H_2/CO_2 Fermentation for Carbon Dioxide Removal from Biogas

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Abstract

The biogas produced from the anaerobic degradation of waste has a calorific value of 21.48 MJ/m^3 (with about 60% methane content). Unfortunately, this promising energy source contains 30% of non-calorific carbon dioxide, which is released in the atmosphere as greenhouse gas when the biogas is used as energy provider.

Convert the carbon dioxide contained in the biogas to methane represents an advantage with double impacts. 1) Solve the problem of the greenhouse gas emission from anaerobic digestion and 2) increase the biogas energy value. The shift of carbon dioxide to methane necessitates finding out a reactor operational characteristics (material balance, dilution rate and temperature) that allow an efficient conversion performance and an economically less cost application process with gas as substrate. The present study proposes to achieve the sub-cited objectives by using H_2/CO_2 fermentation chemostat reactors with acclimated hydrogenotrophic methanogens culture.

Experimental chemostat reactors are regularly fed with minerals salt and limiting trace metals at different dilution rates; in addition, 12 L mix gases H_2/CO_2 (80:20, v/v) is supplied as limiting single substrate. The material balance is determined by the application of the Monod model to the steady state chemostat cultivation. The results show that 0.1/d is the dilution rate at which the cells concentration is maximal and the methanogenic activity, 0.41 LCH_4 /gVSS.d, the highest. The growth yield Y_{CH4} is 11.66 g cells formed /mmol H_2/CO_2 consumed. The maximal specific growth rate μmax and the Monod half saturation coefficient K_S are 0.15/d and 0.82 g/L respectively. The

determined material balance data are computed from the Monod chemostat model and the result predicts the dependence of the H_2/CO_2 concentration, S and the cell concentration, X on the dilution rate and the cells washout is realized when the dilution rate is 0.14/d.

In the H_2/CO_2 fermentation, like all bioprocess researches, the economic considerations play an important role in the plant design. I have investigated an experiment, the impacts of vary the mixing durations, the heat balance and the vitamin B_{12} production on the carbon dioxide conversion to methane process economy. Using four mixing durations (60 min/h, 45 min/h, 30 min/h and 15 min/h) to four reactors set up at 0.1/d dilution rate with two fermentation temperatures (37°C and 20°C). The results show that 60 min/h mixing duration has the maximum H_2/CO_2 gas dissolution rate in the liquid but the best conversion rate of H_2/CO_2 gas to methane is at 45 min/h mixing duration with 37°C (80.8%) and 20°C (39.8%). The continuous mixing rate may induce cells damage in the culture growth. I have measured the vitamin B_{12} presence in the effluent and the maximal production is at 45 min/h mixing, 3 mg/L-effluent for 37°C and 0.61 mg/L-effluent for 20°C cultivations. The application of the obtained experimental results to estimate the carbon dioxide reduction from the biogas produced in an anaerobic wastewater treatment plant (Chikusei City) gives the following results. The release of carbon dioxide in the atmosphere if the biogas produced from Chikusei City plant is use as energy source is reduced from 153.6 Nm3/d emission to 29.5 Nm3/d at 37°C and for 20°C cultivation, the reduction is from 173.9 Nm3/d to 111.6 Nm3/d. The methane content in biogas increases from 268.7 Nm3/d to 392.8 Nm3/d at 37°C and from 279 Nm3/d to 341 Nm3/d at 20°C.The vitamin B_{12} production is 32.7g/m^3 effluent at 37°C and 8 g/m^3 effluent at 20°C.

CHAPTER 1

General introduction

1.1 The carbon dioxide problem

Energy from fossil fuels had constituted an undeniable provider of life style until considered nowadays as correlated to the development; heretofore, it intervenes in human utilities for improving the framework of living, producing goods, etc.

However, the obvious main disadvantage of the fossils fuels is their participation to the greenhouse gas; the combustion of the fossils fuels in the energy sector activities poured out important amount of carbon dioxide (Bungay, 1981) which contribute to 80% of the total carbon dioxide emissions in 1999 as referred in Table 1.1. Moreover, the fossils fuels are not renewable and their depletion had already created serious impact on the world energy supply in several occasions; the well-known example is the energy crisis in the 1970s. From that crisis, the utilization of more reliable and environmental friendly energy source to replace fossils fuels was deeply investigated and promoted. These renewable energies are provided by various sources:

- Solar energy

The sun produces energy by consuming hydrogen during nuclear fusion reactions. The energy is used for heating or cooling, lighting homes and buildings, for electricity generation in commercial and industrial uses. Recently, new solar cells combining organic/inorganic hybrid cells have been experimented to reduce the cost of the conventional silicon solar cells (Günes and Sariciftci, 2008).

- Wind energy

The wind turbines capture the wind energy by the blades rotation. The wind energy is used to turn mechanical machinery to do physical work, such as crushing grain or pumping water. The technology has been improved to achieve a low cost instrumentation using a power controller (Ahshan et al., 2008).

- Hydrogen

As the most abundant element on the earth, hydrogen can be burned as a fuel or converted into electricity. Various systems are generally used to produce hydrogen; these are decomposition, reforming or gasification of carbon fuels (Docekal, 1986; Steinberg and Cheng, 1989) and the production of hydrogen from water electrolysis. Recent technology improvement has shown that biomass is a significant source of hydrogen production (Cheong and Hansen, 2007).

- Geothermal energy

The energy taps the earth's internal heat for a variety of uses, including electric power production, and the heating and cooling of buildings. The energy of the ocean's tides comes from the gravitational pull of the moon and the sun upon the earth.

- Hydropower

Also called the hydroelectric power, the energy derives from the water flow that can be captured and turned into electricity.

- Biomass energy

Three sources of biomass energy are recognized: wood, waste and alcohol fuels. The wood energy is derived both from direct use of harvested wood as a fuel and from the wood waste streams. The largest source of energy from wood is pulping liquor, a waste product from processes of the pulp, paper and paperboard industry.

Waste energy is the second-largest source of biomass energy. The main contributors of waste energy are municipal solid waste, manufacturing waste and landfill gas. The third is the biomass alcohol fuel or ethanol, derived from corn and used as oxygenate in gasoline.

These energy sources have been widely investigated to improve their performance and the utilization cost; because, as predicted by Donohue and Cogdell (2006), the world's energy demands will increase by the end of the 21st century; creating a big impact on the global environmental sustainability and economic stability.

Renewable energies are generally used for electricity generation; heat in industrial processes, heating or cooling buildings and in transportation as vehicle fuel. As example, in 2004, the electricity generation in the US accounted for about 70% of total renewable energy consumption. The total amount of electricity generated from renewable energy was 359 billion kilowatt-hours (kWh), about 9% of total United States of America electricity generation (EIA, 2004) presented in Figure 1.1. Industrial process heat and building space heating accounted for 25% of renewable energy use and the remainder was used as vehicle fuels.

An increasing number of renewable energy projects using biomass have been developed. Most of these use waste products from agriculture, so they solve a waste disposal problem and, at the same time, produce the biogas for use in homes, farms and factories.

Table 1.1 Total greenhouse gas emission in 1999 (International Energy Agency, CO_2 emissions from fuel combustion 1971-2000. IEA Statistics, 2002 Edition.)

Source Category	Total GHG emissions (Gt CO_2-eq.)	Contribution to total GHG emissions (%)	Share of CO_2 in each source category (%)
Fuel Combustion	11.4	80.0	98.2
Fugitive Fuel	0.4	3.0	14.3
Industrial Processes	0.7	4.9	50.5
Agriculture	1.2	8.3	N.A.
Other	0.5	3.8	N.A.
Total	14.2	100.0	N.A.

UNFCC, Report on national greenhouse gas inventory data from annex I parties for 1990 to 1999, FCCC/SBI/2001/13, 25 October 2001 and FCCC/SBI/13/Corr.1.excluding Belarus, Croatia, Romania, Liechtenstein, Lithuania, Russia, Slovenia and Ukraine for which 1999 inventories were not available.

N.A. = Not Available

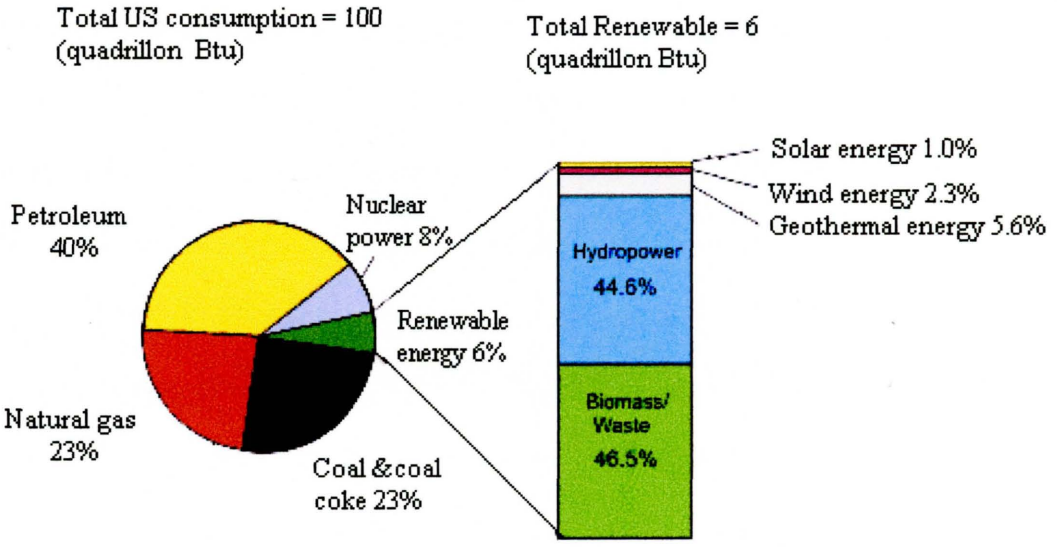


Figure 1.1 Contribution of Renewable Energy to U.S. Energy Consumption, 2004. (Source: Energy Information Administration, EIA)

1.2 Energy potential of biogas

The biogas that is produced from anaerobic digestion of biomass or wastewater contains generally 60% methane and about 30% carbon dioxide. The calorific value content of biogas (60% methane, 38% carbon dioxide and 2% other gas) is 21.48 MJ/m^3 (IEA Bioenergy Task 24, 2001); that capacity of energy production makes the anaerobic digestion a promising renewable energy source substitute of fossils fuels in many applications. Hence, the process utilization started to be actively encouraged to sustainable development in industrialized countries as well as in developing countries (Hall and Scrase, 1998). In Japan, the "Biomass Nippon Strategy" as national strategy has been focused to use 3.08 million kiloliters of biomass heat by 2010 (Suematsu, 2006); also presently, biomass energy supplies at least 3.6% of the primary energy [54.1 EJ with EJ= exajoules (10^{18}J)] in the OECD Europe and estimation of bioenergy potential in 2050 represents 9.0 to 13.5 EJ/year (Hall, 1994).

Unfortunately, the biogas has the disadvantage to contain about 30 to 40% of carbon dioxide, a non calorific component that:

a) reduce the total energy value of the biogas; the flame speed of carbon dioxide was measured to be 25 cm/s as against 38 cm/s for LPG (Liquefied Petroleum Gas).

b) is released in the atmosphere as greenhouse gas.

Therefore, despite the widespread of its utilization, anaerobic digestion still suffered from the proof to be economically profitable compared to fossils fuels and as greenhouse gas provider, there is real fear that our Society has to cope with the increase of the carbon dioxide emission not only from fossils fuels utilization but also from anaerobic fermentation gas emissions (Keller and Hartley, 2003; Cakir

and Stenstrom, 2005).

Varieties approaches have been investigated by authors to remove CO_2 from biogas. The absorption of carbon dioxide in water under pressure (about 7 to 10 bar) called water scrubber technology has demonstrated its capacity to reduce carbon dioxide from 41% in the biogas to 30 and 20% (Porpatham et al., 2007). Water from the resulted water containing dissolved carbon dioxide is regenerated through desorption with air. The dioxide absorption on ceramic particles has developed high efficient carbon removal in Japan (Taro et al., 2002) also for power plants industries.

Indirect methods to reduce the carbon dioxide such as improvement of the methane yields had been tested; Hartley and Lant (2006) have demonstrated a high level energy recovery for about 70% conversion of sewage COD to methane, in an Anaerobic Migrating Bed Reactor (AMBR) by operating with a micro-aeration utilizing biogas at useful power input of 6 W/m^3. Other authors had used an inexpensive biofilm composed by nylon mesh in a continuous stirred tank reactor (CSTR) to recovery the methane yield for less than 20% in an anaerobic dairy wastewater (Ramasamy and Abbasi, 2000).

Some biogas utilization does not have high gas quality requirements; these are gas heater, kitchen stove and Combined Heat Power (CHP) engines. In the other hands, Vehicle fuel and natural gas grid recommend biogas high quality and free from carbon dioxide.

The methanogenic bacteria inhabitants of the anaerobic digester have been used in several researches because of their potentiality to convert, among acetic acid and one-carbon molecules, carbon dioxide to methane. Hansson (1979) have applied a recirculation of CO_2 and CH_4 gases at low and high partial pressures to a

mixed microbial enriched culture from sewage sludge to determine theirs effects on methane production. The author found that a net increase in methane production occurs under supply of 0.05 atm partial pressure CO_2 (pCO_2) and proposed the gas recycling when pCO_2 pressure is high to increase methane production. Moreover, studies confirmed that CO_2 was rapidly reduced to CH_4 in the presence of H_2 until H_2 was exhausted (Ferguson and Mah, 1983). Hence, the possible conversion of the carbon dioxide comprise in the biogas to methane offers a promising procedure for carbon dioxide removal from biogas and increase the biogas energy value. Zhang and Maekawa (1993) have succeeded in producing methane from H_2 and CO_2 gases using a fermentor in batch cultivation.

Unfortunately, the procedure of gas circulation in a reactor lacks of engineering approach to find out and standardize the operational characteristics of digester to achieve the goal of conversion of carbon dioxide from biogas to methane. Furthermore, the economic considerations that play an important role in the plant design must be considered deeply.

1.3 Objectives of the study

The common chemostat reactor type was used because of its several advantages. The chemostat ensure the completely controlled experimental systems for testing microbial growth, competition and a high level mixing (each phase of the reactor contents is uniform). Materials and energy balances are key tools in achieving a quantitative understanding of the behavior of any systems. Generally, the mass balance in environmental system provides information for model and predicts the operation system.

The following objectives were investigated through the study:

Objective 1: Determination of the material balance in steady state chemostat cultivation of hydrogenotrophic methanogens when H_2/CO_2 gas is supply as sole substrate.

The obtained data will serve to generate a mathematical model that can express the hydrogenotrophic methanogens metabolic process and simulate the performance of a digester when H_2/CO_2 mixture gas is used as substrate. The purpose will be achieved by the utilization of a chemostat composed by a mix culture of acclimated methanogenic sludge previously collected from an anaerobic domestic wastewater treatment plant and acclimated during 8 months with H_2/CO_2 gas supply as substrate. Four chemostat operated at different dilution rates indeed D_1= 0.071/d, D_2= 0.083/d, D_3= 0.1/d and D_4= 0.125/d will held the experiment.

Objective 2: Find out the effect of H_2/CO_2 gas mixing duration variation, the heat balance and Vitamin B_{12} production on the cost performance of CO_2 conversion to methane when H_2/CO_2 is used as substrate.

The obtained data will serve to establish the fundamental basic engineering data of the conversion of CO_2 to methane when the feeding substrate is H_2/CO_2. The purpose will be achieved using four reactors stirred at 15min/h, 30 min/h, 45 min/h and 60 min/h at mesophilic (37°C to 40°C) and psychrophilic (20°C) condition. The dilution rate will be set up (0.1/d) constant for all dilutions.

From the resolution of the two objectives, a simulation to estimate the feasibility of conversion the CO_2 present in the biogas to methane was realized. The Chikusei City (TSUKUBA, Japan) domestic wastewater treatment center, will serve as pilot for the large-scale carbon dioxide removal from biogas estimation. Data from the period of April 2006 to March 2007 will be used.

CHAPTER 2

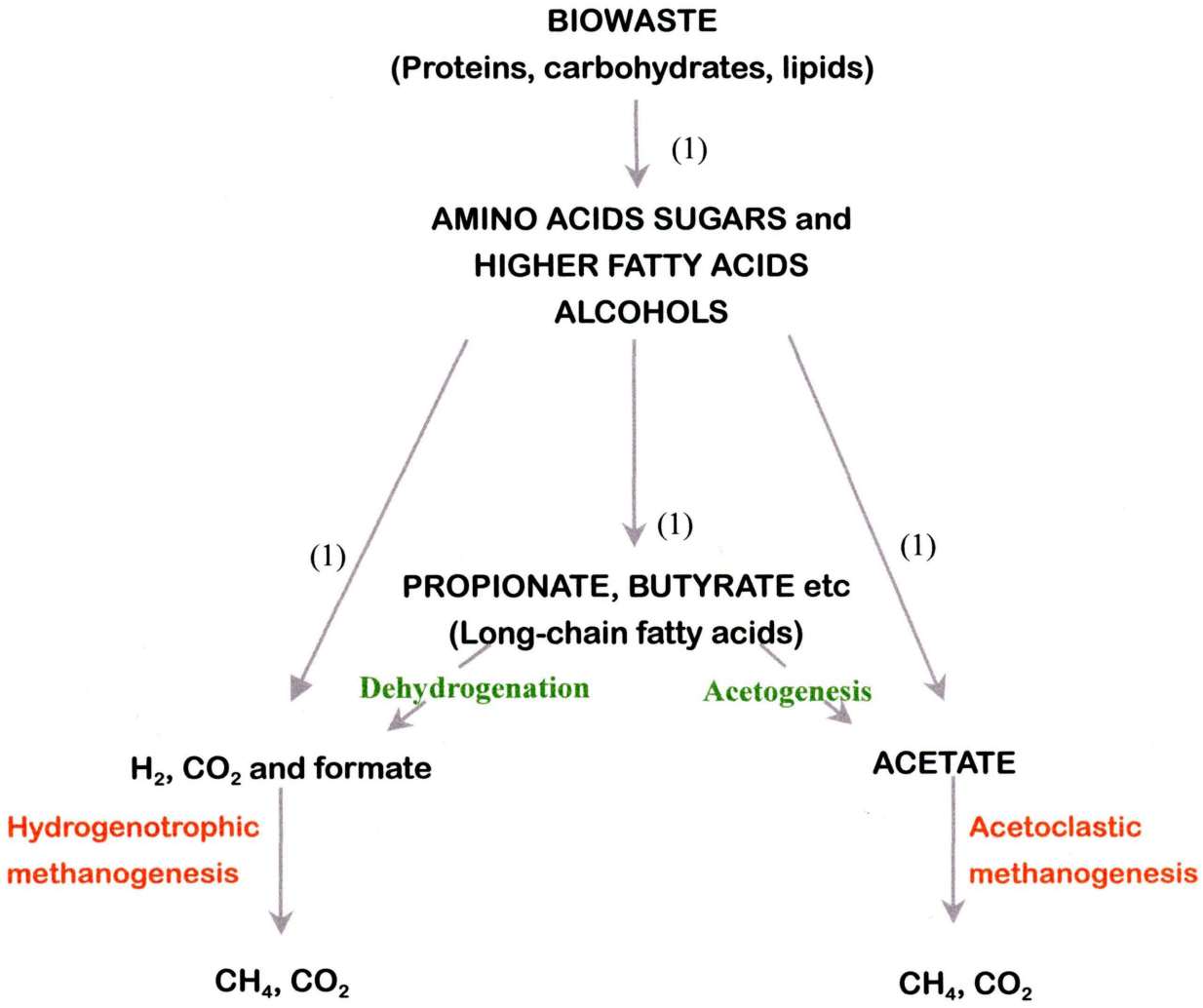
Studies of methanogenesis from H_2/CO_2

2.1 Generalities

The anaerobic digestion technology has been widely investigated due to its great advantages to treat variety of waste and the biogas generated, as end product can be useful as an energy source. The process involves six interacting microbial reactions (Figure 2.1) described by Gujer and Zehnder (1983):

1. hydrolysis of proteins, lipids and carbohydrates;
2. fermentation of sugars and amino acids;
3. anaerobic oxidation of long chain fatty acids and alcohols;
4. anaerobic oxidation of intermediates such as volatiles fatty acids (with the exception of acetate);
5. conversion of acetate to CH_4; and,
6. conversion of H_2 to CH_4.

