



## Control of Methane Emission in Ruminants and Industrial Application of Biogas from Livestock Manure in Korea\*

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**ABSTRACT :** Methane is known to be one of the major greenhouse gases. On a global scale, livestock farming may contribute 18% of total greenhouse gas emissions. Though methane contribution is less than 2% of all the factors leading to global warming, it plays an important role because it is 21 times more effective than carbon dioxide. Methane emission is a direct result of the fermentation process performed by ruminal microorganisms and, in particular, the archaeal methanogens. Reducing methane emission would benefit both ruminant production and the environment. Methane generation can be reduced by electron-sink metabolic pathways to dispose of the reducing moieties. An alternative way for methane control in the rumen is to apply inhibitors against methanogens. Generating methane from manure has considerable merit because it appears to offer at least a partial solution to two pressing problems—environmental crisis and energy shortage. An obvious benefit from methane production is the energy value of the gas itself. Control of methane emission by rumen microbes in Korea has mainly been focused on application of various chemicals, such as BES and PMDI, that inhibit the growth and activity of methanogens in the rumen. Alternatives were to apply long-chain polyunsaturated fatty acids and oils with or without organic acids (malate and fumarate). The results for trials with methane reducing agents and the situation of biogas production industries and a typical biogas plant in Korea will be introduced here. (**Key Words :** Methane, Methanogens, Ruminants, Biogas, Livestock Manure)

### INTRODUCTION

Carbon dioxide (CO<sub>2</sub>) and methane (CH<sub>4</sub>) are major greenhouse gases (Moss et al., 2000). Methane is found in various places in the environment, and its release from agricultural sources was estimated to 205 to 245 million tons per year (Duxbury and Mosier, 1993). At a global scale, livestock farming may contribute 18% of total greenhouse gas emissions (FAOSTAT, 2006). Though methane's contribution is less than 2% of all the factors leading to global warming (Johnson and Johnson, 1995), it plays an important role because it is 21 times more effective than

carbon dioxide (Johnson et al., 1996).

Methane emission is a direct result of fermentation process performed by ruminal microorganisms and, in particular, the archaeal methanogens, which scavenge hydrogen and use it to produce methane. Since its release to the atmosphere represents an energy loss of between 2% and 15% of ingested gross energy (Van Soest and Demeyer, 1996), reducing its emission would benefit both the ruminant production and the environment (Moss et al., 2000). Theoretically, methane generation can be reduced by electron-sink metabolic pathways to dispose of the reducing power (López et al., 1999; Ungerfeld et al., 2003). Therefore, it is desirable to reduce in H<sub>2</sub> production by enhancing the reactions that accept electrons other than H<sub>2</sub> formation. Alternative way for the methane control in the rumen is to apply the inhibitors against methanogens (Mathison et al., 1998).

Meanwhile, livestock manure has been considered waste which makes worse the environment or has been used as soil fertilizer, partially, in many developing countries, but it has become one of the renewable energy sources. Many industries over the world have established plants to produce bio-methane from the livestock manure. The emphasis in

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**Table 1.** Effects of 2-bromoethanesulfonic acid (BES) on *in vitro* methane production and quantification of total methanogen

Items	Timothy (mM) <sup>1</sup>			Mixed feed (mM) <sup>1</sup>			Significance		
	0	1	5	0	1	5	Sub	BES	Sub×BES
Methane (ml)									
24 h	5.7	2.1	0.3	8.7	2.4	0.2	**	**	NS
48 h	7.5	3.8	0.3	11.2	4.3	0.4	**	**	*
72 h	9.3	5.3	0.4	11.5	4.8	0.2	**	**	**
Total methanogen									
24 h	1.38	1.37	1.04	1.47	1.49	0.98	NS	*	NS
48 h	1.57	0.92	0.42	1.92	0.92	0.46	NS	**	NS
72 h	1.11	0.60	0.27	1.17	0.52	0.31	NS	**	NS

<sup>1</sup> Within timothy and mixed (40% timothy+60% concentrates) substrate, BES was treated at the final concentration of 0, 1 and 5 mM.

