percentage of individual VFA species to the total VFAs (TVFAs) in the presence of BES at 37°C on day 7.

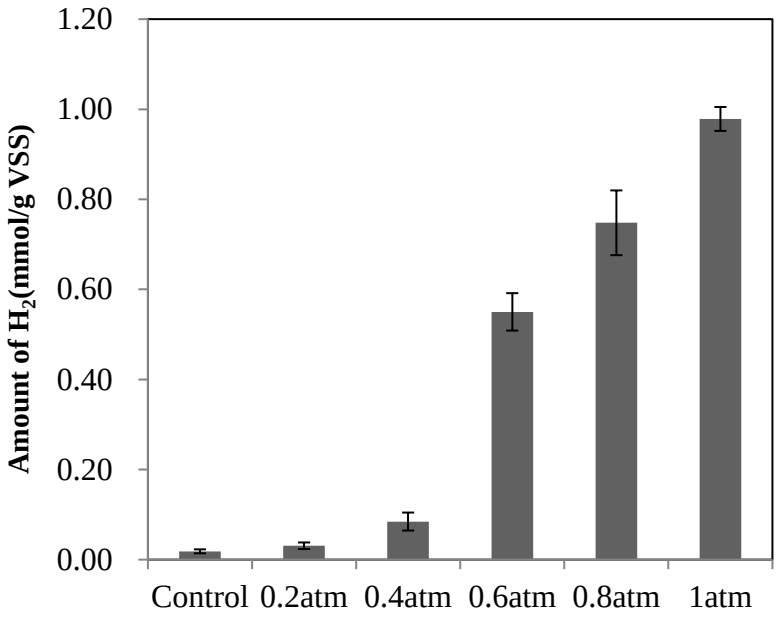


Fig. 3-11 Hydrogen accumulation under different CO partial pressures (atm) in the presence of BES at 37 ℃.

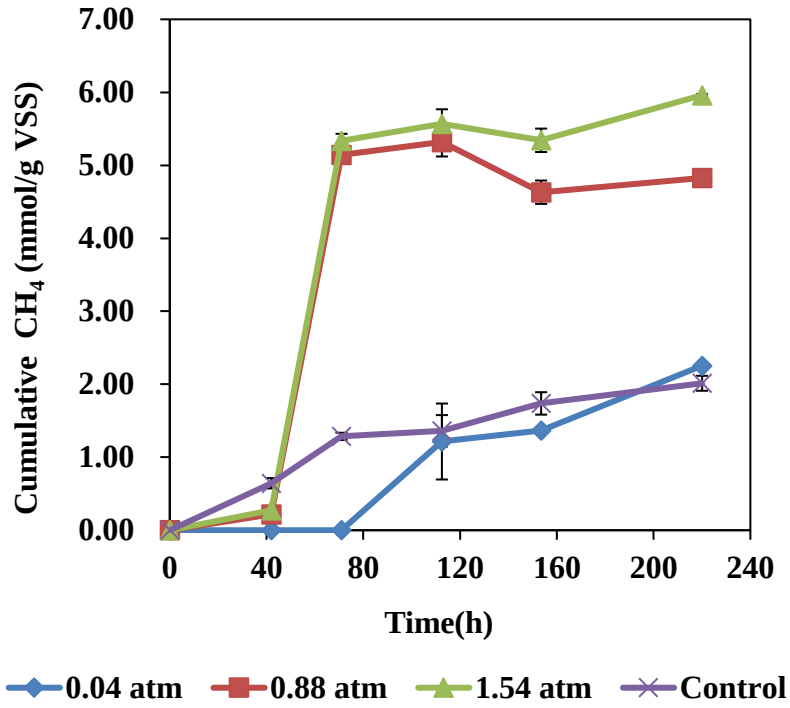


Fig. 3-12 Effects of H_2 partial pressures (atm) on cumulative CH_4 during BFG fermentation by AGS at 37 ℃.