The anaerobic fermentation involves several bacteria strains and understanding the growth of the microbial strains in the different steps is important to operate a fermentor.



(1) hydrolysis and acid fermentation process

Figure 2.1 Anaerobic biodegradation process in an anaerobic digestor.

Figure 2.1 showed that methanogenesis is the part of the anaerobic degradation process that decompose single or two carbons materials (acetate, carbon dioxide or formate) resulting from the degradation of complex organic polymers to methane. Methanogenesis occurs under two bacteria strains type the acetoclastic methanogens and the hydrogenotrophic methanogens. Ferry (1993) has demonstrated that in the anaerobic environment, the methane production originates preferentially from the acetoclastic pathway for about 2/3 of the total methane produced because acetate is an end product of several fermentation pathways; hence much more present comparing to H_2.

The hydrogenotrophic pathway in Figure 2.2 (Shima et al., 2002) and the acetoclastic pathway in Figure 2.3 (Ferry, 1992) have been investigated in several researches and their required enzymes determined because of their importance as last step for methane gas production. The conversion of carbon dioxide in anaerobic degradation involves the hydrogenotrophic pathway.

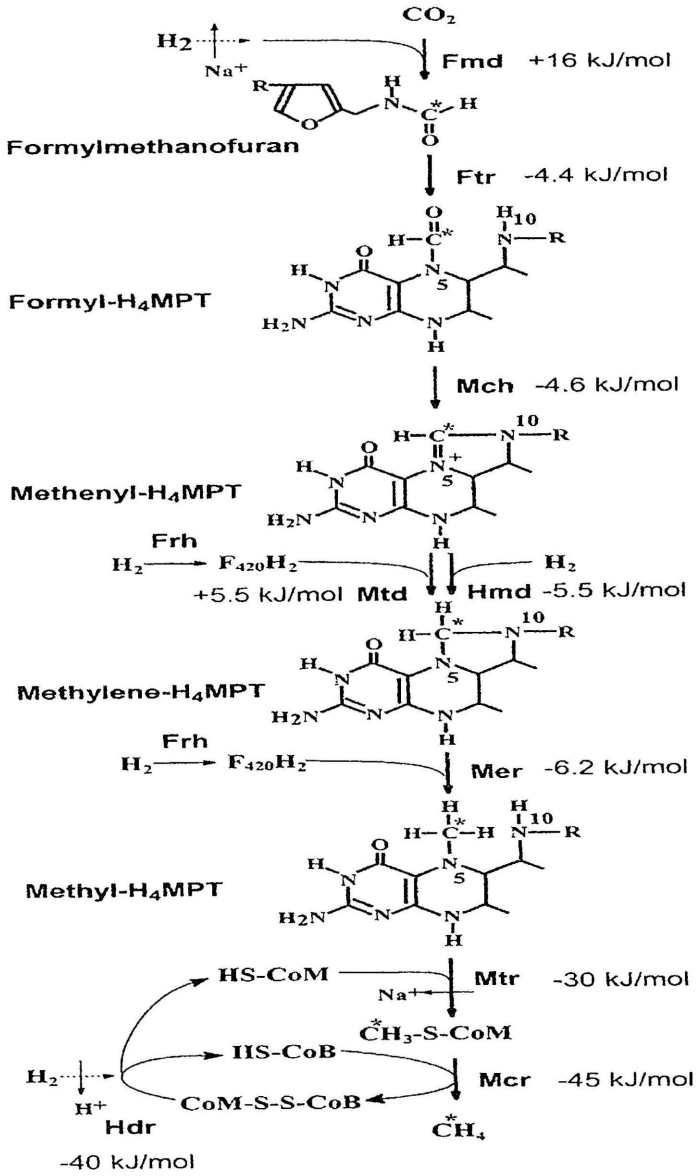


Figure 2.2 Methanogenesis pathway from H_2 and CO_2 with the involved enzymes and the free energy $\triangle G^{0'}$ flow: H_4MPT, tetrahydromethanopterin; HS-CoM, coenzyme M;HS-CoB, coenzyme B; CoM-S-S-CoB, heterodisulfate of coenzyme M and coenzyme B; Fmd, formylmethanofuran dehydrogenase; Ftr, formylmethanofuran: H_4MPT formyltransferase; Mch, methenyl-H_4MPT cyclohydrolase; Mtd, F_{420}-dependant methylene-H4MPT dehydrogenase; Hmd, H_2-forming methylene-H_4MPT dehydrogenase; Mer, methylene- H4MPT reductase; Mtr, methyl-H_4MPT: coenzyme M methyltransferase; Mcr, methyl-coenzyme M reductase; Hdr, heterodisulfide reductase; Frh, F_{420}-reducing hydrogenase. (Shima et al., 2002)

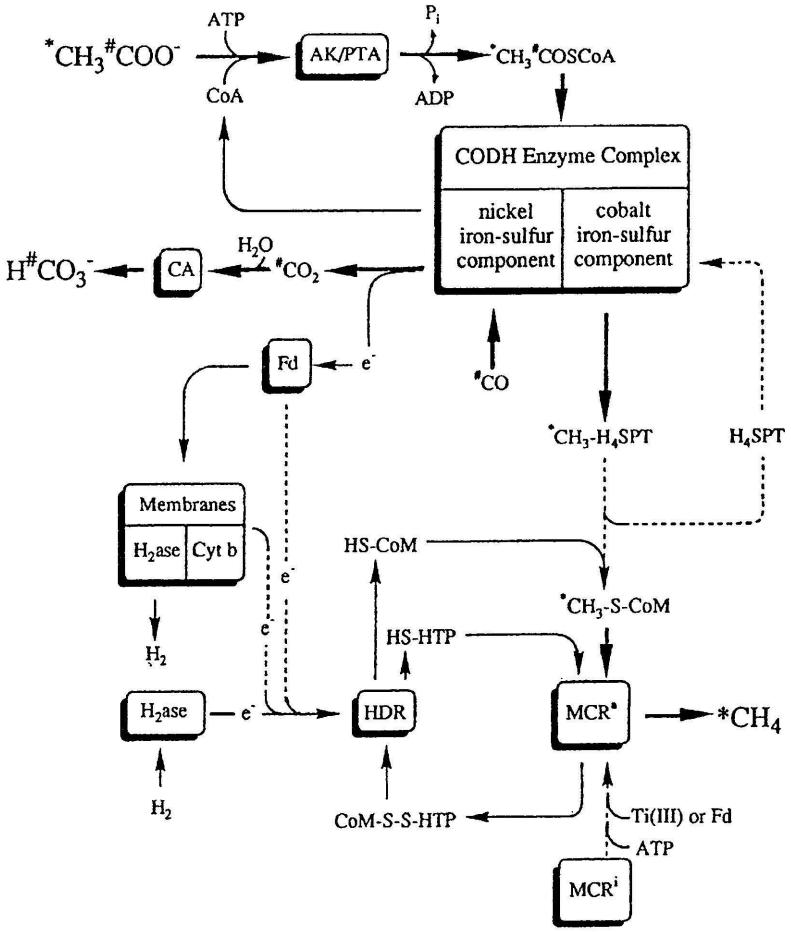


Figure 2.3 Methanogenesis from Acetate: AK, acetate kinase; PTA, phosphotransacetylase; CA, carbonic anhydrase; MCR^i, inactive methylreductase; MCR^a, active methylreductase; HDR, heterodisulfide (CoM-S-S-HTP) reductase; Fd, ferredoxin; Cyt b, cytochrome b; H_2ase, hydrogenase; e^-, electron. Dashed lines represent the not yet well understand matter in the pathway. Carbon marked with* and # symbols distinguish the methyl and carbonyl groups respectively. (Ferry, 1992)

2.2 H_2 and CO_2 as substrate in the methanogenesis

The majority of the methanogenic archaea can derive their metabolic energy for the autotrophic growth from the reduction of CO_2 to methane with H_2 as energy source; exceptions of some strains such as *Methanothrix* spp., which grow only under acetate as substrate; *Methanosphaera stadtmaniae* that reduce methanol in the presence of hydrogen and *Methanolobus tindarius* synthesizes under only methylamine and methanol (Boone et al., 1993). The catalytic relation using CO_2 as carbon source and H_2 as electron acceptor is:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad \Delta G^{0'} = -130.7 \text{ kJ/mol } CH_4 \qquad (2.1)$$

The hydrogenotrophic methanogenesis is a highly exergonic process in standard conditions; however, in natural ecosystem and also in anaerobic digester, the hydrogen partial pressure (10^{-5} to 10^{-4} atm) and concentration was found to limit methanogenesis; H_2 in anaerobic environment never accumulate because it is rapidly utilized even at low concentration (Wolin, 1976).

In order to increase the mass transfer rate of hydrogen and reduce the energy losses inherent to hydrogen gradients, hydrogen producers and methanogens live in close proximity (Conrad et al., 1986).

2.2.1 Factors inhibiting the H_2 and CO_2 dependant bacteria activity

- Metals and compounds

Zhang et al. (2003) have recently showed the necessity of the trace metals to stimulate the bacteria metabolic activity but at suitable amount.

For the formation of 1 g of *Methanobacterium thermoautotrophicum* cells (dry-weight), Schönheit et al. (1979) have determined that approximately 150 nmol $NiCl_2$, 20 nmol $CoCl_2$ and 20 nmol Na_2MoO_4 are required. Others elements

metals Fe, Al, Zn have shown their importance as electron source in the metabolic activity of methanogenesis from H_2 /CO_2 (Belay and Daniels, 1990). However, Van Bodegom et al. (2004) have demonstrated direct inhibition of methanogenesis by amorphous Ferric (III) Iron hydroxide at concentrations between 0 and 10 mM in experiments with pure cultures of *Methanospirillum hungatei* and *Methanosarcina barkeri* that grown with H_2/CO_2. Moreover, copper (Cu^{+2}) at concentration of 8.9 mg/L inhibits 50% of the hydrogenotrophic methanogens activity (Karri et al., 2006).

- Hydrogen partial pressure

Cord-Ruwisch et al. (1988) have examined the H_2 threshold for large range of hydrogenotrophic strains (Figure 2.4 and Table 2.1) because H_2 partial pressure below to 6.5 Pa inhibited several mesophilic hydrogenotrophic methanogens activity in the conversion of hydrogen to methane (Lovley, 1985). Figure 2.4 explains the competition that occurs in natural environment, the addition of 10 to 20 mM sulfate to sediments resulted in a decrease in the hydrogen partial pressure and a concomitant inhibition of methane production (Lovley et al., 1982).

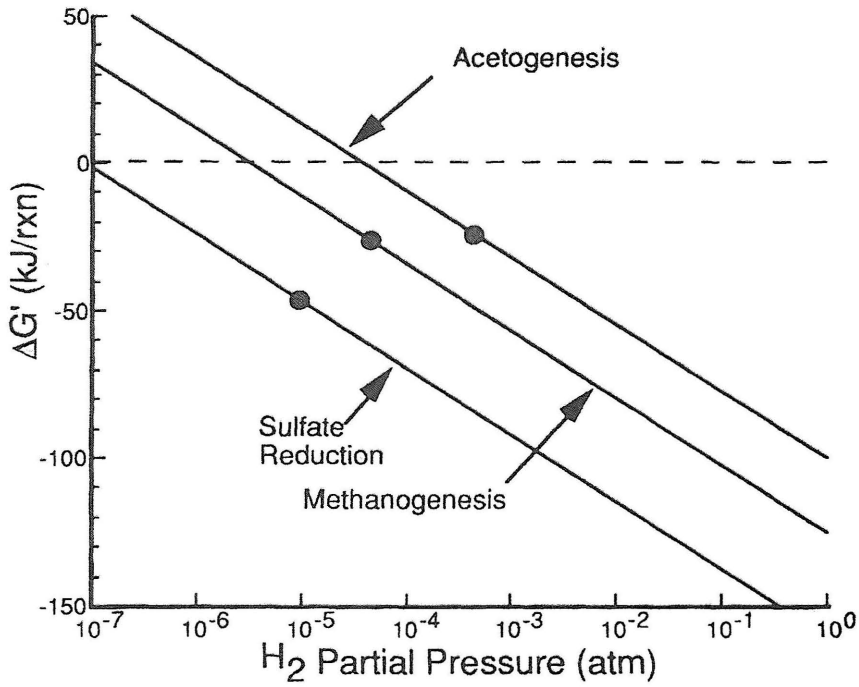


Figure 2.4 Effect of H_2 partial pressure on free energy of methanogenesis, sulfate reduction and acetogenesis using H_2. (Zinder, 1993)

Table 2.1 Thresholds for hydrogenorophic anaerobes. (Cord-Ruwisch et al., 1988)

			H_2 Threshold	
Organism	Electron Accepting Rxn*	$\Delta G^{o'}$ (kJ/ mol H_2)	(Pa)	(nM)
Acetobacterium woodi	CO_2 → acetate	-26.1	52.0	290.0
Methanospirillum hungatei	CO_2 → CH_4	-33.9	3.0	23.0
Methanobrevibacter smithii	CO_2 → CH_4	-33.9	10.0	75.0
Desulfovibrio desulfuricans	SO_4^{2-} → H_2S	-38.9	0.9	6.8
Acetobacterium woodi	caffeate → hydrocaffeate	-85.0	0.3	2.3
Wolinella succinogens	fumarate → succinate	-86.0	0.002	0.015
Wolinella succinogens	NO_3^- → NH_4^+	-149.0	0.002	0.015

*Rxn = Reaction

However, Diamond and Akinfiev (2003) have found discrepancies in the values of the carbon dioxide dissolution in pure water after assembled several literatures data in large ranges temperature and pressure. Someya et al. (2005) have measured the behavior of the carbon dioxide dissolution in pure water; they determined that the solubility decreased with decreasing temperature and with decreasing pressure. The solubility of hydrogen is generally considered to decrease with increasing temperature.

In both case, the pressure is showed to be an important factor in the gas solubility in water.

2.2.2 Substrate availability and physical environment

Lithotrophic synthesis methanogens obtain metabolic energy for their growth and maintenance from hydrogen. Increasing the consumption rate of substrate, by means of the supply of specific amount of trace metals, affect undoubtedly the growth yield then the anaerobic digestion performance. By comparing the community structure of hydrogenotrophic methanogens under low and high hydrogen substrate concentration Leybo et al. (2006) have demonstrated that concentration of substrates also affect the H_2-dependant methanogens strains growth. Ammonia as one of the most present component in the anaerobic digestion phase inhibits at 350mM the hydrogenotrophic methanogens growth at variable pH-levels and temperatures (Koster and Koomen, 1988). Hydrogenotrophic methanogens are present in thermophilic, mesophilic and psychrophilic environment (Bryant and Boone, 1987). The growth yield of some species under variable substrate has been determined by different authors (Table 2.2).

Table 2.2 Typical growth of some methanogenic bacteria.

Organism	Growth yield (g dry weight/mol methane) after growth on			References
	H_2/CO_2	formate	methanol	
Methanobacterium bryantii	2.5	-	-	Robertson and Wolfe (1970)
Methanobrevibacter arboriphilus	2.7	-	-	Zehnder and Wuhrmann (1977)
Methanobacterium thermoautotrophicum[a]	1.6-3[b]	-	-	Schönheit et al. (1980)
Methanobacterium formicicum	3.5	4.8	-	Schauer and Ferry (1980)
Methanosarcina barkeri	6.4	-	7.2	Weimer and Zeifus (1979)
Methanosarcina thermophila[a]	-	-	4.5	Zinder and Mah (1979)

[a] Thermophilic organisms

[b] Observed variations between hydrogen-excess (lower value) and hydrogen limited conditions (upper value).

2.3 Kinetics of substrate utilization for the bioreactor design and operation

Various cells kinetics models have been presented and are dependent on the specific experiments conditions. Two types of models groups can be distinguished:

2.3.1 Structured models

Those models type assumed that the biomass is composed of components based on physiological and morphological functions (Nielsen and Villadsen, 1994) and the cells population composition change significantly until influence the kinetics. Harder and Roels (1982) have reported that different models have been determined, most derived from a general equation which showed that the biophase variables employed in structured models are typically the mass (x_j) or molar (c_j) concentrations per unit of biophase. For a well-mixed reactor, the material balance on component j gives

$$\frac{d}{dt}\left[\frac{1}{\rho_c} V_R x c_j\right] = \frac{1}{\rho_c} V_R x r_{fi} + \frac{1}{\rho_c} \Phi_x c_j \qquad (2.2)$$

where ρ_c is the mass density of cells (cell mass/unit volume cells), r_{fi} is the molar rate of formation of component j [mol j · time^{-1} · (unit volume cells)$^{-1}$], Φ_x is the mass of cells added to the reactor by flow · time $^{-1}$, x is the cell mass concentration (cell mass/ unit volume culture), c_j is the moles j/ unit volume cells, V_R is the culture volume.

Assuming that ρ_c, the cell density and the volume of culture V_R are constant in time, Eq. (2.1) becomes

$$\frac{dc_j}{dt} = r_{fi} - c_j\left(\frac{1}{x}\frac{dx}{dt}\right) + c_j \frac{\Phi_x}{xV_R} \qquad (2.3)$$

2.3.2 Unstructured models

In those models, only the biomass is used as parameter to characterize the kinetics. The change in the biomass composition is ignored and all cells in the reactor are considered as single component, the biomass concentration. The unstructured models described efficiently the steady state microbial growth (Nicolaï et al., 1991; Santoyo et al., 1997).