\* \*\* Significant at p<0.05 and p<0.01, respectively; NS = Not significant.

this overview, therefore, will be put on aspects of methane control and trends to develop the bio-methane from livestock manure in Korea.

### CONTROL OF METHANE PRODUCTION IN THE RUMEN

Control of methane emission by rumen microbes has mainly been focused to apply the various chemicals that inhibit the growth and activity of methanogens in the rumen. They are direct inhibition of methane generation using halogenated methane analogues (Van Nevel and Demeyer, 1995), chlorinated CH<sub>4</sub> analogues (Van Nevel and Demeyer, 1996) and chemical complex of bromo-chloromethane and cyclodextrin (McCrabb et al., 1997). Bromoethanesulphonate (BES), structural analogue of the cofactor mercaptoethanesulfonic acid (coenzyme M) used by methanogenic bacteria also is a potent inhibitor of methane emission (Mathison et al., 1998). Lee et al. (2009) observed a reduced *in vitro* methane production by supplementing the various level (0, 1 and 5 mM) of BES to the culture solution containing timothy or mixed (40% timothy and 60% concentrate) substrate in a dose dependent manner (Table 1). The quantification of total methanogen supported the results of methane production.

Propionate enhancers are also one of the effective alternatives in methane control. Fumarate and malate are four carbon intermediates (dicarboxylic acid) in the

propionate pathway in which they are reduced to succinate. In this reaction, hydrogen ion (H<sup>+</sup>) is needed and therefore, reduction of the dicarboxylic acids may provide an alternative electron sink for H<sub>2</sub>. Addition of fumarate or malate in acid form up to 24 mM each to culture solution containing concentrate (70%) and ground alfalfa hay (30%, DM) reduced *in vitro* methane generation by 65.6% and 47.5%, respectively for 12 h incubation compared to control (Li et al., 2009a, Table 2). Thus the organic acid such as malate and fumarate may be put to practical use for ruminant diets since it has the dual benefit of decreasing CH<sub>4</sub> production and increasing net energy retention.

It was also found that the addition of up to 500 umol of sodium fumarate *in vitro* decreased CH<sub>4</sub> production by 6% after 48 h incubation (Lopez et al., 1999). Asanuma et al. (1999) also showed that the addition of 20 mM of fumarate to cultures that were fermenting hay powder and concentrate incubated for 6 h significantly decreased CH<sub>4</sub> production by 5% while with the addition of 30 mM fumarate, CH<sub>4</sub> declined by 11% compared to the control.

The unsaturated fatty acids (UFAs) in the added fat were widely identified to reduce CH<sub>4</sub> emission through hydrogenation of them (Johnson and Johnson, 1995; Dohme et al., 2000; Li et al., 2010). Li et al. (2010) conducted an *in vitro* experiment with 60mg linoleic acid (LA, C<sub>18:2</sub>) in associated with organic acids, and found that 24 mM of C<sub>18:2</sub> alone decreased methane generation compared to control, and malate (24 mM) with C<sub>18:2</sub> (M-

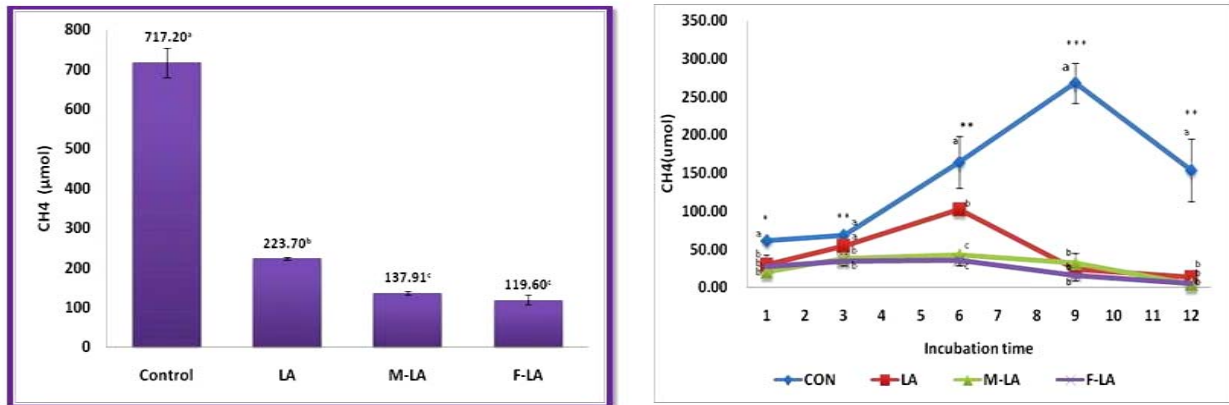
**Table 2.** Methane production (μmol) as influenced by addition of fumarate or malate in acid form for 12 h incubation