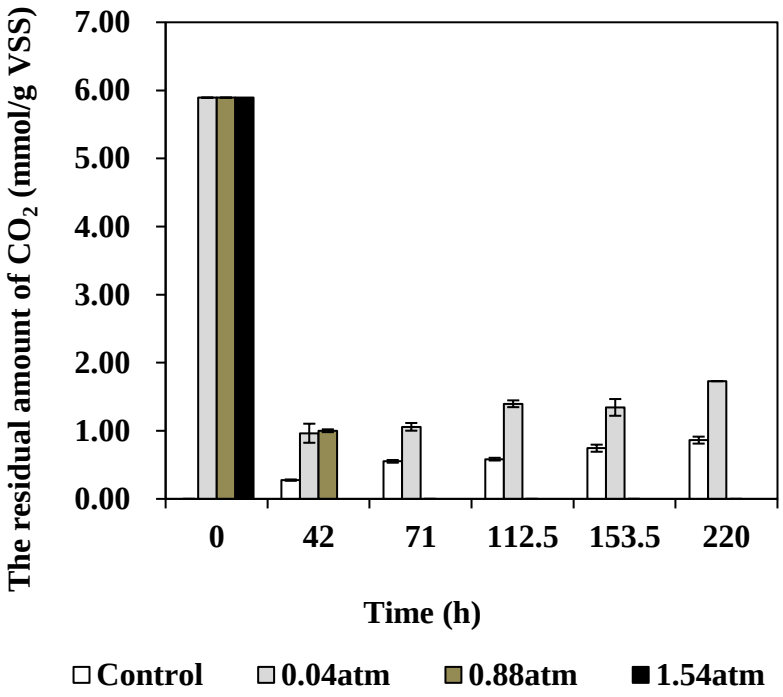


Fig. 3-13 Effects of H_2 partial pressures (atm) on residual CO_2 during BFG fermentation by AGS at 37℃.

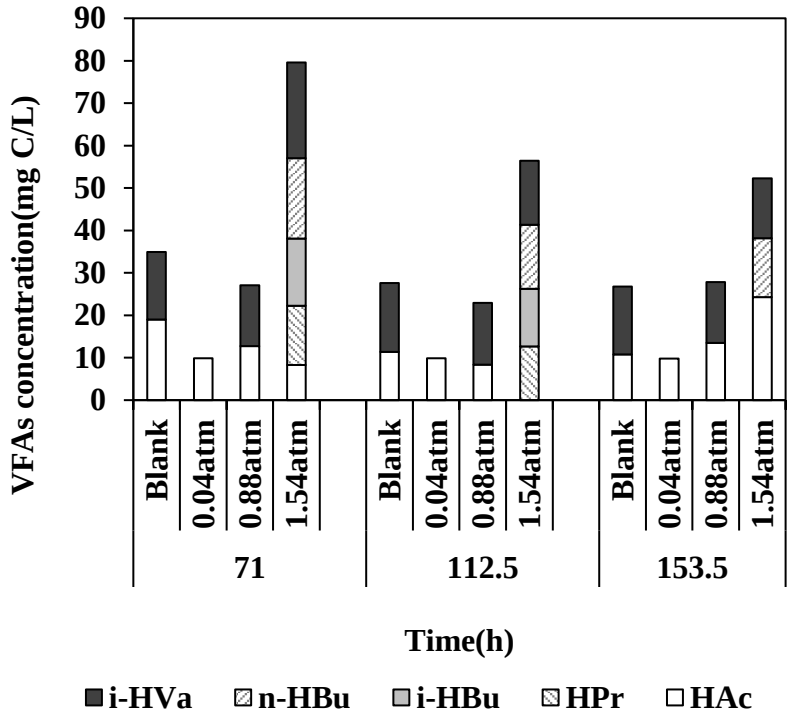


Fig. 3-14 Effect of H_2 partial pressures (atm) on VFAs distribution during BFG fermentation by AGS at 37°C.