Monod model

Monod model is applicable to bacterial growth in pure culture chemostat when the specific growth rate is dependent of x, the cell mass and s, the substrate concentration (Monod, 1973).

$$\mu = \frac{\mu_{\max} s}{K_s + s} \tag{2.4}$$

where K_s is the half velocity coefficient (g/L), $\mu_{\max}$ is the maximum specific biomass growth rate (g/L), s the limiting substrate concentration (g/L), μ is the microbial specific growth rate (g/L).

Contois model

The model is used when the biomass is inhibited by the substrate concentration (Beba and Atalay 1986; Hu et al., 2002); the specific growth rate is considered as a function of the growth-limiting nutrient in the both influent and effluent substrate concentration by utilizing a constant B related to microbial concentration.

$$\mu = \frac{\mu_{\max} s}{Bx + s} \tag{2.5}$$

where B is the Contois apparent saturation constant (g substrate/g- cell), x is the biomass amount in the liquid growth (g/L).

Tessier model

The model is used in the case of a double substrates addition to the anaerobic culture growth (Andrews, 1968; Sonmezisik et al., 1998).

$$\mu = \mu_{\max}\left(1 - e^{-s/K_s}\right) \tag{2.6}$$

where K_s is the Tessier apparent saturation constant (g/L)

Moser model

$$\mu = \mu_{\max}\left(1 + K_s s^{-\lambda}\right)^{-1} \tag{2.7}$$

where λ is the exponential constant in Moser equation (dimensionless).

2.3.3 Ideal reactors for bacterial kinetics measurement

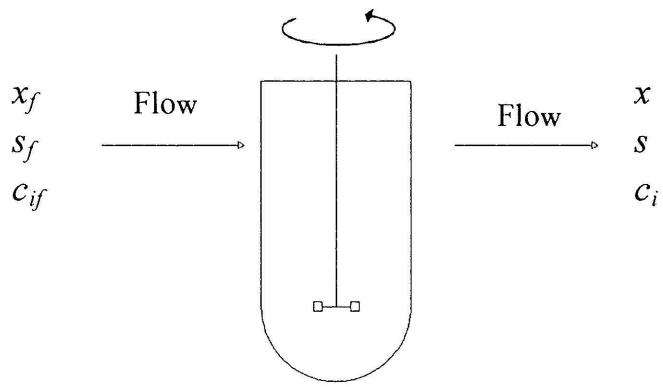
The microbial populations' kinetics information in anaerobic fermentation can be achieved by using ideal type reactors.

Ideal continuous stirred tank reactor or chemostat

The concentration of dissolved substrate is assumed to be the same throughout the bulk liquid phase and at the steady state, the material balance which are considered to be the key tools to achieve a quantitative understanding of the environment systems behavior is:

$$[Rate\ of\ addition\ to\ reactor] - [Rate\ of\ removal\ from\ reactor] +$$
$$[Rate\ of\ formation\ within\ reactor] = 0$$

By representation of the schematic chemostat as:



x_f and x represent the cell mass per unit culture volume in the feeding and in the effluent respectively, s_f and s represent the substrate per unit culture volume in the feeding and in the effluent respectively.

The steady state CSTR material balance can be written as:

$$F(C_{if} - C_i) + V_R r_{fi} = 0 \tag{2.8}$$

V_R represents the total volume of culture within the reactors (L), F is the volumetric flow rate of feed and effluent liquid streams (L/ time unit), C_{if} represent the component I molar concentration in the feed stream (g/L), C_i is the component i concentration in the reaction mixture and in the effluent stream (g/L).

Ideal batch reactor

The concentration of the nutrients, cells, and products vary with the time; the rate of accumulation of a reaction product i in time function is equal to the rate of formation of i product due to chemical reactions in the fermentor; thus

$$\frac{d}{dt}(V_R \cdot c_i) = V_R \cdot r_{fi} \tag{2.9}$$

where V_R is the culture volume (L), c_i is the moles of i per unit culture volume (mol/L), r_{fi} is the mol of i formed by reaction per unit volume in unit time (mol/L s).

2.4 Mixing and temperature in the methanogenesis

2.4.1 The mixing parameter

Poulsen and Iversen (1998) have related the gas-liquid mass transfer to the mixing intensity; several others authors have showed the importance of the mixing in the anaerobic treatment (Lin and Pearce, 1991; Holland and Chapman, 1966). From the various methods for mixing inside the reactor, Lee et al. (1995) have investigated and showed that, when the biogas pressure recirculation is used as mixing power, the performance of the reactor is increased. The authors had therefore, proposed the utilization of the gas recirculation as low cost operational method.

2.4.2 The temperature parameter

Until recently, the mesophilic (24 - 45°C) and the thermophilic (45 - 65°C) temperature ranges have been widely investigated because of the process instability at psychrophilic (< 20°C) temperature (Zeeman et al. 1988). Increasing authors have interested in the anaerobic degradation at low temperature because the majority of industrial effluents are discharged at low-ambient temperatures (Lettinga et al., 2001). Kotsyurbenko et al. (1996) have shown that the H_2/CO_2-utilizing methanogens have better activity at low temperature compared to the acetotrophic methanogens. However, the microbial structure have been investigated at temperature variation and Fey and Conrad (2000) observed that the temperature affect not only the methanogens activity but also changed the structure and the function (carbon and electron flow) of a complex methanogenic system.

CHAPTER 3

Model an operational condition for hydrogenotrophic methanogens growth in chemostat under H_2/CO_2 substrate

3.1 Introduction

Numerous researches have extensively demonstrated the capability of methanogenic bacteria to grow on molecular hydrogen (H_2) and utilize carbon dioxide (CO_2) as an energy source (Daniels et al., 1984; Jones et al., 1985). The methanogens are inhabitants of the anaerobic fermentor, which is currently considered as an attractive process for degradation of various types of organic matters into methane and other byproducts. The construction of mathematical model continues to be a crucial step in order to design, control and operate the fermentor (Batstone, 2006). Based on previous literature, several models have been proposed to simulate and improve the fermentor performance through the supply of organic substrates (Siegrist et al., 2002; Nopharatana et al., 2003). Monod kinetics model is the most simple and commonly used approach; it is based on the bacterial growth parameters and constants. According to the model, the specific growth rate of biomass (μ) [1/d] is assumed to be related to the residual substrate concentration (S) [g/L].

The relation has been used to model the single substrate H_2 consumption in the presence of another substrate (Robinson and Tiedje, 1982); additionally, it has been used to model the anaerobic acidogenesis (Demirel and Yenigün, 2002). However, there is no report regarding the use of Monod kinetics model for acclimated hydrogenotrophic methanogens under steady state continuous

cultivation with recirculation of H_2/CO_2 as a single substrate. The chemostat, which is an important system for the study of microbial population dynamics (Tang et al., 1997), was associated with steady state cultivation that provided as proposed by Valentine et al. (2004), a better ratio of H_2/CO_2 conversion to methane and carbon dioxide during the growth of a moderate thermophilic methanogens under conditions of H_2/CO_2 gas. The present investigation was performed on 4 chemostat reactors with varied inorganic medium flow rate. The objectives of this study were as follows: firstly, to realize the steady-state condition at limiting substrate supply of H_2/CO_2-dependant methanogens during chemostat cultivation; subsequently to apply the Monod relation to investigate the characteristics of H_2/CO_2 gas fermentation. The obtained parameters would provide data for the production of a generic model for dynamic simulation of biogas production in a mixed H_2/CO_2-dependant methanogens culture using gas as the substrate.

3.2 Materials and Methods

3.2.1 Acclimation of hydrogenotrophic methanogens

Anaerobic activated sludge (2.5 L) obtained from domestic waste water treatment plant (Ibaraki, Japan) was acclimated in duplicate 5 L reactors at mesophilic temperature for 7 months; H_2/CO_2 (80:20, v/v) gas was used as the solitary source of energy and carbon. Using a 2 L gas bag, the gas substrate was recirculated at 0.08 MPa for each reactor, and small amount of H_2/CO_2 (80:20, v/v) gas was supplied daily to the gas bag in order to maintain the viability of the cells (Figure 3.1). Archaea cultivation procedure (Sowers et al., 1995) was implemented and the acclimation was continued until the H_2/CO_2-dependant methanogens were considered to be predominant in the culture. The physical and chemical

compositions of the obtained acclimated sludge were averaged from the 2 reactors and are shown in Table 3.1; according to the results, the measured acetic acid concentration was found to be nil, and 56.6% methane was produced. Generally, the H_2-consuming methanogenic strains in the anaerobic fermentor comprise the acetotrophic methanogens and the H_2-consuming methanogens (Zinder, 1993). In the present investigation, the absence of acetic acid indicates the absence of acetotrophic methanogens in the growth culture. Thus, the possibility of methane production from substrates other than H_2/CO_2 gas can be excluded. The elimination of acetic acid from the reactor was achieved after 7 months of acclimation and using H_2/CO_2 as the sole source of carbon and energy.

3.2.2 Epifluorescent microscopy analysis

The acclimated sludge samples were filtered using a polycarbonate black filter of 0.22 μm pore size diameter. Using ethidine bromide (Sigma-Aldrich, Poland) as the stain, the sludge samples obtained after filtration were placed on a slide and observed under ultraviolet using ultra microscopy (OLYMPUS, BX 50, Japan). Under optical microscopic view, the uniform blue-autofluorescence colour of $factor_{420}$ that is a characteristic feature of the methanogens verified the purity of the culture. Morphological examination of the bacteria revealed long rod-shaped cells and cocci (Figure 3.2).

3.2.3 Experimental procedure for continuous chemostat reactors

A modified version of the media used by Yang et al. (2004) was applied (Table 3.2). The mineral nutrients and trace metals were separately, boiled and cooled individually under H_2/CO_2 (80:20, v/v) gas to remove any traces of O_2. The

estimated pH values of mineral nutrients and trace metals were 7.95 and 6.93, respectively. The media were stocked in two 5 L Duran vials; in order to maintain an anaerobic environment, the headspaces were gassed with H_2/CO_2 (80:20, v/v) for 15 min at 0.1 MPa.

Chemostat cultivation

Using different dilution rates (D), i.e. $D_1 = 0.071/d$, $D_2 = 0.083/d$, $D_3 = 0.1/d$ and $D_4 = 0.125/d$ with nutrients and fixed amount of trace metals (10 mL/L-culture), the acclimated sludge was grown in 4 chemostat semi-continuous stirred tank reactors of 1 L each with 500 mL working volume (Figure 3.3). The recirculation of H_2/CO_2 (80:20, v/v) gas feed assumed a uniform composition throughout the reactor by the utilization of an airtight pump connected to a timer working at 1 min interval; the flow rate of H_2/CO_2 (80:20, v/v) gas was maintained at 0.83 mL/min at 0.08 MPa.

During the experiments, the reactors were disposed in an incubator chamber and maintained at 37 ± 2°C. The liquid growth culture was taken from the reactors and replaced anaerobically with inorganic nutrients and trace metals at the abovementioned dilution rates. A new aluminum tedlar bag CCK from by GL Sciences (Figure 3.4) containing pure H_2/CO_2 gas was connected to the recirculation gas system at 24 h intervals to obtain a daily estimate of the CH_4 gas produced during the experiment. A steady state control over the fermentation systems was considered to be achieved when there was no significant change in the amount of bacteria during the 4 time operation of the reactors. No pH adjustment was made for the media during the experiments.

Table 3.1 Characteristics of acclimated methanogens under H_2/CO_2 as sole substrate after 7 months acclimation representing the average of the duplicate reactors.

Parameters	Values average	Standard deviation
COD (g/ L)[a]	9.4	0.387
TS (g/L)[b]	120.7	4.000
VSS (g/L)[c]	116.6	0.550
Total N (g/L)	2.2	0.243
pH	7.7	0.200
Acetic acid (mg/L)	0.0	0.000
Methane production (%)	56.6	1.700

[a] COD, chemical oxygen demand.

[b] TS, total solids.

[c] VSS, volatiles suspended solids.

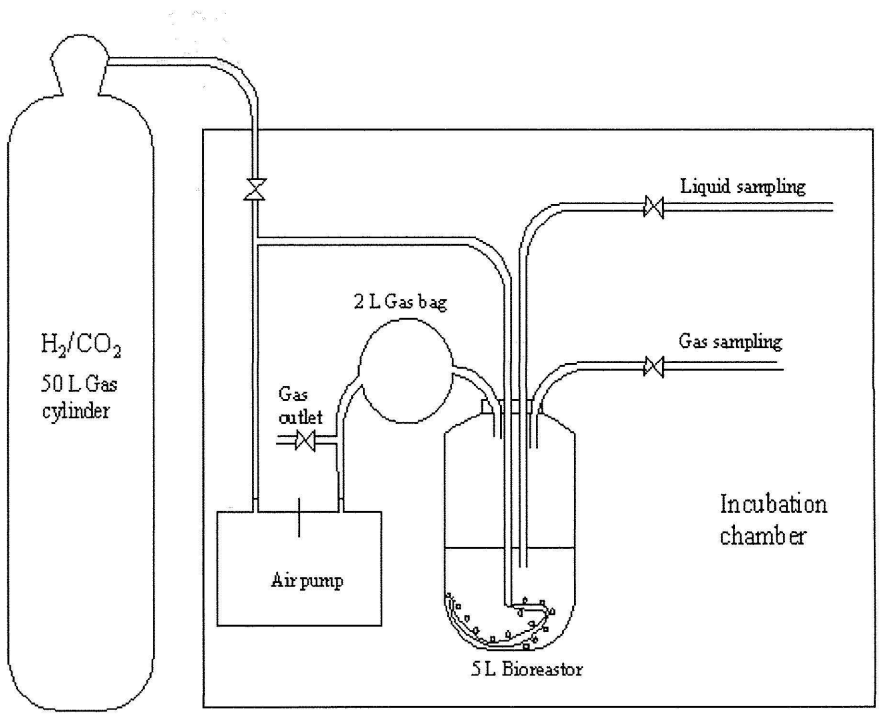


Figure 3.1 Schematic diagram for hydrogenotrophic methanogens acclimation.

Figure 3.2 Illustration of the hydrogenotrophic methanogens acclimation. (Schematic see Figure 3.1)

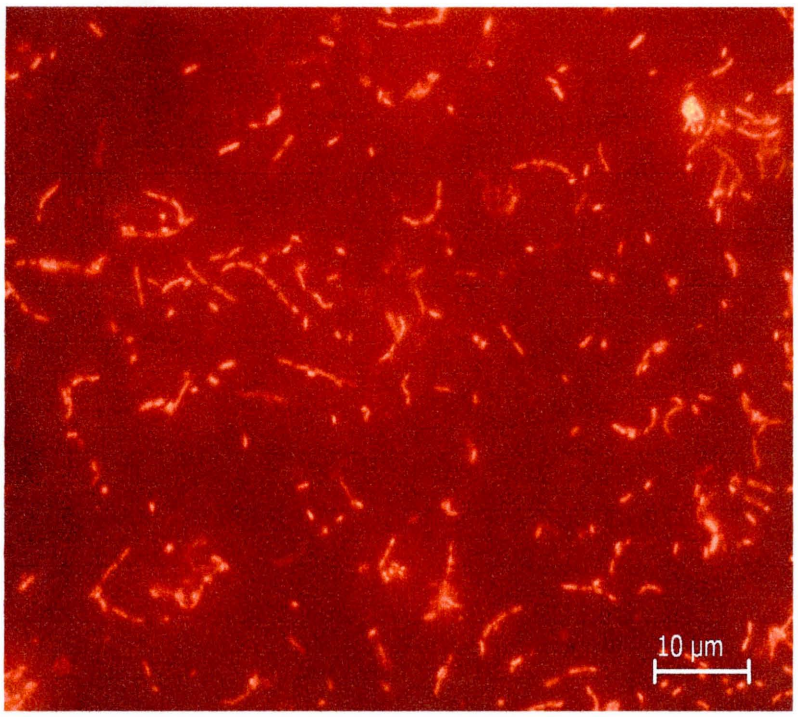


Figure 3.3 Hydrogenotrophic methanogens after 7 months acclimation. Magnitude 1000 Olympus BX 50

Table 3.2 Composition of the sterile inorganic media for methanogens growth.

Nutrients (g/L)		K_2HPO_4	3.4
		KH_2PO_4	3.4
		Na_2CO_3	2.54
		NH_4Cl	2.13
Trace metals solution (mg/L)			
$MgSO_4 \cdot 7H_2O$	600	$CaCl_2 \cdot 2H_2O$	40
$MnSO_4 \cdot 5H_2O$	5.3	$ZnSO_4 \cdot 7H_2O$	30
$NiCl_2 \cdot 6H_2O$	125	$AlK(SO_4)_2 \cdot 12H_2O$	1
$FeSO_4 \cdot 7H_2O$	28	H_3BO_3	1
$CoCl_2 \cdot 6H_2O$	10	$Na_2MoO_4 \cdot 2H_2O$	1.5

(Source Zhang et al 1994; Yang et al, 2004)

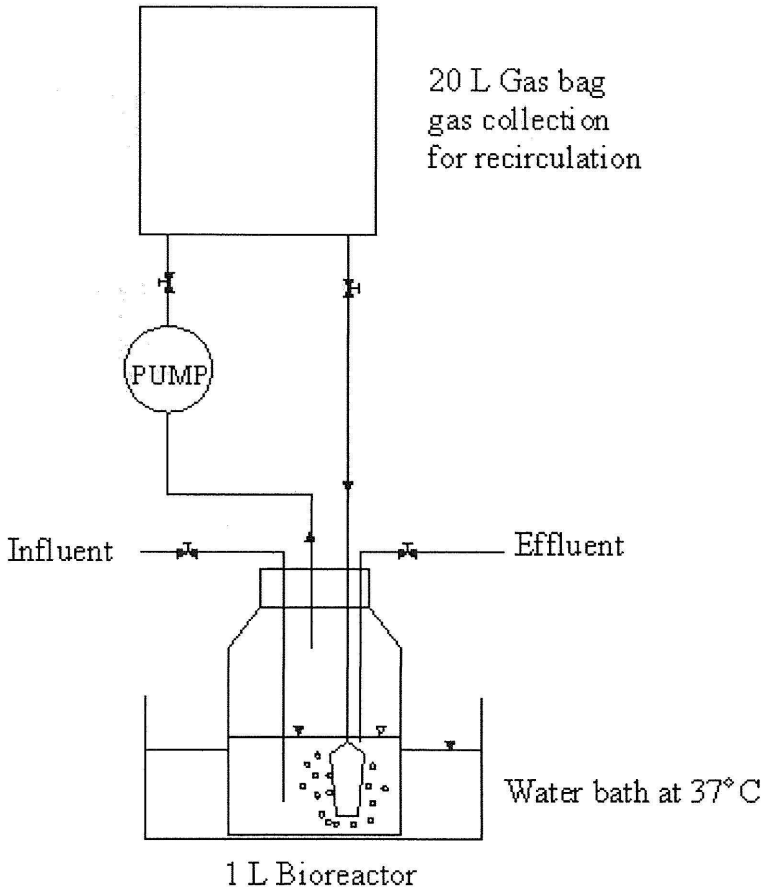


Figure 3.4 Schematic diagram for experimental bioreactor.