Items	Fumarate (mM) <sup>1</sup>				Malate (mM) <sup>1</sup>				Effects <sup>2</sup>		
	0	8	16	24	0	8	16	24	Source	Level	S×L
Total gas (ml)	125 <sup>c</sup>	149 <sup>ab</sup>	153 <sup>ab</sup>	171 <sup>a</sup>	125 <sup>a</sup>	154 <sup>ab</sup>	184 <sup>a</sup>	181 <sup>a</sup>	NS	**	NS
CH <sub>4</sub> (μmol)	328 <sup>a</sup>	187 <sup>b</sup>	148 <sup>c</sup>	112 <sup>d</sup>	328 <sup>a</sup>	266 <sup>b</sup>	182 <sup>c</sup>	172 <sup>c</sup>	***	***	**

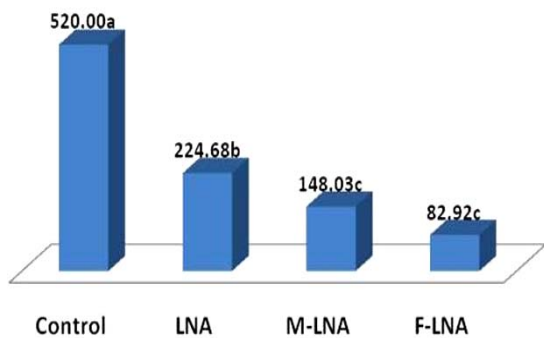
<sup>1</sup> Means in the row with different letters differ.

<sup>2</sup> Source, fumarate vs malate; level, addition level of organic acid; S×L, interaction between fumarate and malate.

\* p<0.05; \*\* p<0.01; \*\*\* p<0.001; NS = Not significant.



**Figure 1.** Methane production (µmol) as influenced by malate (M) or fumarate (F) when incubated with C<sub>18:2</sub> (LA). \* p<0.05; \*\* p<0.01; \*\*\* p<0.001.



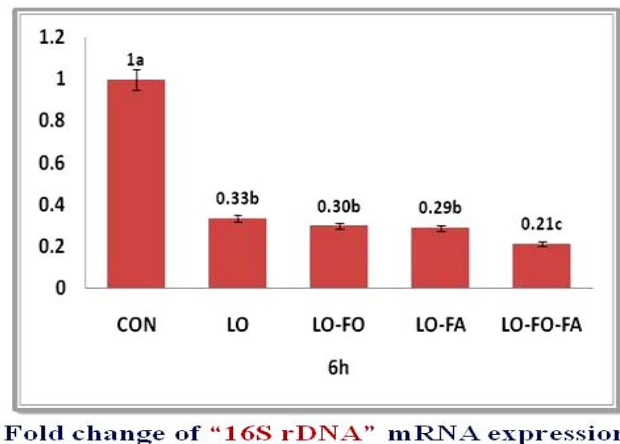
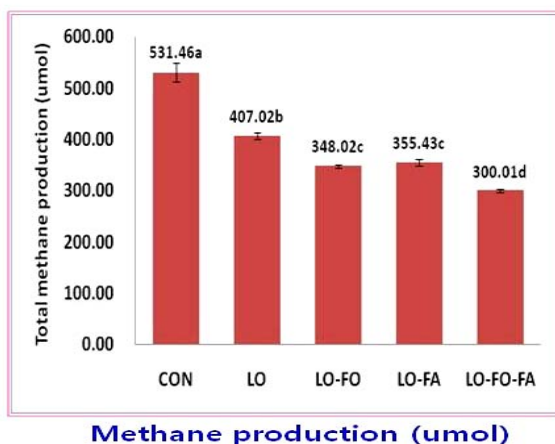
**Figure 2.** Methane production (µmol) as influenced by malate (M) or fumarate (F) when incubated with C<sub>18:3</sub> (LNA). p<0.01.