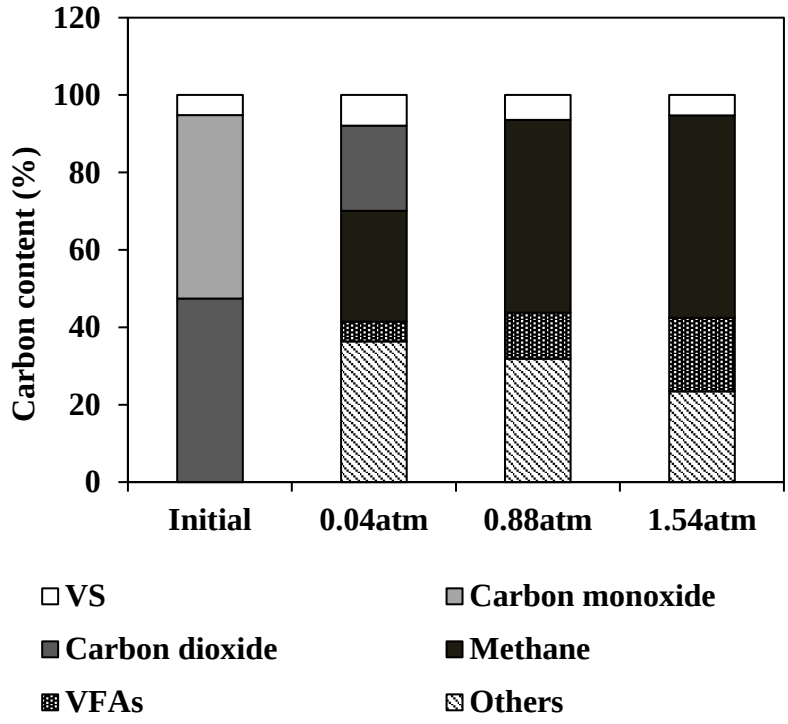


Fig. 3-15 Carbon balance analysis at different H_2 partial pressures during BFG fermentation by AGS on day 7.

Chapter 4 Conclusions

During the process of operation of a blast furnace in steel mills, reducing gases from coal and air combust are used to reduce iron ore to produce iron. The resulting exhaust gas (especially BFG) has a certain calorific value, and are usually used as fuel for power and steam generation. Even though it is a method to use this gas, but not effectively due to the low calorific value. Biological fermentation of BFG is an effective method for greenhouse gas (CO_2) or hazardous gas (CO) emission reduction and biofuels production. In this study, two kinds of inoculums were used for converting BFG to methane, which were acclimated methanogens under H_2/CO_2 and anaerobic granular sludge.

Firstly, for the acclimated methanogens under H_2/CO_2 as inoculum, the conclusions were draw as follows:

(1) A semi-continuous bioreactor presented good performance using acclimated methanogens for methane production in the H_2/CO_2 conditions. Among the different factors, besides basic medium, trace metal element can maintain the performance of the system's ability to stabilize methane yield. A steady-state was achieved with CH_4 production rate of 9.56 L/L_{liquid} d and 13.2 L/L_{liquid} d when the inlet flow rate was 54.72 L/d and 89.28 L/d.

(2) This research also provides insight into that acclimated methanogens under H_2/CO_2 was not suitable to use CO in industry exhaust (BFG) as sole energy and carbon source for CH_4 synthesis, since that it could just tolerate the CO partial pressure lower than 0.1 atm.

Secondly, when using the AGS as inoculum to convert BFG to methane, the following conclusions were obtained:

(1) AGS possesses potential for anaerobic conversion of substrates like H_2/CO_2, and acetate to methane. The corresponding hydrogenotrophic and acetoclastic methanogenic potentials (18.31 ± 1.2 mmol/g VSS d and 6.58 ± 0.38 mmol/g VSS d) were obvious. While, even though AGS might not adapt to CO as energy source, it also exhibited a promising carboxydotrophic potential (1.19 ± 0.03 mmol/g VSS d and 5.56 ± 0.26 mmol/g VSS d) under CO/N_2 and $CO/CO_2/N_2$. Compared with only CO as substrate, the co-presence of

hydrogen resulted in VFA accumulation. For the effect of P_{CO} to methane production, the methane production rate was found to be obviously inhibited when P_{CO} was higher than 0.4 atm. Compared with above mentioned acclimated methanogens, AGS presented obvious advantages to utilize CO for CH_4 production.

(2) BES was used to explore the pathways from CO to methane under mesophilic conditions, the intermediates mainly included acetate, propionate and H_2/CO_2. And they accumulated with the increase of P_{CO}.

(3) After H_2 was added together with BFG to reactor, VFAs accumulated at P_{H_2} up to 1.54 atm, which was mainly composed of acetic acid, butyric acid and valeric acid. Meanwhile, the remaining VFAs species and their concentrations under P_{H_2} = 0.88 atm were almost similar with those of the control. Therefore, P_{H_2} at 0.88 atm was more suitable to sustain the stable gas fermentation system even though methane yield was 4.70% lower than P_{H_2} up to 1.54 atm. This observation suggests that proper exogenous hydrogen can improve the biomethanation of BFG by using AGS.

Further work is necessary to improve the BFG and H_2 utilization and methane production efficiencies by controlling the mixing intensity or optimization of the anaerobic reactors systems. All the experiments were conducted in batch study and laboratory scale, in order to evaluate the real application, large scale test study is needed in the future. In addition, the changes in microbial communities during a long-term test should be paid attention shedding light on the mechanisms involved in this biological process.

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Appendix

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