Figure 3.5 Aluminum sampling gas bag CCK (20 L) used in the different experiments. The two outlets disposed two mini valves.

3.3 Analytical methods

The composition of the produced gas was determined using a GC-14B (Shimadzu) gas chromatograph, equipped with a thermal conductivity detector, connected to a C-R8A data analyzer. A high performance column packed with 50/80 mesh Porapak Q was used. The temperature of the injection, column and detector was set at 100, 50, 100°C, respectively. Argon was used as the carrier gas at flow rate and pressure of 50 mL/min and 0.5 MPa, respectively. In order to obtain the calibration of the gas chromatograph, pure H_2, CH_4 and CO_2 gases were injected into the gas chromatograph injector port separately with 3 duplicate analyses to determine the related peak area accuracy.

Total solids (TS), volatile suspended solids (VSS), volatile solids (VS) and total N_2 (TN) were determined with 5 duplicate analyses to ensure accuracy of the results obtained, according to the Standard Methods for Examination of Water and Wastewater (APHA, 2005). The data were subsequently averaged; and the obtained deviations were found to be less than 4%. The pH was monitored *in situ*.

3.4 Results and Discussion

3.4.1 Steady state characteristics of chemostat H_2/CO_2-dependant methanogens

The chemostat cultivation of acclimated hydrogenotrophic methanogens at different dilutions rates reached the steady state at 11.14 g/L of H_2/CO_2 gas supply. According to the general views, the differences in the mass transfer rates for H_2 and CO_2 in the liquid (0.8 and 15.6 mmol/L for H_2 and CO_2, respectively at 60°C) were overcome by consideration of H_2 and CO_2 as 2 independent substrates in the

gas mixture during the estimation of kinetic growth parameters for methanogens growth (Schönheit et al., 1980). In a continuously stirred tank reactor, it is assumed that complete mixing conditions were present inside the reactor. Moreover, it was suggested that the application of vigorous agitation to the reactor increases the mass transfer of gas to liquid (Coates et al., 1996). Considering these findings, an airtight pump at 0.08 MPa and speedy recirculation at 6 L/min was used for the gas recirculation during the experimental procedure inside the continuous stirred tank reactors. Analysis of the decrease in the gas concentration inside the replaced gas tedlar bag shows, as presented in Table 3.3, that the percentage consumption rates of H_2 and CO_2 which were individually estimated are approximately equal, and the ratio equilibrium did not change with the variations in the dilution rates; the results are suggestive of the fact that the H_2/CO_2 complex gas mixture can be considered as a single substrate during the determination of the kinetics parameters of bacteria.

The pH, which acts as an important external factor that inhibits bacterial activity within the continuously flowing reactor (Keshtkar et al., 2003) was controlled by regular monitoring during the determination of the growth parameters. The data are presented in Figure 3.5. According to the results, the pH data in the chemostat reactors showed a progressive decrease from 7.67 at the start of the experiment to 6.63, 6.5, 6.4 and 6.35 respectively, for dilution rates of 0.125, 0.1, 0.084 and 0.072/d under steady state conditions. Unexpectedly, the results demonstrated that pH values were higher at high dilution rates, and lower at decreased dilution rates. The data obtained were compared with those obtained from the pre-growth acclimation cultivation at neutral pH, and it was concluded that dilution rate affects the pH value. It is possible that at low dilution rates, the

trace elements limited the hydrogenotrophic growth, thereby affecting the pH value. However, according to the literature, the inhibitory effect of pH on the physiological activities of hydrogenotrophic methanogens varied among different species. While certain studies demonstrated that the hydrogenotrophic methanogens participated in the methane production in an anaerobic digester at low pH and in an acidic environment (Savant et al., 2002; Kotsyurbenko et al., 2004), others verified that the methanogenic strain was best metabolized at neutral pH range of 6.7 - 8 (Wolfe and Higgins, 1979).

It is presumed that the Monod kinetics can thus be regarded as a mechanism for correction of the pH inhibition during the determination of kinetics parameters (Costello et al., 1991). Figure 3.6 represents the cells production under these conditions; it illustrates the plot demonstrating the evolution of biomass at different dilution rates for a constant rate of substrate supply. It shows that the biomass concentration decreases with an increase in the dilution rate. No bacteria carrier was used in the different reactors; thus, the dilution rate effect explains the obtained data.

During the experiments, the amount of trace metals supplied was maintained constant (10 mL trace metal/ L-culture growth) at varied dilution rates in order to avoid their toxicity effects on the data. During the exponential growth of bacteria, there is a corresponding increase in the requirement of trace metals; however, in the present study, no adjustment was required between the growth rate of bacteria and the supply rate of trace metals. Under steady state conditions during continuous cultivation, limited supply of H_2/CO_2 substrate resulted in limited growth of the biomass as the dilution rate varied; this was similar to the results obtained by Haydock et al. (2004). With the regard to bacterial growth, it was

difficult to isolate the effects of limited supply of the trace metals from those due to the limited supply of substrate in the present study.

The cell activities were examined by measuring the specific methanogenic activity (SMA) at different dilution rates. The SMA was determined according to the relation between the rate of methane production and the VS which is the biomass expression (Ahring and Sorensen, 1993) and the results are presented in Figure 3.7. Figure 3.7 shows an exponential activity within the 10^{th} and 20^{th} day due to the trace metals and the limiting substrate, which provide sufficient nutrient supply for bacterial metabolism. Among the 4 dilution rates that were used in this experiment, the highest activity for methanogens was observed at dilution rates of 0.1/d after the 20^{th} day. It is well known that the trace metals stimulate the activity of the methanogens during the process of degradation. In the present study, limited amount of the trace metals induced maximal methanogenic activity at the dilution rate of 0.1/d, 0.24 L CH_4/g VSS·d, and the value conforms to previous explanations regarding the low quantities of available trace metals at low dilution rates. Figure 3.6 and 3.7 suggest a decoupling mechanism of anabolism and catabolism with regard to the metabolic activity of hydrogenotrophic methanogens (Schönheit et al., 1980; Fardeau et al., 1987).

From the data obtained, it is evident that increasing the H_2/CO_2 utilization by the bacteria via acclimation increases the conversion rate of H_2/CO_2 to methane and using inorganic nutrients as the dilution liquid results in a high rate of biogas production rate during short-term cultivation. The methanogenic activity appears to be related more strongly to the dilution rate than to the bacterial growth rate.

3.4.2 Kinetic evaluation in chemostat reactor using H_2/CO_2 as substrate

In chemostat cultivation, the expression of Monod equation is expressed as follows:

$$\frac{1}{D} = \frac{K_S}{\mu_{\max}} \frac{1}{S} + \frac{1}{\mu_{\max}} \tag{3.1}$$

where D is the dilution rate (1/d). The substrate balance (S) is expressed as follows:

$$\frac{dS}{dt} = D(S_0 - S) - \mu . \frac{X}{Y_{X/S}} \tag{3.2}$$

S_0 represents the substrate concentration at the start of the experiment (g/L) and X represents the biomass concentration (g/L). Under steady state conditions where $dS/dt = 0$, the growth yield ($Y_{X/S}$) is expressed as follows:

$$Y_{X/S} = \frac{X}{S_0 - S} \tag{3.3}$$

Eq. (3.3) determined Y_{CH_4} as the straight line that was derived by plotting S_0–S versus X (Kun, 2003).

Considering the complex gas H_2/CO_2 as a single substrate, the steady state shows a correlation between the limited H_2/CO_2 substrate and the bacterial growth rate. Lee (1992) proposed the application of the Monod kinetics to predict the cell growth from the start of the exponential growth phase to the stationary phase. K_s and μ_{max} were determined when the straight line obtained by plotting $1/D$ versus $1/S$ was derived from Eq. (3.1) (Lee, 1992), and the experimental result in the chemostat cultivation enhanced a maximum yield at exponential growth of 11.66 g cells (a value obtained from Figure 3.9) for each mmol of substrate H_2/CO_2

consumption. It was found to be higher than the ranges of 0.0053~0.0095 g/mmol and 0.00279~0.0079 g/mmol that were obtained using pure mixture of *Methanosarcina* sp. strain 227 and *Methanosarcina mazei* under H_2/CO_2 in media containing minerals where *Methanosarcina mazei* was presented a low growth rate under such conditions (Ferguson and Mah, 1983).

During the development of the technological process, it is desirable to maintain a high growth yield in order to achieve efficient conversion of the substrate to biomass. The designed operation used in the experiment shows a good productive fermentation system. The saturation constant K_S was 0.82 g/L, and was higher than 0.116 g/L, the necessary value for methanogenic bacteria when they compete with sulphate-reducing bacteria for acetate (Bhattacharya et al., 1996) and other previous report (Gilardo-Gomez et al., 1992); when the cultivation is solely operated using an inorganic substrate, it is suggested that high concentration of H_2/CO_2 is necessary to achieve complex substrate-cells for biomass production. However, the maximum specific growth obtained due to the use of H_2/CO_2 as a unique substrate was 0.15/d, as observed in Figure 3.8, and it is 10-fold lower than 1.5/d that was reported for H_2 and CO_2 using acclimated mesophilic methanogens culture using separate organic substrates for H_2 and CO_2 (Zhang and Maekawa, 1994). According to the study, the mixture gas flow rate was estimated as 8.3 mL/min; this was substantially lower than that applied by Zhang and Maekawa (1994). The presented results correspond well with those of previous literature regarding the maximum specific growth rate in relation to the gassing rate (Schill et al., 1996).

3.4.3 Simulation

To simulate the substrate (S), the cells production rate (DX) and the cells concentration (X) behavior in chemostat reactor under steady state conditions, the following relations are used:

$$S = \frac{K_S D}{\mu_{\max} - D} \tag{3.4}$$

$$X = Y_{X/S}\left(S_0 - \frac{K_S D}{\mu_{\max} - D}\right) \tag{3.5}$$

$$DX = Y_{X/S} D\left(S_0 - \frac{K_S D}{\mu_{\max} - D}\right) \tag{3.6}$$

While approaching the washout period, the reactor is sensitive to the variations in the dilution, D; the rate of cell production in terms of the dilution factor is as follows:

$$\frac{d(DX)}{dD} = \frac{(S_0 + K_S)D^2 - 2\mu_{\max}(S_0 + K_S)D + S_0\mu^2{}_{\max}}{(\mu_{\max} - D)^2} \tag{3.7}$$

Direct computation can be used for expressing the maximal cell output rate by solving

$$\frac{d(DX)}{dD} = 0$$

While considering maximum productivity, the dilution ratio D_m is required; from Eq. (3.7)

$$D_m = \mu_{\max}\left(1 - \sqrt{\frac{K_S}{S_0 + K_S}}\right) \tag{3.8}$$

the washout occurs when the cell concentration is equal to zero

$$D_{crit} = \mu_{\max} \frac{S_0}{K_S + S_0} \tag{3.9}$$

The slow growth of the CO_2-reducing methanogens showed the importance of the retention time inside the fermentor when the treatment occurred. The retention time must be long enough to enhance the efficient activity of the bacteria as demonstrated by most of the engineering studies, which demonstrated that the retention time must be greater than 10 d for efficient and stable operation (Zinder, 1993). The kinetic parameters derived from the application of Monod equation at steady state cultivation are used to simulate and predict the performance of a chemostat type fermentor.

Figure 3.10 summarizes the prediction plots for the hydrogenotrophic methanogens growth and substrate supply at different dilution rates. The graphs indicate the maximum bacterial growth at low dilution rates. The amount decreases as the dilution increases until it reaches D_m, the maximal dilution where the amount of bacteria in the reactor due to the dilution rate is in equilibrium with the limited supply of nutrients and trace metals; subsequently, the cells washout begins to reach D_{crit} at the high dilution rate of 0.14/d; the predicted D_m was estimated as 0.11/d. However, the cell production rate (DX) increases with increasing dilution rate. The accuracy of the model was determined by comparing the theoretical data with the experimental results; the experimental data were in agreement with the predicted plots. The proposed operation system shows that dilution rates must be around the dilution rate 0.11/d in order to have a short retention time and a high substrate conversion rate at relatively low cells concentration in order to obviate the problems due to long retention time and toxicity effects of the trace elements. It is known that a long retention time always necessitates a large

capacity-operating reactor. In the present study, the methanogenic bacteria reach an efficient activity under short retention time. This prediction was verified by the previously determined specific methanogenic activity as shown in Figure 3.7; the highest methanogenic bacterial activity was 0.24 L CH_4 /g VSS· d, which was detected at 0.1/d; the value was found to be around the predicted D_m, which was 0.11/d. The predicted critical dilution (D_{crit}) value was 0.14/d. CH_4 production effectively dropped when the dilution tended to move upward of D_{crit}. Additionally, the measured activity was found to be strongly related to the dilution rate.

Table 3.4 presents the suitable range of parameters in the chemostat cultivation under H_2/CO_2 (80:20, v/v) as substrate to design a reactor plant.

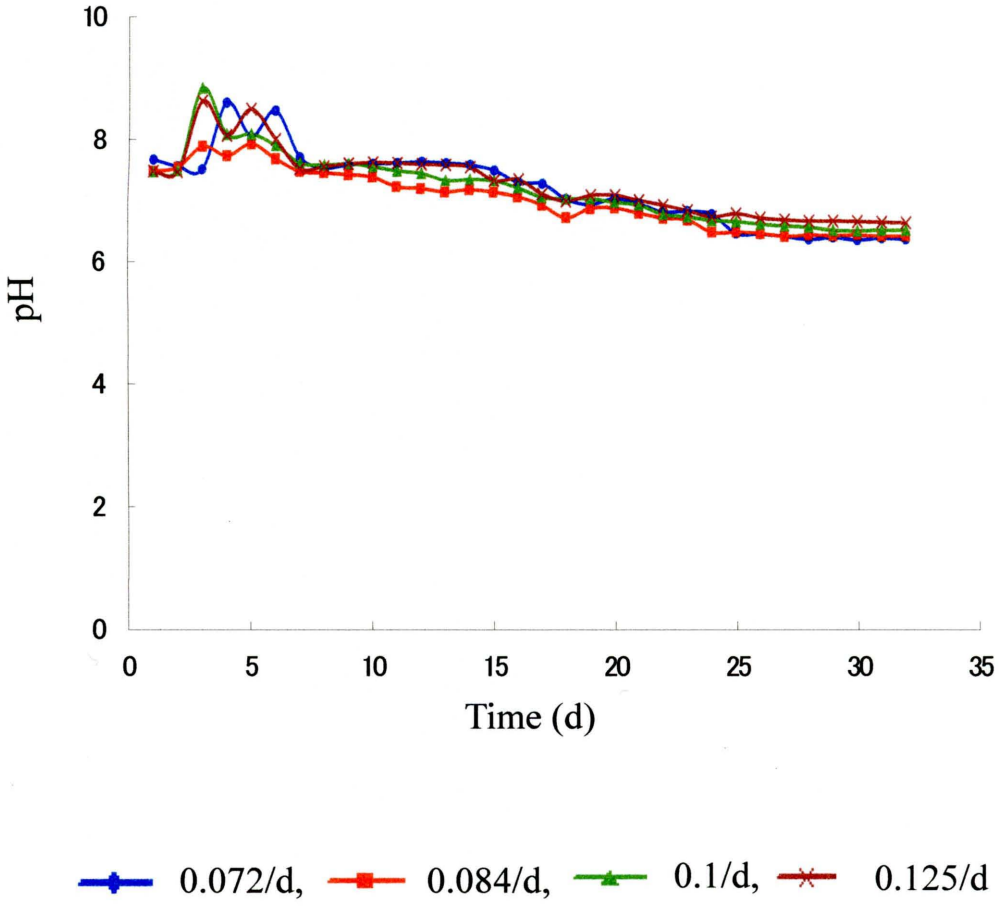
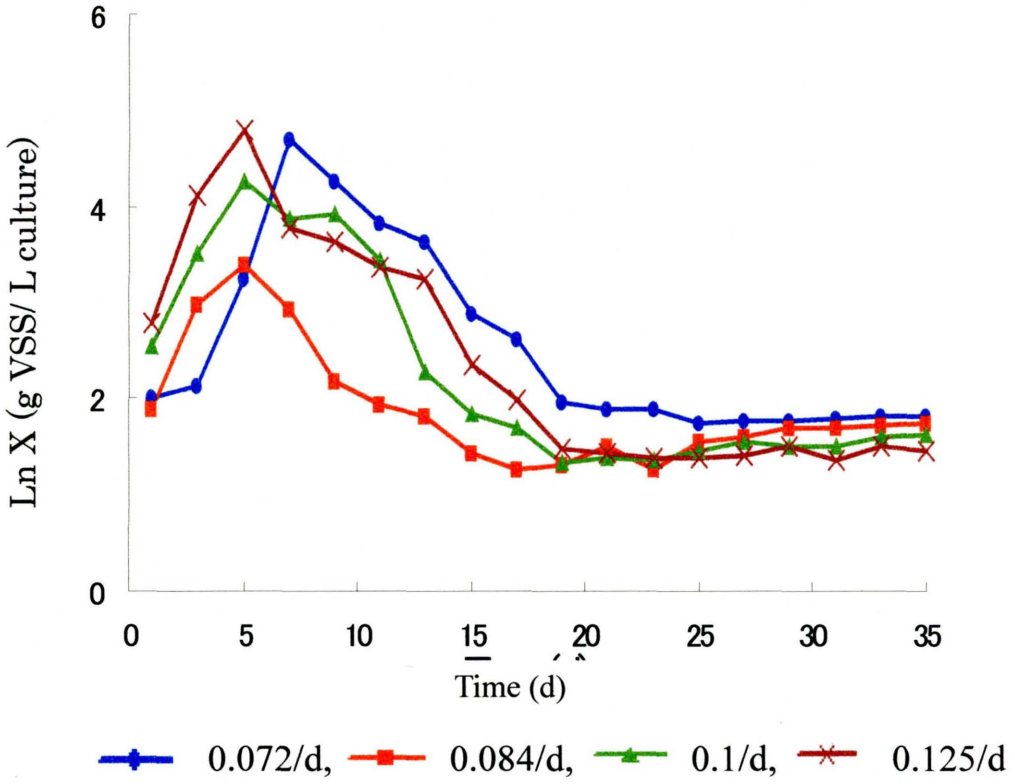


Figure 3.6 Time course of the reactor pH at different dilutions rate (1/d).



Ln X: expression of the exponential concentration of bacteria in the chemostat

Figure 3.7 Time course of bacteria growth during the experiment at different dilutions rate (1/d).