LA) reduced methane emission by 38% and fumarate (24 mM) with C<sub>18:2</sub> (F-LA) by 47% compared with addition of C<sub>18:2</sub> alone for 12 h (Figure 1). Incubation of 60 mg linolenic acid (LNA, C<sub>18:3</sub>) in associated with 24 mM malate or fumarate each in culture solution supplemented

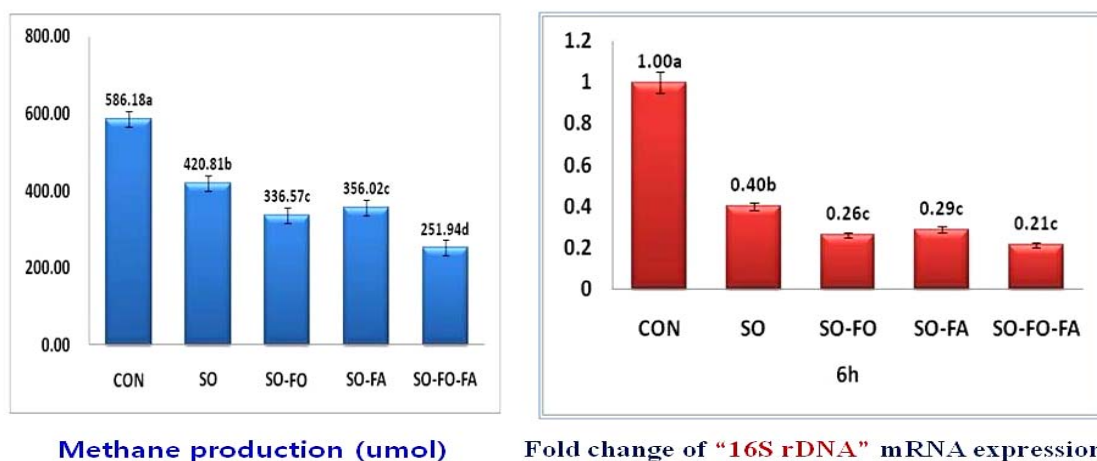
with 2 g feed also reduced *in vitro* methane production compared to control, and malate with C<sub>18:3</sub> (M-LNA) decreased methane production by 34.12% and fumarate with C<sub>18:3</sub> (F-LNA) by 63.09% compared to C<sub>18:3</sub> alone (Li et al., 2009b, Figure 2). Reductions in CH<sub>4</sub> emissions in the rumen have been widely reported when a variety of plant oil was added to the diet (Cieslak, 2003; Jordan et al., 2004), and the level of methane emission in ruminants is directly proportional to bio-hydrogenation of unsaturated fatty acid (UFA, Plascencia et al., 1999).

Linseed oil and fish oil as oil rich in UFAs, and the bio-hydrogenation of UFAs may provide an alternative hydrogen sink to compete with methanogens for the available H<sub>2</sub> (Czerkawski et al., 1972). Li et al. (not published) observed the decreased *in vitro* methane production by rumen microbes when incubated with C<sub>18:3</sub> rich linseed oil (LO) and fish oil (FO) in association with fumarate (FA, Figure 3).

Added oils greatly decreased mRNA expression of



**Figure 3.** Methane production (µmol) and mRNA expression of methanogens as influenced by fish oil (FO) and fumarate (FA) when incubated with linseed oil (LO). p<0.01.



**Figure 4.** Methane production ( $\mu\text{mol}$ ) and mRNA expression of methanogens as influenced by safflower seed oil (SO) and fish oil (FO) in association with fumarate (FA).

archaeal 16S rDNA relating to methanogens and fumarate decreased it further (Figure 3). Incubation of  $C_{18:2}$  rich-safflower seed oil (SO) and fish oil (FO) with fumarate (FA) also reduced methane production *in vitro* and methane reduction was supported by clearly reduced mRNA expression of archaeal 16S rDNA (Li et al., not published, Figure 4).

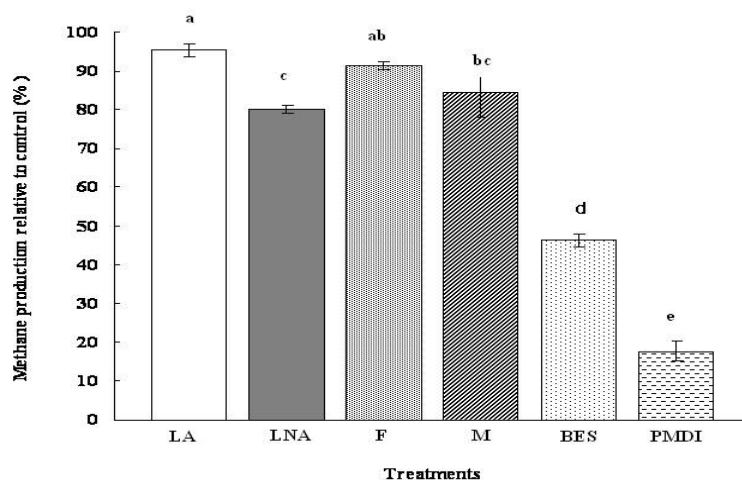
Fievez et al. (2003) conducted *in vitro* experiments with two types of fish oil and they found that  $\text{CH}_4$  inhibition seems proportional to the relative amount of polyunsaturated fatty acids and their rate of lipolysis. Fish oil was reported to increase concentration of propionate (Keady and Mayne, 1999; Wachira et al., 2000) which also requires  $\text{H}_2$  in the rumen, and thus could depress the  $\text{CH}_4$  emission (Fievez et al., 2003).

Meanwhile, Choi et al. (2004) conducted *in vitro* trial with various methane reducing agents (30  $\mu\text{mol}$  BES, 10 ppm PMDI, 10 mM fumarate or malate, 5%  $C_{18:2}$  or  $C_{18:3}$ )

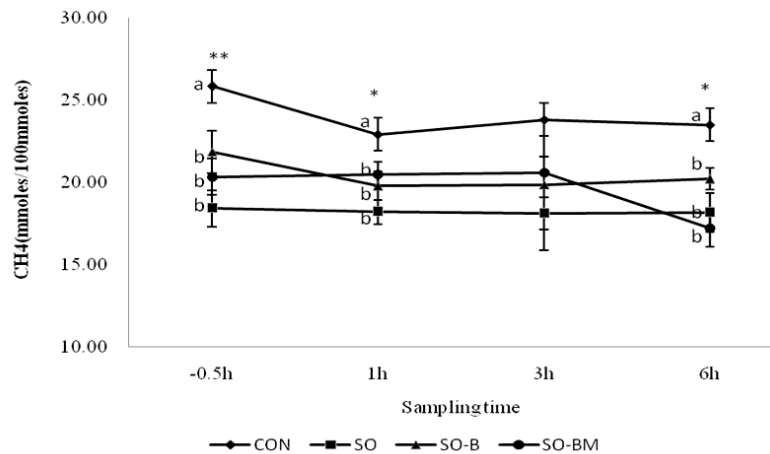
and they found that all the agents reduced methane emission compared to control, and effect in methane reduction was greater for BES and pyromellitic diimide (PMDI) as halogenated compound than for UFAs ( $C_{18:2}$  and  $C_{18:3}$ ) and organic acids (malate and fumarate, Figure 5).

Many other *in vitro* studies with methane reducing agents have been conducted in Korea. One of them was cyclodextrin (CD) complex of fatty acids, and it decreased methane production at 8 h and 12 h incubation compared to control and CD alone (National Institute of Animal Science, Korea, not published). Addition of Resveratrol and iodo propane-CD complex also reduced *in vitro* methane emission by up to 64% and 50%, respectively, compared to control (Oh et al., National Institute of Animal Science, Korea, not published).

Methane emission in the ruminant animals, in general, is closely related with feed but dietary manipulation itself has been limited in reducing it. Direct application of the



**Figure 5.** Comparison of the effect of methane reducing agents *in vitro*. LA, linoleic acid; LNA, linolenic acid; F, fumarate, M, malate; BES, bromoethanesulfonic acid; PMDI, pyromellitic diimide.