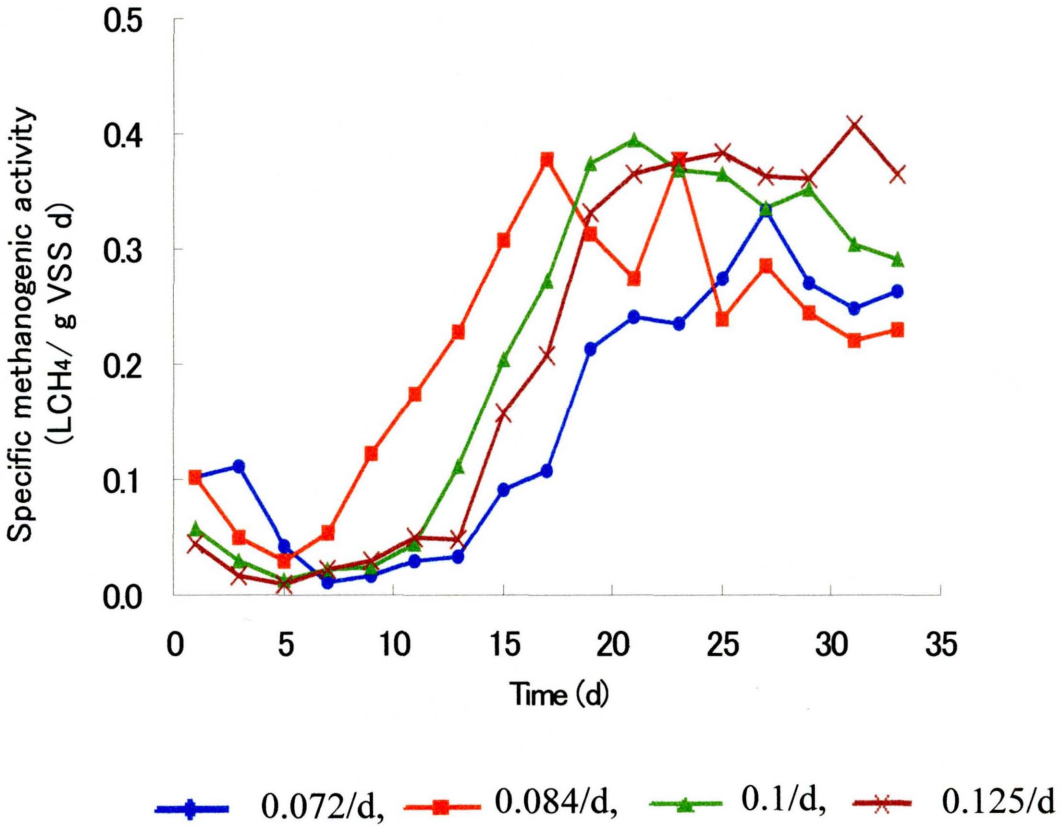


Figure 3.8 Time course of the specific methanogenic activity during the experiment at different dilutions rate (1/d).

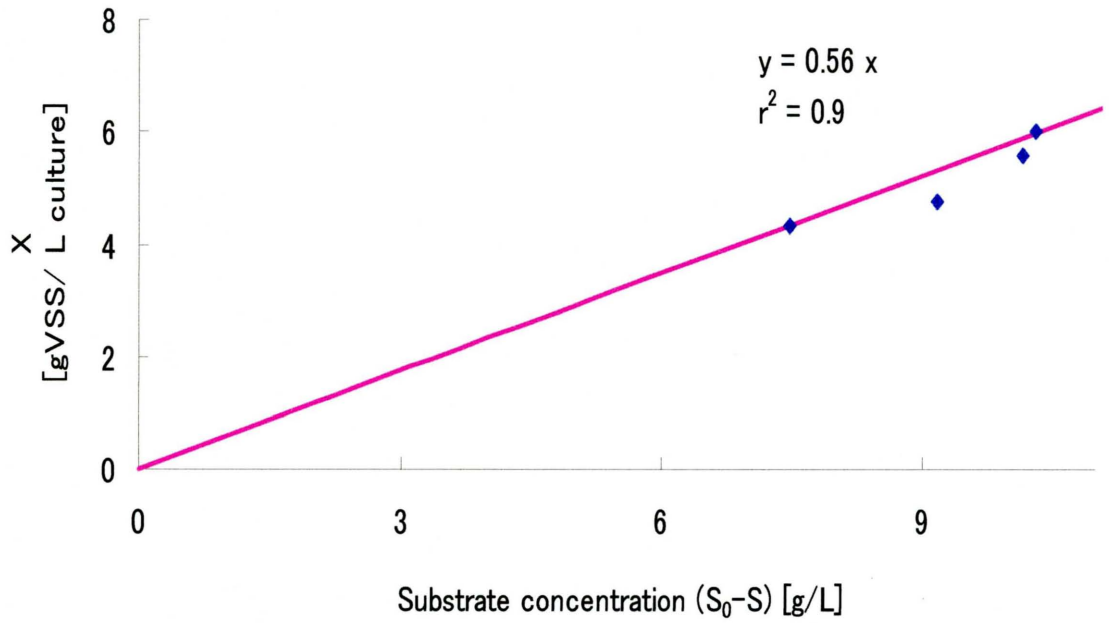


Figure 3.9 Determination of the growth yield in chemostat culture for hydrogenotrophic acclimated methanogens.
X is the bacteria concentration, S_0 the initial substrate concentration and S the concentration at t time.

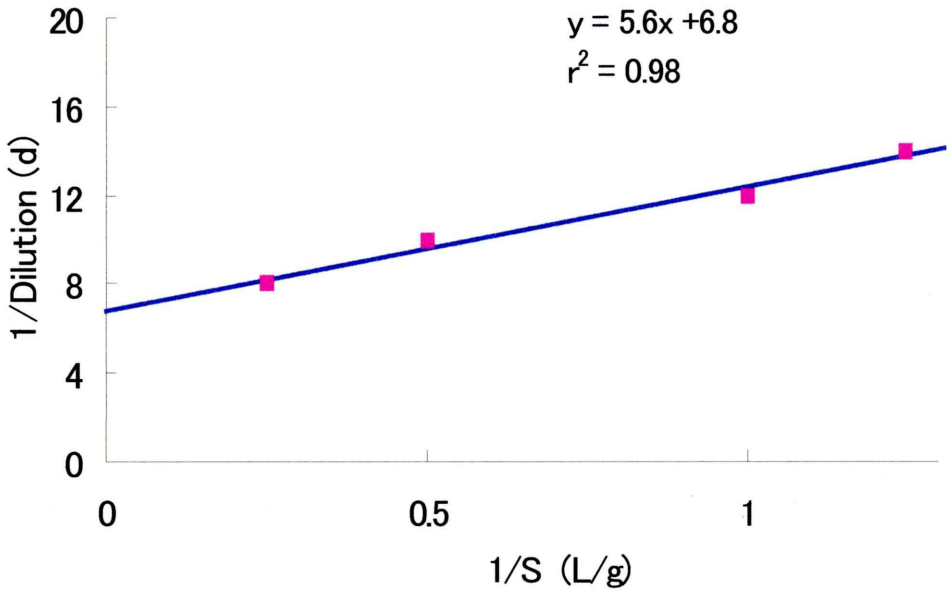
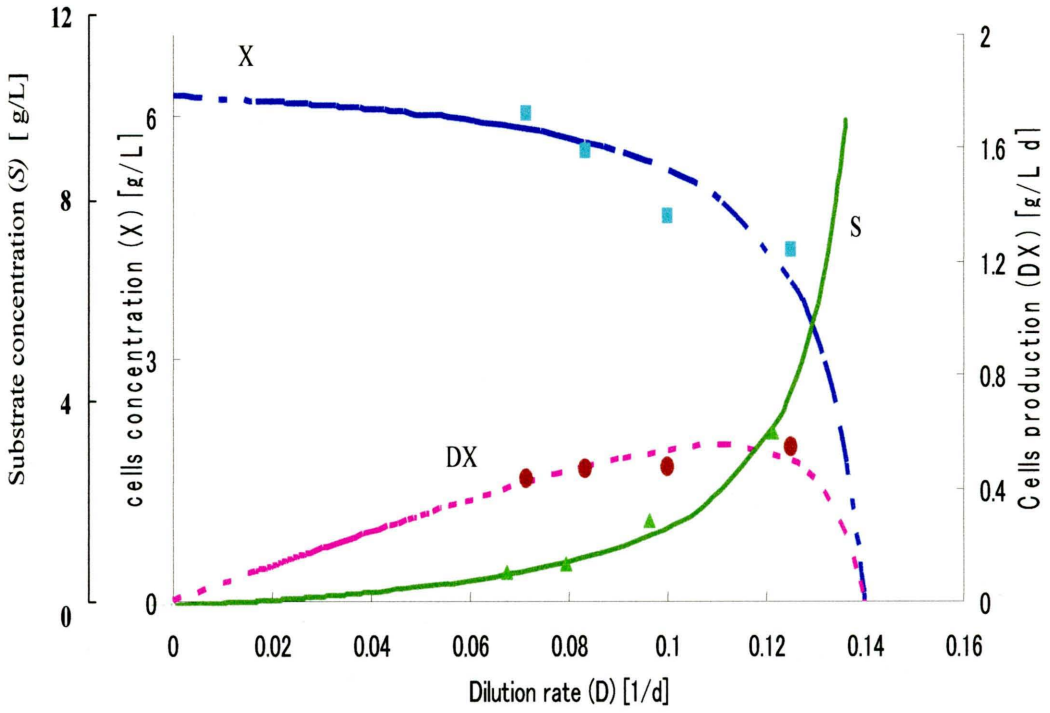


Figure 3.10 Lineweaver-Burk plot of hydrogenotrophic methanogens growth on H_2/CO_2; S is the concentration of H_2/CO_2.



■ Cells concentration X; ▲ H_2/CO_2 concentration S; ● cells production rate DX; (—) model prediction. D_{crit} (0.14 /d)

Figure 3.11 Dependence of cells concentration X, cells production rate DX and supply substrate concentration on the continuous culture dilution rate D as simulated from Monod chemostat model.

Table 3.3 Experimental consumption rate of H_2 and CO_2 calculated when assumed that the gases are perfect.

Reactor		Input substrate per day S_0 (g/L)	Daily output substrate average S (g/L)	Variance coefficient s (%)	Consumption rate (%)
I	H_2	1.71	0.13	2.5	92.0
	CO_2	9.43	0.71	1.8	92.0
II	H_2	1.71	0.15	5.5	91.2
	CO_2	9.43	0.83	6.2	91.0
III	H_2	1.71	0.29	3.7	83.0
	CO_2	9.43	1.69	5.1	82.0
IV	H_2	1.71	0.52	3.0	70.0
	CO_2	9.43	3.14	4.4	67.0

Table 3.4 Proposed operational condition using hydrogenotrophic methanogens.

Parameters	Proposed values
Reactor working volume, V (mL)	500
Dilution, D (1/d)	0.10
Hydrogenotrophic methanogens concentration, X (gVSS/ L –culture)	5±0.12
pH	Controlled at 6.40-7.67
H_2/CO_2 gas supply (L)	12.00
Temperature (°C)	37±2
Mixing	1min interval at 0.08 MPa
Daily methane production (L/d)	1.21

3.5 Conclusion

Four anaerobic chemostat reactors containing the growth culture of acclimated hydrogenotrophic methanogens obtained from a domestic anaerobic wastewater treatment plant were continuously fed with inorganic medium. The operation was done at different dilution rates in order to obtain engineering data that was necessary to simulate the steady state chemostat cultivation under conditions of inorganic substrate supply. The bacteria were cultivated using H_2/CO_2 as substrate until the steady state was achieved at 11.14 g/L of gas supply. Subsequently, Monod model was applied to determine the kinetics parameters, μ_{max} and K_S; the values were estimated to be 0.15/d and 0.82 g/L, respectively.

From these results, a simulation was performed to predict a chemostat type digestor performance when H_2/CO_2 is used as sole substrate. The simulation permit also to obtain the dilution rates at which bacteria washout began and at which maximum bacterial activity could be achieved. In the present experiment at a concentration of 11.14 g/L of H_2/CO_2 daily supply, the critical dilution rate of the chemostat was 0.14/d, and the maximum bacterial activity occurred at 0.11/d. The predictions were in conformity with the data obtained; additionally, maximum methane gas production of 1.21 L/d was achieved at a dilution of 0.1/d.

Thus, the engineering results can be summarized and applied to the large-scale use of H_2/CO_2-dependant methanogens for biogas production under using only inorganic substrate. The results demonstrated that at steady state, the concentration of cells was related more strongly to the dilution rate than the H_2/CO_2 concentration.

CHAPTER 4

Effect of two physical parameters: mixing duration and temperature on chemostat fermentation under H_2/CO_2 feeding as substrate

4.1 Introduction

Mixing time is defined as the time required for a reactor composition to achieve a specific level of homogenization following an addition of nutrient. The circulation time interval in which a cell in the agitated culture liquid circulates through different regions of the reactor to accomplish a possibly reaction condition is presented as an important factor which influences the efficiency of the anaerobic degradation operation (Verhoff et al., 1974; Baxter, 1988). Different types of reactors and agitations give rise to different circulation and mixing time characteristics; mixing can be realized by biogas recirculation, slurry recirculation or mechanical mixers. Brade and Noone (1981) have reported that among all processes, mechanical mixers are the most efficient in terms of power consumed per gallon mixed. The gas recirculation has been presented as the most efficient process in term of performance and cost (Konstandt and Roediger, 1977).

The effect of mixing on anaerobic degradation has been widely investigated in anaerobic digestion (Hashimoto, 1982; Lin and Pearce, 1991). Monteith and Stephenson (1981) have listed the benefits of utilization of mixing: minimum solids deposition, reduction of dead space and uniform repartition of material inside the reactors. Moreover, in the case of using gas as substrate, the dissolution

of the gas in liquid growth for bacteria activity is assumed to be dependant on the rate of mixing (Pauss et al., 1990) and the mode of mixing (Khursheed et al., 2005). Unfortunately, the economics of the process utilization is reduced because of the cost of the electricity used during mixing procedure in anaerobic digestion.

To surround the process economics, two factors will be investigated in addition:

The temperature

As influencing factor in anaerobic digestion of organic wastes, Pfeffer (1974) and Bouallagui et al. (2004) have shown the temperature importance because most of the bacteria involving the methanogenesis have their optimal activity at mesophilic range. Biogas technology poses many challenges in countries particularly for the optimization of process. The major reason for their failure seems to be the climatic conditions. Biogas production is temperature dependent with optimum at 32 – 38°C in the mesophilic range and at 50 – 55°C in the thermophilic range. The temperature in the hilly regions during winters dips very low, therefore affecting the process drastically. Kanwar and Guleri (1994) have shown a decrease of biogas production of about 70% in high altitude installations of biogas plants during winter in northeast in India when the demand for energy is high. Some authors assumed that the decreased in biogas production at low temperature is probably due to the inability of mesophilic bacterial biomass producing gas to survive under low temperature conditions. The relative low temperature for the growth of these organisms results in an exhaustion of cell energy, a leakage of intracellular substances or complete lysis (Gounot, 1986). At temperatures below their optimum for growth, microorganisms are unable to sequester substrates from their environment; due to the decrease of their affinity consistently as temperature drops

below the optimum temperature for growth (Nedwell, 1999).

Vitamin B_{12}

The vitamin has been widely investigated in the anaerobic degradation process (Bainotti et al., 2000). Unfortunately, the extraction procedure still suffer in the lack of standard procedure but the possibility of large scale production of this important vitamin constitute a promising wastewater treatment cost reduction.

Thus, we will investigate the effects of mixing using H_2/CO_2 gas recirculation on hydrogenotrophic methanogens culture at two temperatures range under four different mixing durations. The CO_2 consumption and conversion to methane will be monitored.

4.2 Materials and Methods

4.2.1 Anaerobic sludge acclimation

Anaerobic sludge was acclimated under H_2/CO_2 feeding as unique substrate for 8 months until the hydrogenotrophic methanogens were found to be dominant in the culture growth.

4.2.2 Experimental procedures

At mesophilic range or 37^0C

The four chemostat reactors with 500 mL acclimated hydrogenotrophic methanogens content each, are connected to four aluminum gas bag CCK with H_2/CO_2 (80:20, v/v) supply at the rate of 12 L /reactor/ d (Figure 4.2). Mixing was realized by four pumps at 0.08 MPa and different mixing durations (Figure 4.3):

Pump I: 45 min/h

Pump II: 60 min/h

Pump III: 30 min/h

Pump IV: 15 min/h

Daily gas production and composition are monitored until the steady state cultivation. The dilution was set up at 0.1/d using sterile nutrient and trace metals (composition identical to the previous experiment). At steady state under mesophilic conditions biogas production and composition, the pH and bacteria amount (VSS) were measured.

At psychrophilic range or 20°C

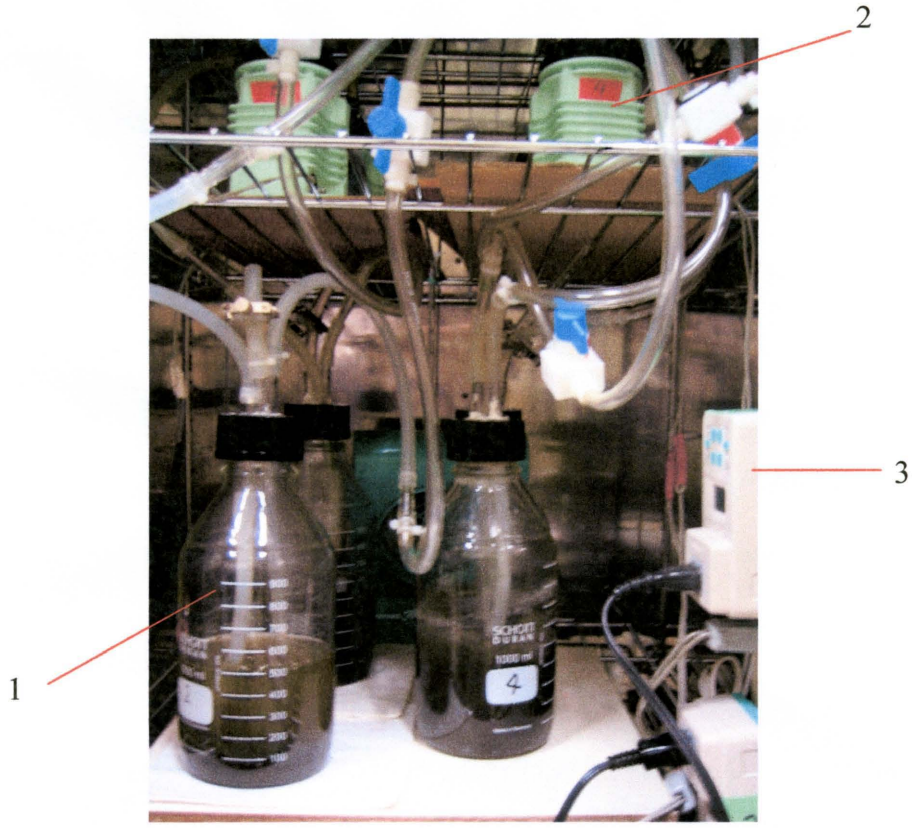
The chemostat reactors temperature was progressively decreased until psychrophilic range (20°C). Under 20°C, the chemostat steady state was realized; the biogas production and composition, pH and VSS were monitored.