**Figure 6.** Effect of soybean oil with buffer and/or monensin on estimated methane emission at various sampling times of rumen fluid. CON, control; SO, soybean oil supplementation; SO-B, supplementation of soybean oil with buffer; SO-BM, supplementation of soybean oil with buffer and monensin.

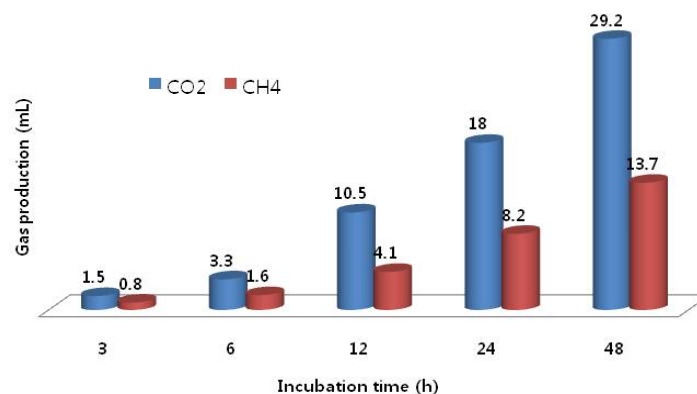
materials that were found to be effective in *in vitro* methane reduction to the ruminants has not been fully successful in consistent effect, mainly due to the microbial adaptation to those materials. Li et al. (2009c) conducted metabolism study with milking goats to examine the effect of soybean oil (5% of DM intake) in association with sodium bicarbonate (0.5%) and/or monensin (30 ppm) on methane production, and they found the clearly decreased methane production as calculated based on VFA concentration at all sampling times of rumen fluid after morning feeding (Figure 6).

### BIOGAS PRODUCTION FROM LIVESTOCK MANURE

Generating methane from manure has considerable merit because it appears to offer at least a partial solution to two processing problems-environmental crisis and the energy shortage (Fulhage et al., 1993). Livestock manure contains portion of organic solids such as proteins, carbohydrates and fats that are available as food and energy

for growth of anaerobic bacteria. Obvious benefit from methane production is the energy value of the gas itself. But the gas production from manure depends mainly upon the efficiency of operating system for it. Gas yield can be a certain amount of gas produced per unit of solids degraded by the anaerobic bacteria (Fulhage et al., 1993). They estimated the average potential methane production from the livestock manure, and found the production of 692, 946, 125 and 6.4 cm<sup>3</sup> daily from dairy cattle (545 kg), beef cattle (450 kg), swine (68 kg) and poultry (1.8 kg), respectively. Thus, their energy production rates (kcal/h/animal) were 143, 195, 30 and 1.3, respectively. They further speculated that the number of animal heads which require energy for the use of kitchen range for 2h daily from livestock manure will be 14, 11, 77 and 1,547, respectively.

Song (2010, not published) made some *in vitro* trials for the estimation of methane production from cattle manure. Four cattle were fed 8 kg feeds (6 kg concentrate and 2 kg ryegrass hay, DM basis) daily. The chemical composition of the diet was 13.2, 3.8 and 45.1% (DM) for CP, EE and NDF, respectively, and its whole tract digestibility of DM was



**Figure 7.** *In vitro* gas production for 48 h from cattle feces.



**Figure 8.** Chang Nyung biogas plant (Easy Bio System) in Korea.

65.3%. Cattle feces contained 15.5% CP, 2.8% EE and 61.5% NDF (DM). *In vitro* degradation of cattle feces for 48 h was 48.4, 61.4 and 36.1% for DM, CP and NDF, respectively. Gas production for 48h was 29.2 and 13.7 ml for CO<sub>2</sub> and methane, respectively (Figure 7). Annual manure production from livestock in Korea has been approx. 40 to 42 million tons on a fresh basis for the past 6 years since 2003 (NIAS, 2008). Thus, overall estimation of methane production from manure for each animal would be possible indirectly when *in vitro* method is applied.

The Korean government announced low carbon green growth as a new dynamic growth engine, and Tokyo Protocol will be expected to apply in Korea for reducing green house gases from 2013. The livestock manure and food leftover, therefore, should be developed to either fertilizer or biogas energy source. Especially, eco-friendly energy production from manure will give a solution for reducing green house gas emission. For this purpose, both the Korean government and private sectors need to cooperate together to lead the biogas project as an enterprise for public utility.

**Table 3.** Gas and electricity production in Chang Nyung biogas plant, Korea

Yield	Unit	Day	Month	Year
Biogas	M3	4,800	144,000	1,728,000
Electricity produced	kWh	9,600	288,000	3,504,000

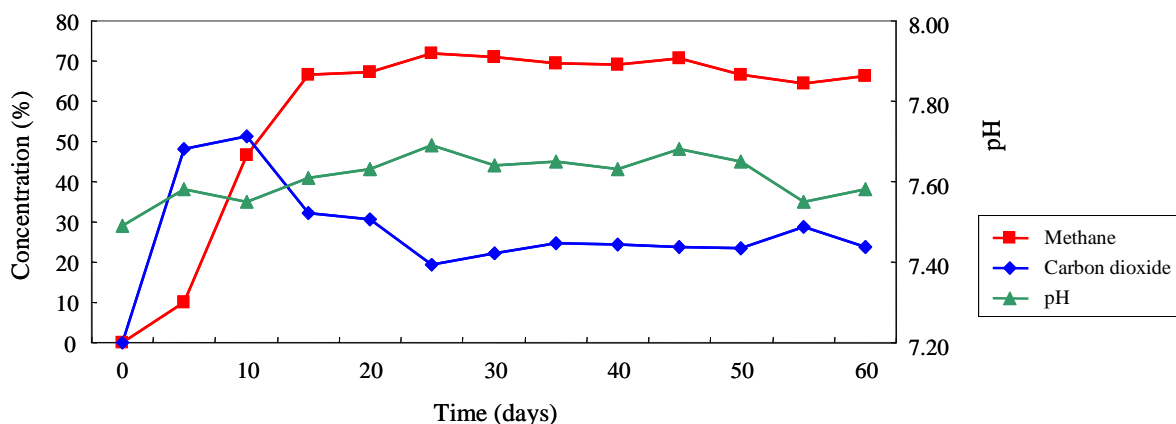
Adapted from Easy Bio System, Korea, 2010

In Korea the biogas project has been developed since 1979 but most of 13 biogas plants have small capacity (10 to 20 tons per plant). They had low capacity of electricity production and are closed or shut down due to low economic value and lack of operating skill. The government supported the plants for construction but not for technology development. Electricity production from renewable energy was only 1% of total power in Korea. There are not many biogas plants which are commercially operated. Korean government and local industries are getting more interested in this project (Hyun, 2010). Pilot plants being supported by government funds will be resulted in increased biogas production from manure. Low economic value from manure, however, still exists mainly due to less incentive price for electricity from biogas energy compared to other energy.

One of the typical biogas plants in Korea is located in Chang Nyung as established in 2008 and has been operated by Easy Bio System, a private company (Figure 8).

The operation of biogas plant has been consulted by NIRAS, Denmark. Production of electricity from manure and food leftover began from October 2008. Daily electricity production was planned to 9,600 kW from 4,800 cm<sup>3</sup> biogas per day (Table 3). Total 785,970 kW was produced by February 2009 and the company has sold the electricity to the KEPCO, Korea total US\$ 98,582 during the period.

Daily gas emission was evaluated by the company, and it was found that methane concentration in the biogas produced was approx. 60 to 70% which is considered to be highly efficient (Figure 9). Despite the successful



**Figure 9.** Normal analysis of data of methane fermentation (Easy Bio System, Korea, 2010).

management of biogas plant in a few industries in Korea, technical limitations and lack of operating skill still exist. As well, wrong preconception on manure handling facility including biogas plants also exists mainly due to odor problem. Positive understanding for the facility of biogas production and cooperative relationship between government and industry, therefore, are necessary to satisfy the needs to reduce the greenhouse gas and energy production from livestock manure.

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