4.2.3 Analysis methods

The pH was measured in situ with pH meter TPX-90 (Toko Chemical Laboratories Co. Ltd) and volatiles suspended solids (VSS) using the standard Methods protocol (APHA, 2005). The biogas composition was measured by GC 8A Shimadzu.

The statistical analysis of the methane production during the 7 d sampling, by means of 7 samples was done using the one-way ANOVA statistical data analysis. The least significant difference (LSD) between any four means (four different mixing durations) at $p = 0.05$ was applied to determine the differences.

Figure 4.1 Experimental incubation chamber with the four reactors systems



1 Chemostat reactor; 2 Airtight pump; 3 Timer for mixing duration control

Figure 4.2 Experimental set up of the four reactors connected to four pumps and timers inside the incubation chamber.

4.3 Results and Discussion

4.3.1 Time course of process parameters

The pH and the bacteria concentration in the growth culture were monitored all long experiments (Figures 4.3 and 4.4). The general configuration of pH results shows that using a constant high pressure for the chemostats cultivation at 0.1/d dilution rate, the carbon dioxide dissolution in the liquid growth culture have a neglect effects on the culture pH. The bacteria concentration results in Figure 4.4 showed a decrease of about 94%, 93%, 94% and 92% for mixing duration of 60 min/h, 45 min/h, 30 min/h and 15 min/h, respectively between the steady state under mesophilic condition and the steady state under psychrophilic condition. The results demonstrated that the utilization of organic wastes (Chae et al., 2008) or inorganic H_2/CO_2 (80:20, v/v) gas, in the present experiment, as substrates in anaerobic digestion obtains an activity reduction under temperature decrease.

In Figure 4.5, the exposition of hydrogenotrophic methanogens culture to H_2/CO_2 gas mixture shows an active methane production when continuous inorganic nutrient is supplied as substrate. Under different mixing durations conditions, the methane production ratio was 80, 83, 71 and 60 mL-CH_4/h for mixing duration of 60 min/h, 45 min/h, 30 min/h and 15 min/h, respectively during the mesophilic cultivation period. The values were low at psychrophilic cultivation range 28, 39, 30 and 27 mL-CH_4/h for mixing duration of 60 min/h, 45 min/h, 30 min/h and 15 min/h, respectively. The plots for methane production were in adequate with the bacteria metabolic activity (Figure 4.5).

The predominant operating full-scale anaerobic digesters within the mesophilic range have shown in various researches to achieve the optimum methane production. Running a digester at 20 instead of 37 °C, results in a

remarkable saving of energy, if the produced gas is fairly comparable between both temperatures.

However, with digestion at 20 C, the methane yield is 4-fold lower than that produced at 37 C. It led to a lesser calculated net energy recovery of the digester of 20 compared to 37 C, even with a lower energy demand for heating the digester of 20°C than the one at 35 C. Consequently, it becomes important to determine the net energy balance between the energy demands to heat the digester and improved energy production from the increased methane yield as the temperature is increased when deciding the optimum operating temperatures.

The net energy balance is also directly dependent upon the feed concentration by means of the H_2/CO_2 concentration in the present experiment as the absolute methane potential depends on the H_2/CO_2 amount supply.

Chae et al. (2008) have been demonstrated that if the input concentration of feed volatiles solids (VS) is higher than about 45000 mg/L, a temperature of 35 C is more economical than 30 C since the improved methane yield at 35°C can overcome the additional heating energy demands. However, at a VS concentration of less than 45000 mg/L, digestion at 30°C is more favorable than at 35°C. Therefore, with respect to the net energy recovery, the optimum temperature might be between 30 and 35°C, as the usual VS content is approximately 40000 mg/L. The present experiment was using 12 L H_2/CO_2 daily supply which is equivalent of less than 11 g/L H_2/CO_2 mix gas concentration.

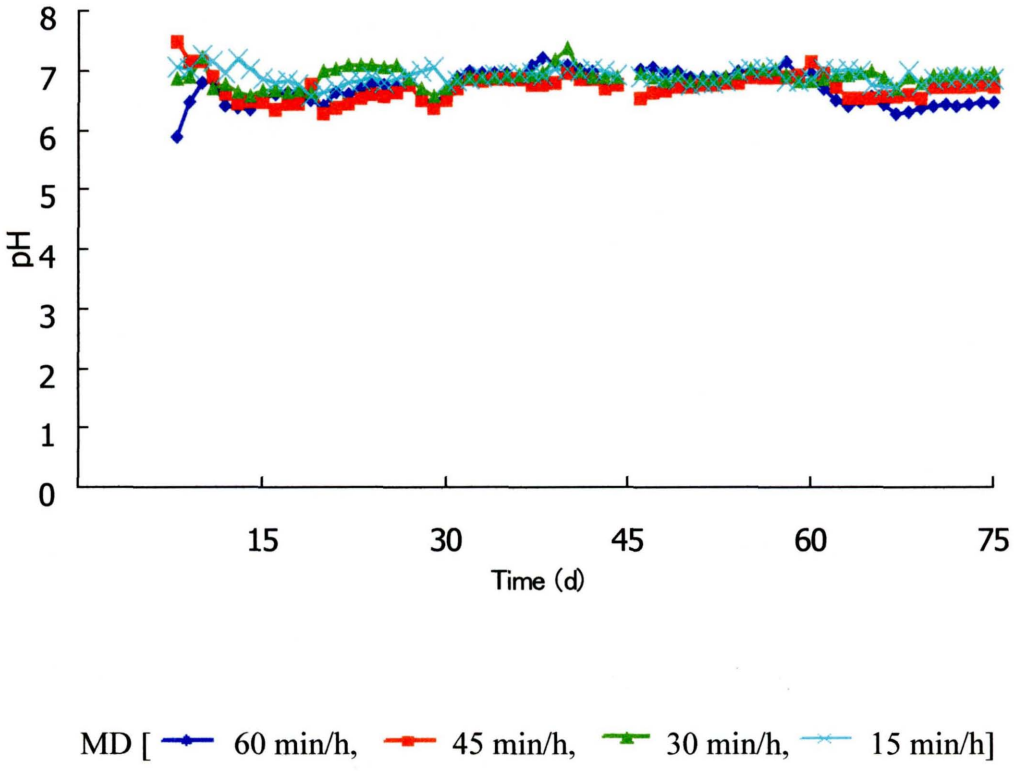


Figure 4.3 Time course of the pH evolution during the experiment at four different mixing durations (MD).

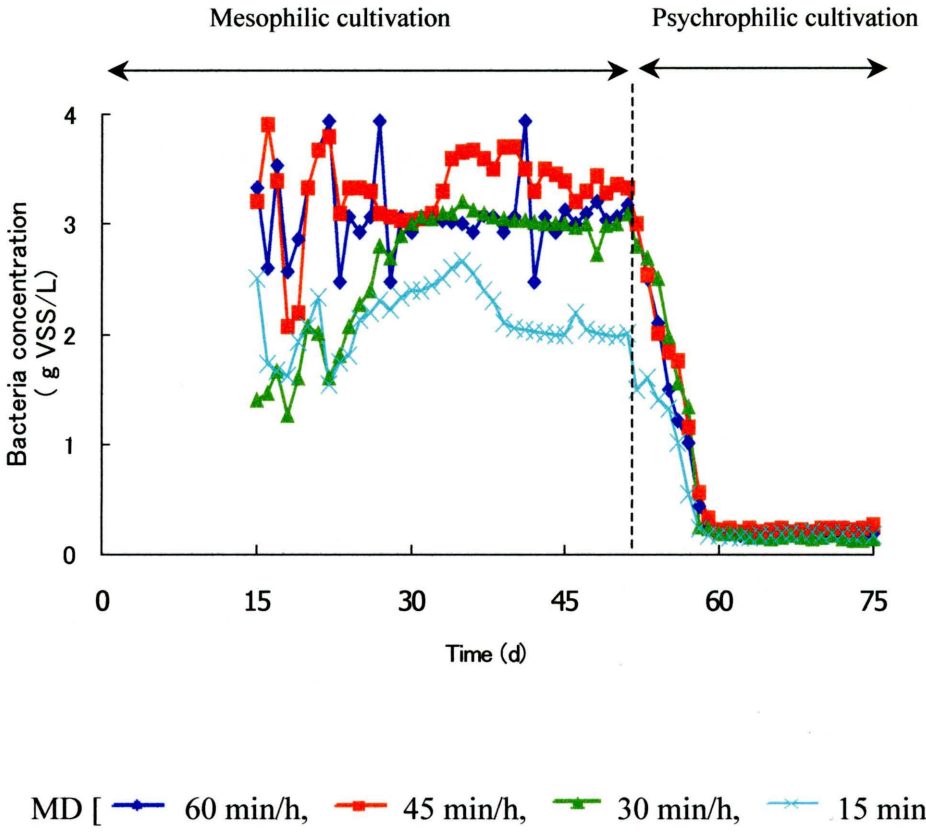


Figure 4.4 Time course of bacteria concentration during the experiment period under four different mixing durations (MD).

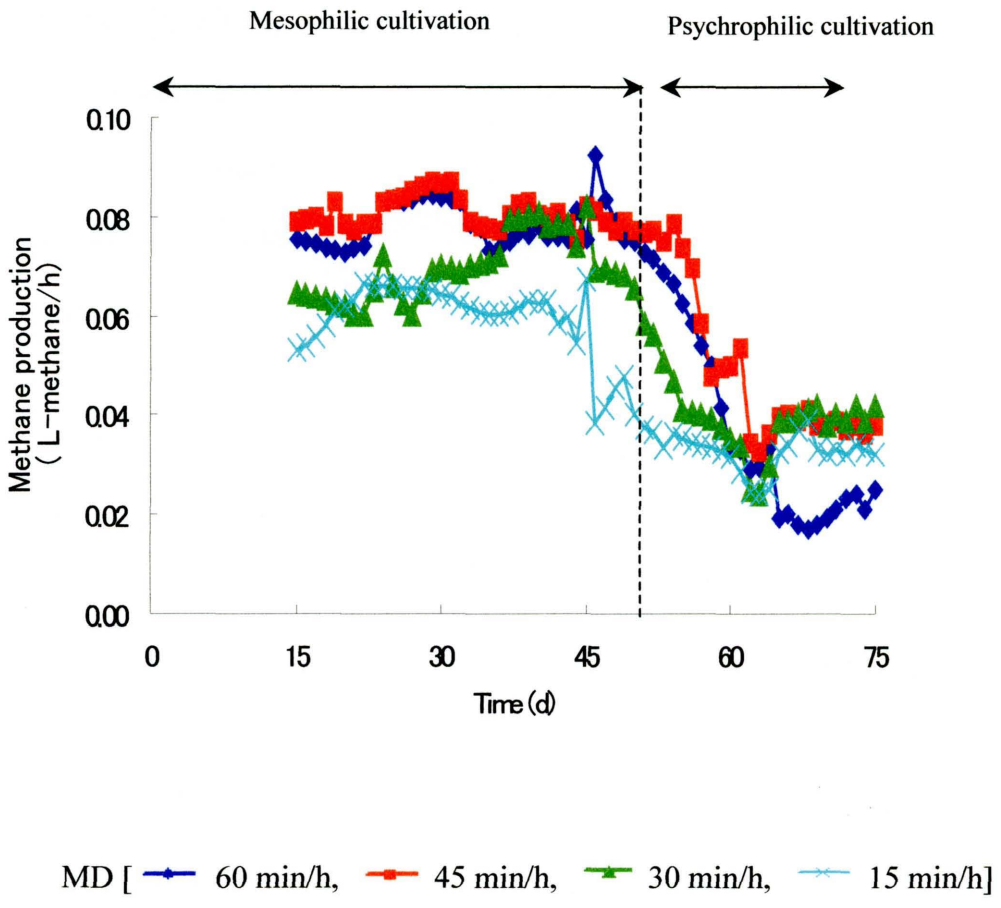


Figure 4.5 Time course of methane production for different mixing durations (MD).

4.3.2 Effect of mixing under mesophilic temperature condition

Results demonstrated that intermittent and continuous mixing produce variable amount of methane. The methane daily production was found to be 1.83 ± 0.05, 1.94 ± 0.06, 1.6 ± 0.08 and 1.4 ± 0.04 L/d at 60 min/h, 45 min/h, 30 min/h and 15 min/h respectively.

The highest methane production was found at 45 min/h mixing duration as referred to the Figure 4.8. It seems that the continuously mixing rate increases easily the cell damage hence even with the highest gas dissolution in the liquid for continuous mixing (Figure 4.9), the methane production was lower during the continuous mixing than the intermittent mixing 45 min/h.

Due to the reason that carbon dioxide is the only carbon source, the determination of the quantity of carbon dioxide effectively utilized by the hydrogenotrophic methanogens to convert into methane is equivalent to the stoichiometric ratio from the relation

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \qquad (4.1)$$

It is resulted that the steady state percent removals of carbon dioxide by means of conversion to methane, was 76.2%, 80.8%, 67.5% and 61.2% for dilution 60 min/h, 45 min/h, 30 min/h and 15 min/h respectively at mesophilic temperature range.

We proposed for further simulation to use the 45 min/h mixing duration.

4.3.3 Effect of mixing under psychrophilic range

The conversion rate of carbon dioxide to methane was found to decline due to the bacteria amount in each mixing duration range (Figure 4.11) compared to the mesophilic data (Figure 4.7). The temperature inhibits drastically the bacteria metabolic activity compared to the mixing duration. The methane production was

admittedly lower but the production ratio within the different mixing durations was identical between mesophilic and psychrophilic range. Others authors have suggested a long period of acclimation to optimize the methanogens activity under psychrophilic conditions (Kurosu et al. 1997; Torsten and Cavicchioli, 2000).

The capacity of CO_2 reduction was affected by the temperature change at steady state cultivation for psychrophilic range, the carbon dioxide conversion rate to methane was lowered from 76.2% to 27.9%, from 80.8% to 35.8%, from 67.5% to 29.6% and from 61.2% to 26.7% for 60 min/h, 45 min/h, 30 min/h and 15 min/h respectively; in other words, an activity reduction of about 45%. The choice of 45 min/h as mixing duration during psychrophilic condition can be made; the proposed mixing duration can then cover a long period model reactor activity under H_2/CO_2 as unique substrate.

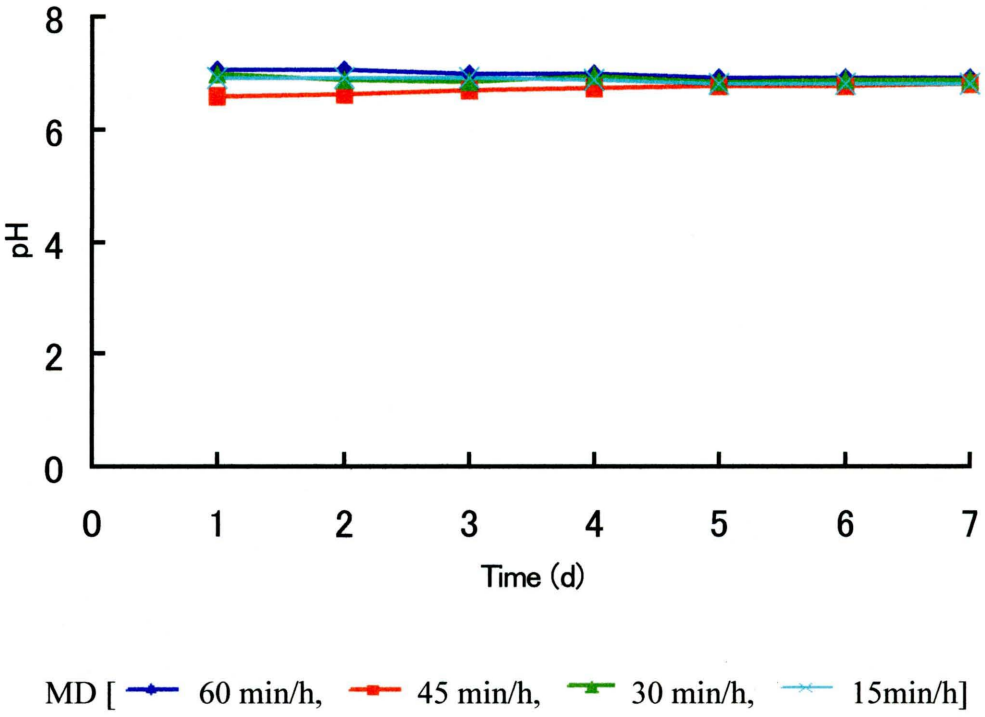


Figure 4.6 Time course of pH during the steady state cultivation at 37°C for different mixing durations (MD).

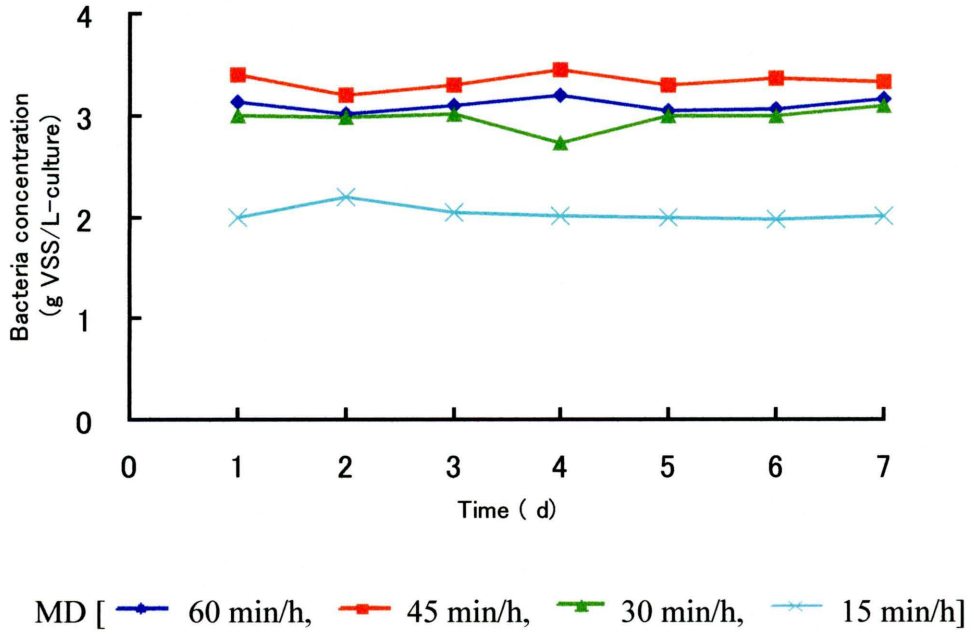


Figure 4.7 Hydrogenotrophic methanogens concentration during the steady state cultivation at 37°C for different mixing durations (MD).

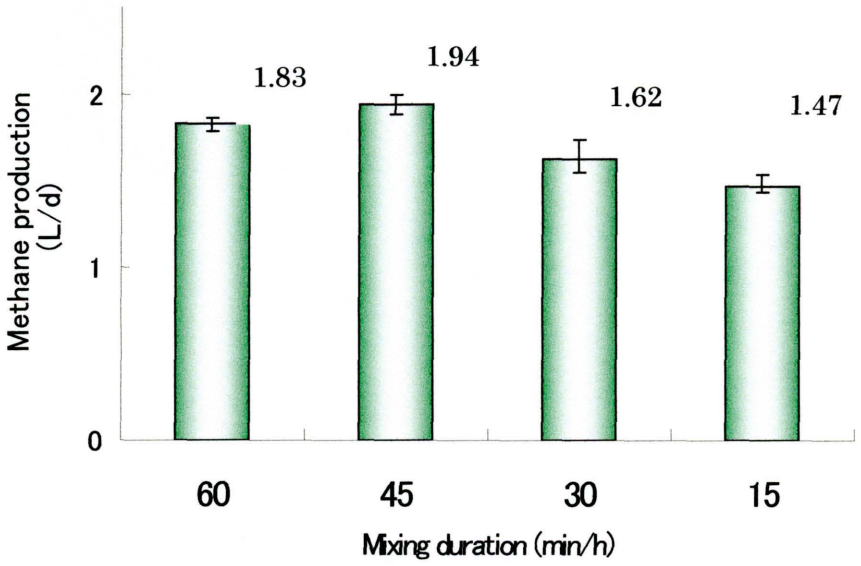


Figure 4.8 Average methane production at different mixing durations at mesophilic range.

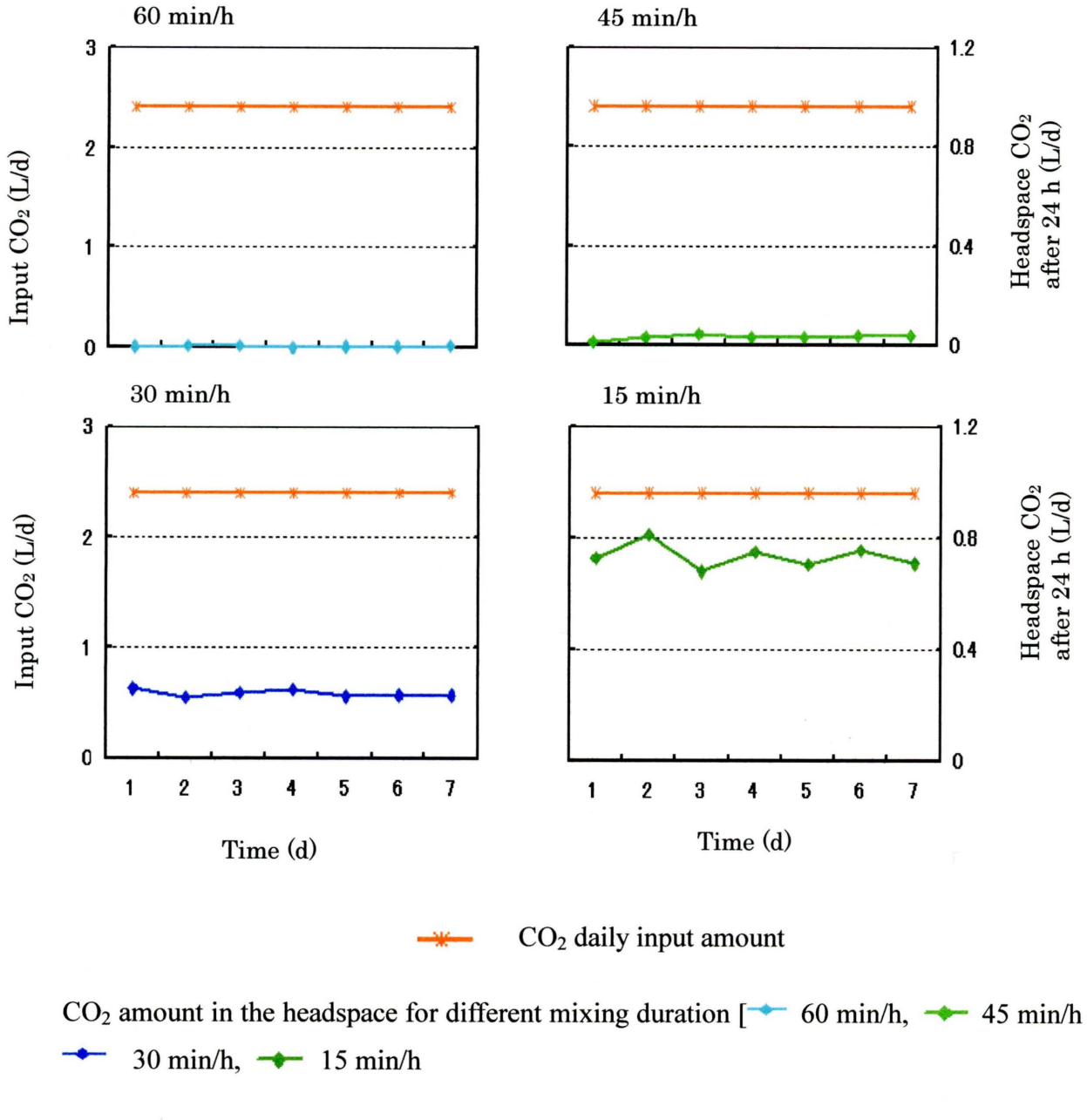


Figure 4.9 Capacity of CO_2 utilization at different mixing duration at 37°C.

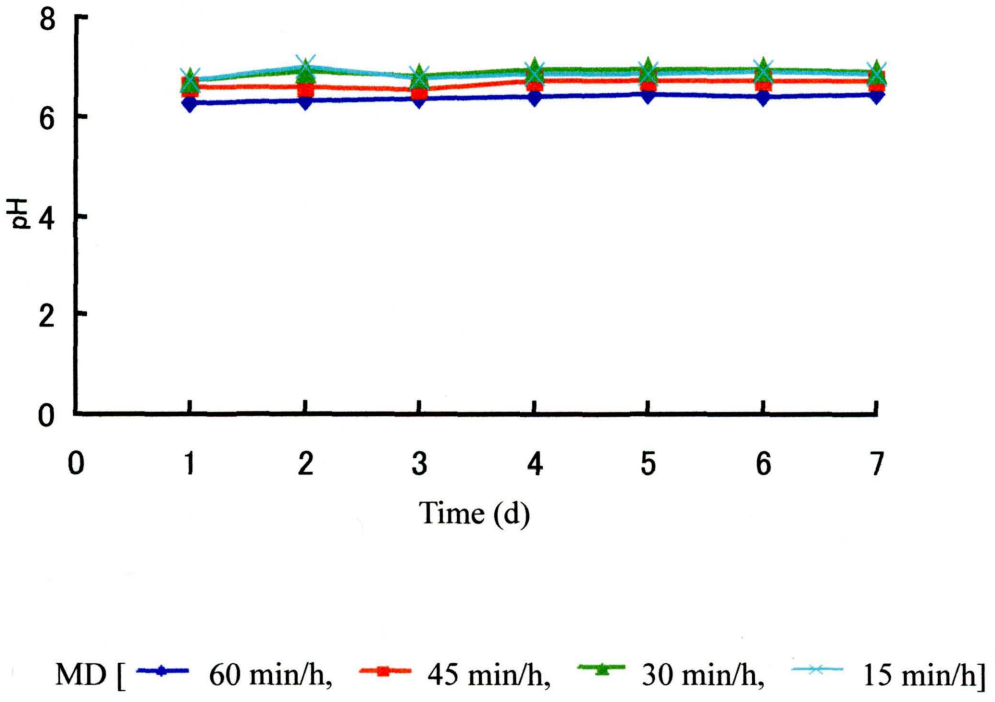


Figure 4.10 Time course of pH at steady state under psychrophilic (20°C) condition for different mixing durations (MD).

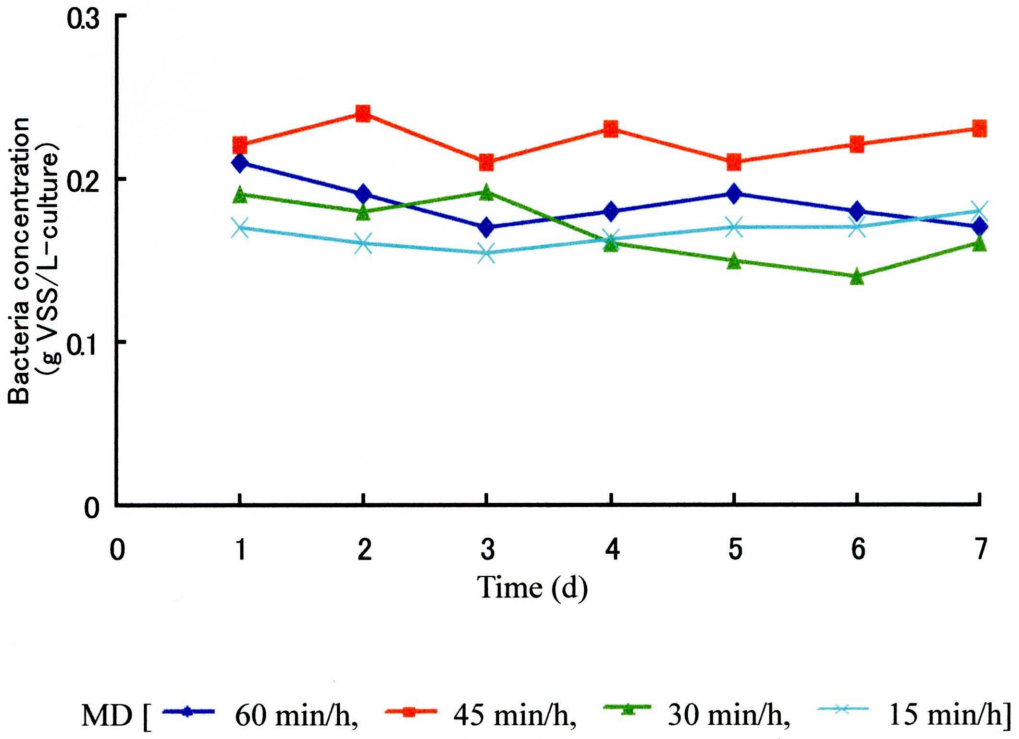


Figure 4.11 Time course of bacteria concentration at steady state under psychrophilic condition.

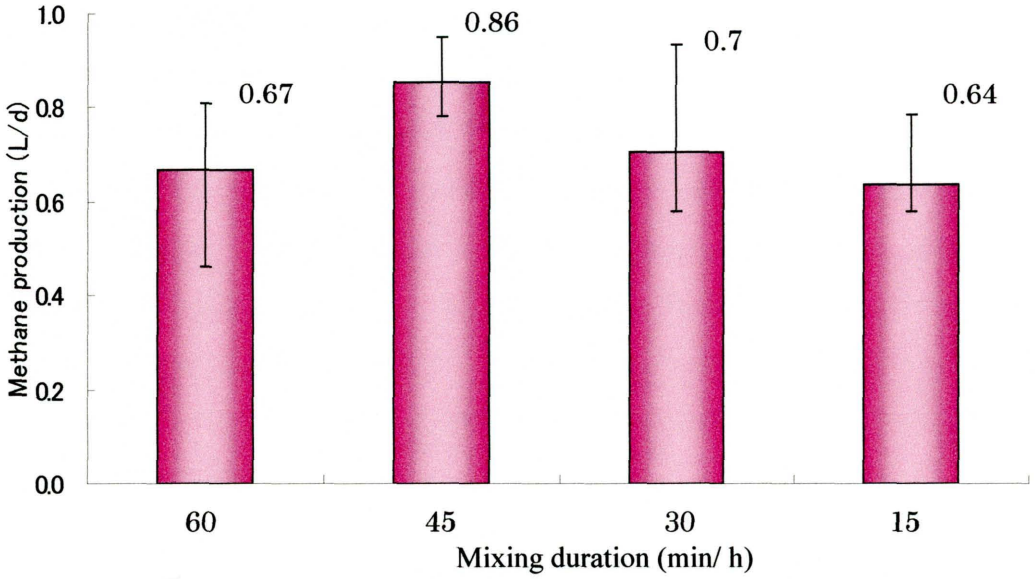


Figure 4.12 Average methane production at steady state psychrophilic condition.

4.3.4 Nitrogen production

Generally, less than 1% of N_2 content in the biogas resulted from the degradation of organic materials and 5 to 15% in the landfills produced biogas (Jönsson et al., 2003). During all experiments, no external N_2 gas was sparged and no organic material that might contain nitrogen in their complex molecules was added to the growth culture. The origin of the presence of N_2 in the bioreactors headspace might be from the cells proteins (decay) and from the media used. From Figures 4.12 to 4.15, averages calculation show that 0.14, 0.11, 0.1 and 0.15 L/d of N_2 are collected from the headspace of the reactors working at mixing duration 60 min/h, 45 min/h, 30 min/h and 15 min/h respectively. The highest amounts of nitrogen in the gas phase was found at mixing rate of 60 min/h (Figure 4.12) and at mixing rate of 15 min/h (Figure 4.15). As concerning the duration of 60 min/h, it has been already asserted that the cells damage was the reason of the relatively lower methane production when a comparison is made to the mixing duration of 45 min/h. Hence, the high N_2 level in the gas phase belongs to the dead cells materials.

Concerning the mixing duration of the mixing duration of 15 min/h, the low amount of substrate dissolution in the liquid phase creates an inhibition of the hydrogenotrophic methanogens growth. The cells death occurs in that case, with the continuous input of sterile inorganic nutrient.

When the temperature was lowered, the same amount of N_2 ratio was found in the headspace of the mesophilic cultivation compare to the psychrophilic cultivation, this for all mixing durations (Figure 4.12 to 4.15). The temperature variation seems to have no effect on the release of N_2 gas in the gas phase of the reactors.

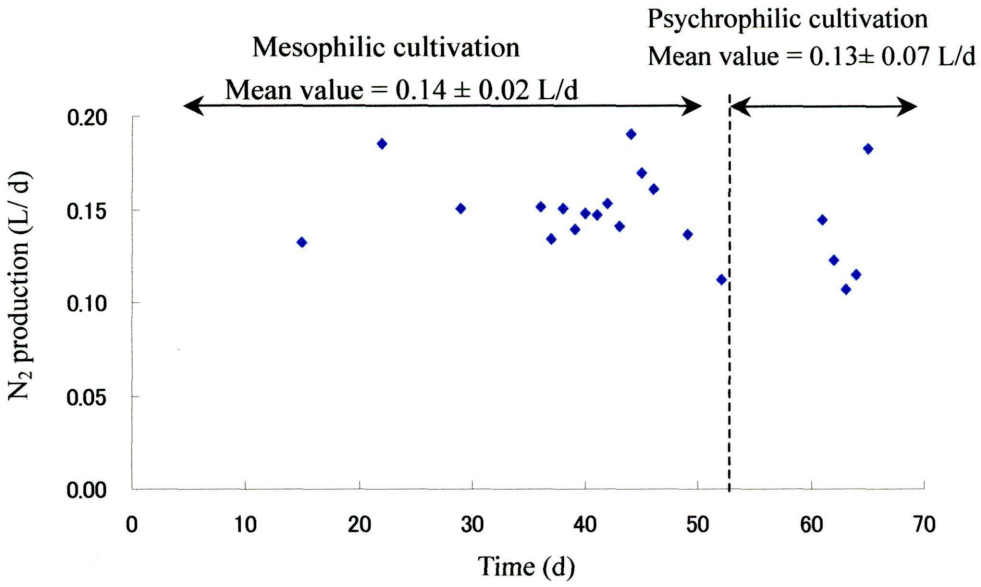


Figure 4.13 Nitrogen production under continuous mixing duration of 60 min/h.

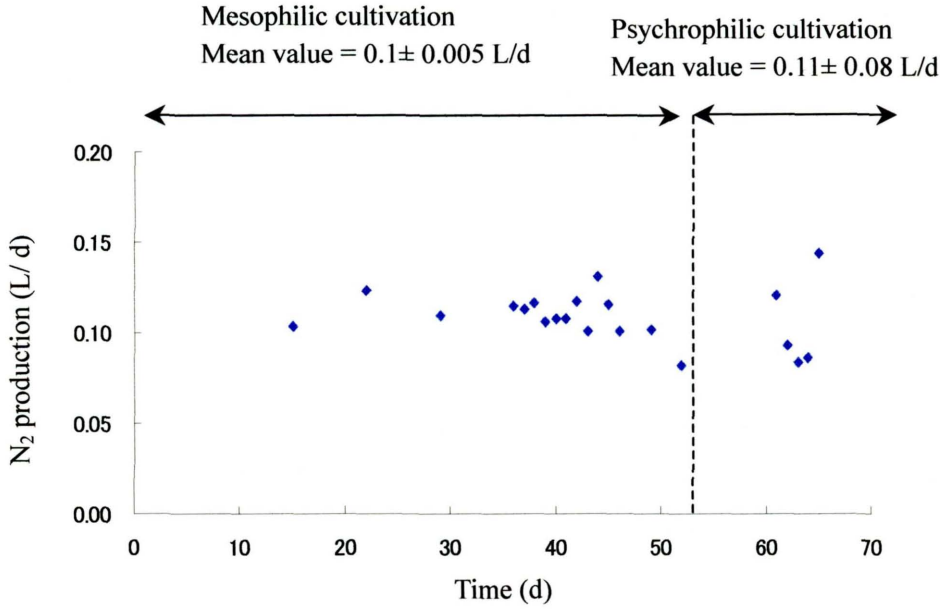


Figure 4.14 Nitrogen production under mixing duration of 45 min/h.

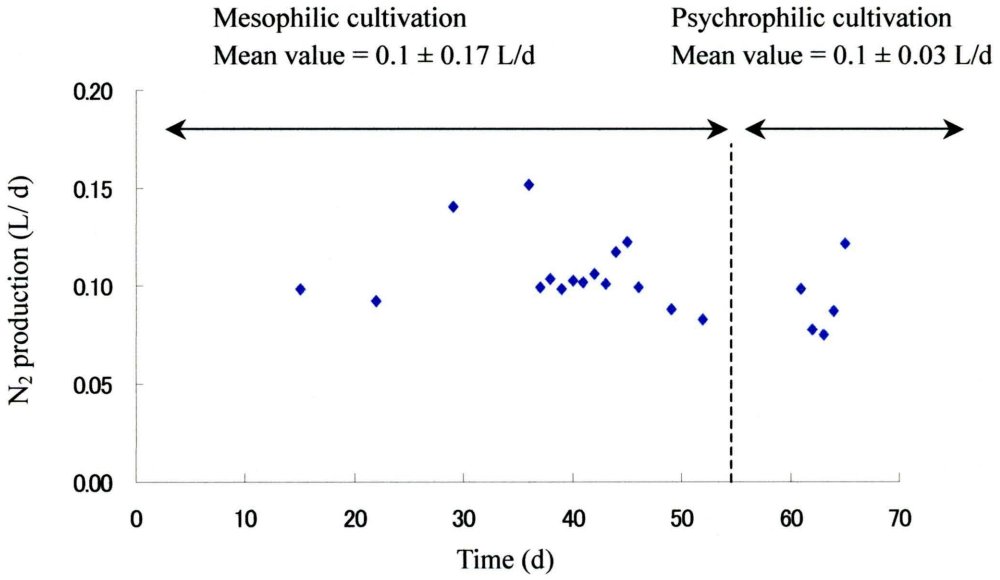


Figure 4.15 Nitrogen production under mixing duration of 30 min/h.

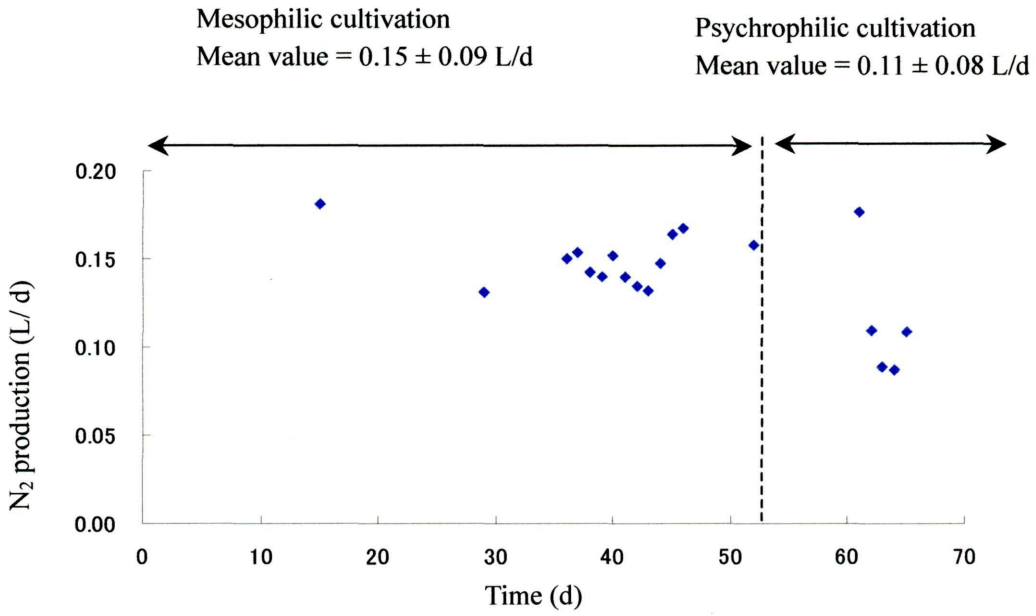


Figure 4.16 Nitrogen production under mixing duration of 15 min/h.

4.3.5 Vitamin B_{12} production by hydrogenotrophic methanogens

Several researches have demonstrated the capability of anaerobic digestion to produce Vitamin B_{12} (Mazumder et al., 1987; Yang et al., 2004). In the present study, the quantity of Vitamin B_{12} was determined to estimate the potentiality of a chemostat reactor working at defined conditions and parameters (0.1/d dilution rate; 12 L H_2/CO_2 gas recirculated at 0.08 MPa pressure) to produce the vitamin compound.

As expected by the results of methane production rate, the vitamin B_{12} production follows the same rate (Figure 4.17 and Figure 4.18). The maximum vitamin B_{12} production was 3 mg/L- effluent at the mesophilic cultivation range and only 0.61 mg/L at the psychrophilic cultivation range. Despite the large amount of trace metals as suggested by Maekawa et al. 1997, the set up value of 10 mL/L-growth culture/day in the present experiment did not enhance a high rate vitamin B_{12} production. Gounot (1986) had presented the reduction of the affinity bacteria - substrate when the growth temperature is lower than the optimal necessary for growth. In other hands, there is a lack of trap to fix the bacteria (Yang et al. 2004) and the utilization of hydrogenotrophic methanogens during the cultivation. In the general anaerobic processes, vitamin B_{12} production is higher when the acetoclastic and the hydrogenotrophic pathways are combined when comparing data from the present experiment and others results and the hydrogenotrophic methanogens have a slow growth rate compared to the acetoclastic methanogens. Vitamin B_{12} production was found to be related much more to the bacteria presence inside the reactor than the substrate availability.

The potentiality of vitamin B_{12} extraction from the effluent of an anaerobic digestor presents an important advantage in the economics of fermentation processes if much research is done.

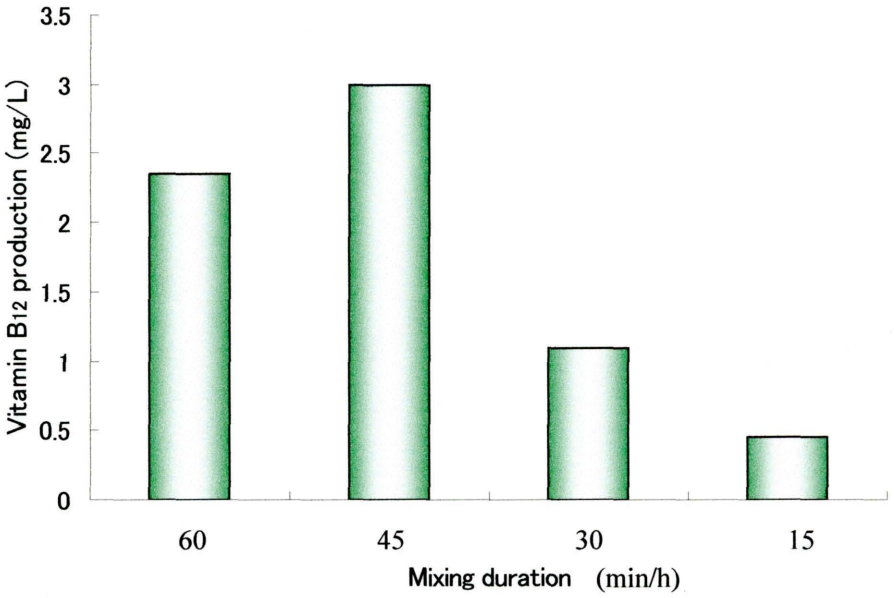


Figure 4.17 Maximum vitamin B_{12} production for different mixing times at 37°C.

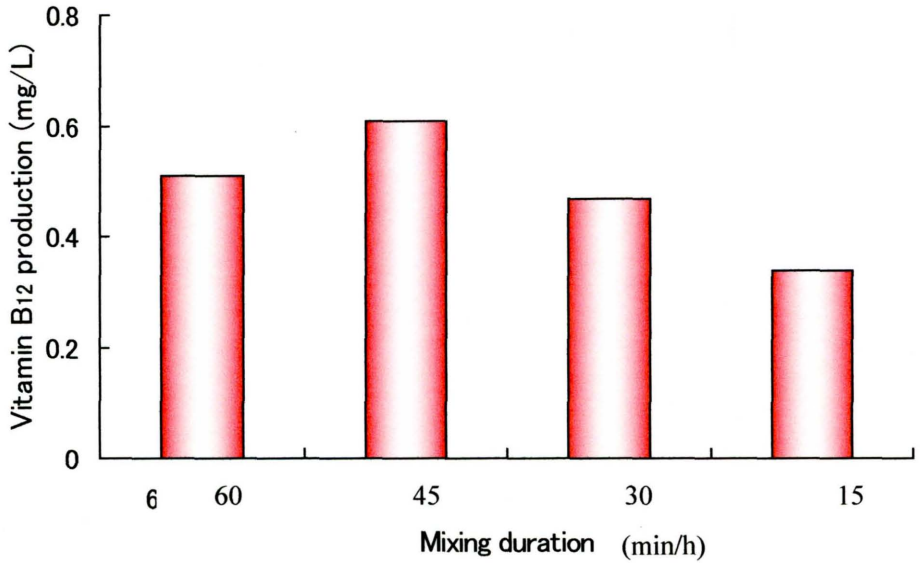


Figure 4.18 Maximum vitamin B_{12} production for different mixing times at 20°C.

4.4 Plant scale simulation of carbon dioxide removal from biogas for two temperatures range (37°C and 20°C)

The biogas resulting from the anaerobic degradation of sewage usually contains about 55% to 65% methane, 35% to 45% carbon dioxide and less than 1% nitrogen while the biogas from organic waste digesters usually contains from 60% to 70% methane, 30% to 40% carbon dioxide and less than 1% nitrogen. Typically, the carbon dioxide is the main non calorific component in the biogas despite the presence of H_2S, other sulfur compounds, such as siloxanes, aromatic and halogenated compounds in amounts of trace compounds.

The carbon dioxide removal from biogas has been experimented in several ways such as absorption separation in liquid (Van Loo et al., 2007), biogas purification using the membranes separation (Harasimowicz et al., 2007; Sawahara et al., 2006), cryogenic separation as well as adsorption separation, the latter playing a vital role if suitable adsorbent material and adsorption-desorption devices are available. The previously cited technologies improve the biogas quality but did not enrich the methane amount in biogas and the cost of regeneration must be take in account. An experimental study on the feasibility of enrichment using indigenously developed coconut shell based active carbon for the carbon dioxide adsorption system was realized by researchers (Jana et al., 2001). Unfortunately, the problem of regeneration still unsolved.

Some studies using biogas recirculation as mixing mode in an anaerobic digestor have demonstrated the efficiency of the operation to recover methane production in the biogas (Morgan and Neuspiel, 1958; Lee et al., 1995). The solubility of biogas component in liquid during the mechanisms such as mixing was investigated elsewhere. Since carbon dioxide has a higher solubility in water than methane, some treatment to remove the carbon dioxide from biogas utilize the water scrubbing to dissolve the CO_2 gas and the gas leaving the top of the column has a high methane content. In addition, the latter water regeneration must be considered.

Therefore, using the stoichiometric relation of conversion of CO_2 to methane in the presence of H_2 and considering the biogas component as ideal gas. We

attempted a simulation of carbon dioxide removal from biogas.

With the data previously obtained in the case of H_2/CO_2 a simulation of a biogas treatment to remove the carbon dioxide design is investigated.

Data from Chikusei City Sewage treatment Center in Ibaraki Prefecture were collected in two periods (one correspondent to the mesophilic range and the other to the psychrophilic range). The data are presented in Tables 4.1 and 4.2. The resulted experimental reactor characteristics are summarized in Table 4. 3; the data will be used for the simulation.

From the stoichiometric of the relation $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$ and the previously determined conversion rate of carbon dioxide to methane at mesophilic and psychrophilic range, we estimate the amount of carbon dioxide conversion in plant scale.

Simulation of Carbon dioxide reduction to methane

The data are presented in Table 4.3; it shows that at 37°C, an increase of 31.6 % in methane can be realized. When the system works during 20°C cultivation, the methane increases only about 18.2 %.

Vitamin B_{12} production and cost

Vitamin B_{12} extracted from anaerobic digestion can participate to the cost reduction of a reactor working in our experiment predefined criteria as presented in Table 4.4.

Table 4.1 Data received from Chikusei City wastewater treatment plant (2006).

Date		5/23	8/15	12/5	2/6
Biogas composition	CO_2 (%)	38	36	37	38
	Methane (%)	61	63	60	60
	H_2S (%)	0.055	0	0.006	0.012
Biogas production rate (Nm^3) *		15,540	12,640	11,790	2,700

* Nm^3 means normal cubic meter and represents the volume of gas measured under the standard conditions of 0 degrees Celsius, and 1 atmosphere of pressure

Table 4.2 Standard of the characteristic for the design chemostat reactor resulted from the laboratory experiments.

Parameters	Mesophilic range	Psychrophilic range
Temperature (°C)	37-40	20
Dilution D, (1/d)	0.1	0.1
H_2/CO_2 gas supply (L/d)	12	12
Mixing duration (min/h)	45	45
CO_2 conversion rate (%)	80.8	35.8
Vitamin B_{12} production (mg/L)	3	0.61

Table 4.3 Carbon dioxide reduction to methane calculated from the experimental results presented in Table 4.2.

Parameters	37°C	20°C
Biogas yields, Gp (Nm^3/d)	426.6	457.6
Methane yields (Nm^3/d)	268.7	279.1
CO_2 in the biogas (Nm^3/d)	153.5	173.9
CO_2 convert to methane (Nm^3/d)	124.1	62.3

Table 4.4 Vitamin B_{12} production simulated from the experimental data summarized in Table 4.2.

Parameters	37°C	20°C
Biogas yields, Gp (Nm^3/d)	426.60	457.60
Methane yields (Nm^3/d)	268.70	279.10
CO_2 in the biogas (Nm^3)	153.60	173.90
CO_2 convert to methane (Nm^3)	124.10	62.30
Input H_2 (Nm^3)	262041.60	318261.40
Feeding rate, F (Nm^3/d)	10.90	13.30
Dilution, D = 1/HRT (1/d)	0.1	0.10
Reactor working volume, V (m^3)	131.0	159.10
Effluent rate, V (m^3)	10.90	13.30
Mixing rate (min/h)	45	45
Experimental vitamin B_{12} amount (mg/L)	3	0.61
Vitamin B_{12} production rate (g/m^3-effluent)	32.80	8.09
Vitamin B_{12} cost (yen/g)	3,520,982	3,520,982
Produced vitamin B_{12} sales (yen)	115,326,266	28,481,968

4.4 Conclusion

In order to determine the suitable mixing duration to achieve a high conversion rate of carbon dioxide to methane under two temperature ranges, different mixing durations were applied to a standardized chemostat reactor at dilution rate of 0.1/d. The mixing duration and the two ranges temperatures analysis emphasize the importance those parameters when a reactor is designed to run under gas substrate for hydrogenotrophic methanogens strains. For the carbon dioxide removal, the choice of 45 min/h mixing at pump pressure of 0.08 MPa was found to be the suitable range for different temperatures (37°C and at 20°C). The mixing duration of 45 min/h at 0.08 MPa can be used under mesophilic and psychrophilic conditions to achieve a high rate carbon dioxide removal from gas. The production of vitamin B_{12} at 45 min/h high at mesophilic and psychrophilic cultivation condition was high. It might be an advantage if the vitamin B_{12} extraction protocol can be standardized for large-scale extraction of the molecule in anaerobic digester.

The observation made in this study shows that hydrogenotrophic methanogens constitute a provider of advantages of high methane production rate. The application of the obtained data to simulate a bench scale experiment demonstrates a good carbon dioxide removal rate by means of conversion to methane in summer season as in temperate climate.

CHAPTER 5

Overall conclusions

In order to determine the operational conditions for an anaerobic bioreactor utilizing the carbon dioxide as carbon source for energy metabolism and simulate a carbon dioxide removal from the biogas, four investigations were conducted: (1) Determination of the mass balance of an acclimated hydrogenotrophic methanogens culture in a chemostat supply with inorganic media to stimulate the bacteria activity and H_2/CO_2 gas mixture (80:20, v/v) as substrate, (2) Simulation of a digester performance under the conditions of H_2/CO_2 gas as substrate, (3) Application of different mixing duration in chemostat to find out the highest conversion rate of CO_2 to CH_4 and (4) Utilization of the experimental results to simulate a large scale conversion of the carbon dioxide content in the biogas to methane. From this dissertation, the following conclusions were obtained:

Chapter 1: Introducing the dissertation, the carbon dioxide problem as greenhouse gas and its different emissions sources were overviewed; various renewable energy source are increasingly used to replace the fossils fuel that are the mean carbon dioxide producers. The energy capacity of the biogas, one of the promising energy sources, was examined and methods used to increase the energy value of the biogas were overviewed. In addition, the method of biogas recirculation in an anaerobic reactor was introduced.

Chapter 2: The methanogenic degradation process that is involved during the biogas recirculation was investigated. Methanogenesis involves two strains of microbes, the acetate dependent methanogens and the H_2/CO_2 dependent methanogens. Studies that utilized H_2/CO_2 as inorganic substrate or as only mixing factor were presented. Then the different types of simulations were summarized.

Chapter 3: The determination of the mass balance of hydrogenotrophic methanogens cultivated in the chemostat reactors was realized with the H_2/CO_2 gas recirculation system at various dilution rates. H_2/CO_2 mixture gas was found to be considered as single substrate independently of their different solubility in liquid. The Monod model applied to the chemostat steady state data determine the

mass balance; the growth yield yield (Y_{CH4}) reached 11.66 g cells formed/ mmol of H_2/CO_2 consumed. The maximal specific growth rate (μ_{max}) and the Monod half-saturation coefficient (K_S) were 0.15/d and 0.82 g/L, respectively. Using the obtained results, a digester performance was simulated. During the steady state, the simulation predicts the dependence of the H_2/CO_2 concentration (S) and the cell concentration (X) on the dilution rate. The model fitted well with the experimental data and was able to yield maximum methanogenic activity, 0.24 L CH_4/g VSS·d; the dilution rate was estimated to be 0.1/d. At a dilution rate of 0.14/d, the exponential cells washout was obtained.

Chapter 4: The proposed digester characteristics were run under different mixing durations to maximize the methane production. The investigation demonstrated that the continuous mixing achieved a high CO_2 gas dissolution rate but not the highest methane production rate. In fact, the suitable mixing duration was 45 min/h, which attend a methane production of 1.94 ± 0.057 L (80.8% of carbon dioxide convert to methane) at mesophilic temperature. The cells damage due to the vigorous continuous mixing is the plausible explanation; results conform by the N_2 level measured in the gas headspace. The psychrophilic temperature range (20°C) obtained also the highest methane conversion at 45 min/h mixing duration 39.8% conversion of CO_2 to methane.

In addition, the possibility of vitamin B_{12} production under the standardize digester run was determined to initiate an economical cost.

The fundamental engineering parameters for a reactor using H_2/CO_2 as substrate obtained in the Chapters 3 and 4 were used to simulate at plant scale the methane content of biogas. Apply the data obtained for H_2/CO_2 mix gas to the biogas was made because of the low solubility of methane gas in liquid. The simulation had shown an improvement of the methane content in the biogas from 268.7 Nm^3/d to 392.8 Nm^3/d in summer and from 279 Nm^3/d to 341 Nm^3/d in autumn season. The vitamin B_{12} was 32.7 g/m^3 effluent and 8 g/m^3 effluent respectively. Both summer and temperate temperature anaerobic digestion condition could be used to re convert the CO_2 generate by the biogas to methane.

Further research

Alternatively, biogas quality recovery by using the hydrogenotrophic methanogens represents a great potentiality for less cost treatment. In further experiment, a study using a reactor with fix bed should be used to increase the trap of hydrogenotrophic methanogens. Furthermore, careful consideration of the net energy balance between the increased heating energy demands and improved additional methane production at different operating temperatures must be simultaneously taken into account when deciding the economical digesting temperature.

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