DETERMINATION OF MICROBIAL BIOMASS GASES USING TITRATION AND HEADSPACE GAS CHROMATOGRAPHY

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DETERMINATION OF MICROBIAL BIOMASS GASES USING TITRATION AND HEADSPACE GAS CHROMATOGRAPHY

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ABSTRACT

Microbial biomass gases, carbon dioxide (CO₂) and methane (CH₄), are generated by a microbial respiration process. They can be used as environmental indicators for assessing degree of a pollutant exposing to the environmental system. These gases are considered as green house gases which directly affect the change of the earth’s climate. The respiration process plays a major role for observing those gases and their content in environment can be measured by many analytical approaches.

This work focused on CO₂ and CH₄ gases analysis in soil using titration method and headspace gas chromatography (HS-GC). Titration method was studied with the adjusted respirometer by flushing CO₂ gas with N₂ gas from the reaction chamber for CO₂ trapping by KOH solution. This method is simple and inexpensive for determining CO₂. Detection limit, linearity range, and %RSD in titration method were 14.12 mg m⁻³ CO₂, 50-180 mg m⁻³ CO₂, and 3.98 - 9.63 %, respectively. Percentage efficiency of trapping CO₂ was in the range 61.41 ± 4.26 %. Comparative measurement using HS-GC was applied by studying packing materials for packing the column. The optimum packed column was silica gel (3.1 mm x 101 cm, 6.5 g) which could separate CO₂ and CH₄ using the optimum HS-GC condition. The HS-GC gave high sensitivity and low detection limits which were 2.29 µg m⁻³ for CO₂ and 0.02 µg m⁻³ for CH₄, respectively. Soil and sediment samples applications using both techniques to determine CO₂ and CH₄ were selected from a vegetable garden at Salaya campus and a canal at Phayathai campus. The biomass gases were 7.51 g CO₂ m⁻² d⁻¹ for soil samples and 8.01 g CO₂ m⁻² d⁻¹ for sediment samples. CH₄ gas was not found in all samples. CO₂ and CH₄ contents are significantly different with the sample niches. These measurements will help us to understand the environmental system better.

KEY WORDS: BIOMASS / CARBON DIOXIDE / METHANE / HEADSPACE GAS CHROMATOGRAPHY

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การหาปริมาณของแก๊สชีวมวลจุลินทรีย์ด้วยเทคนิคไทเทรตและเฮดสเปสแก๊สโครมาโทกราฟี (DETERMINATION OF MICROBIAL BIOMASS GASES USING TITRATION AND HEADSPACE GAS CHROMATOGRAPHY)

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บทคัดย่อ

แก๊สชีวมวลจุลินทรีย์คือแก๊สคาร์บอนไดออกไซด์และแก๊สมีเทนซึ่งเกิดจากการหายใจของสิ่งมีชีวิตขนาดเล็ก。

การหาปริมาณแก๊สชีวมวลจุลินทรีย์ใช้เทคนิคไทเทรตและเฮดสเปสแก๊สโครมาโทกราฟี โดยทั่วไป แก๊สคาร์บอนไดออกไซด์และแก๊สมีเทนเป็นแก๊สที่มีความสำคัญในการวิเคราะห์สิ่งแวดล้อม และการวิเคราะห์การเปลี่ยนแปลงของภูมิอากาศ ทำให้เกิดการปฏิกิริยาที่สำคัญต่อสิ่งแวดล้อมและ ArgumentException: นักวิทยาศาสตร์เคมี.

ในการวิจัยนี้ นักวิทยาศาสตร์เคมีมีการวิจัยเกี่ยวกับการหาปริมาณแก๊สชีวมวลจุลินทรีย์โดยใช้เทคนิคไทเทรตและเฮดสเปสแก๊สโครมาโทกราฟี โดยทั่วไป แก๊สคาร์บอนไดออกไซด์และแก๊สมีเทนเป็นแก๊สที่มีความสำคัญในการวิเคราะห์สิ่งแวดล้อม และการวิเคราะห์การเปลี่ยนแปลงของภูมิอากาศ ทำให้เกิดการปฏิกิริยาที่สำคัญต่อสิ่งแวดล้อมและArgumentException: นักวิทยาศาสตร์เคมี.

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3.1 The carbon cycle of microorganisms

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<table>
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<th>Description</th>
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<tbody>
<tr>
<td>HS-GC</td>
<td>Headspace gas chromatography</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared absorption spectroscopy</td>
</tr>
<tr>
<td>IRGA</td>
<td>Infrared gas analyzers</td>
</tr>
<tr>
<td>TGA</td>
<td>Trace gas analyzer</td>
</tr>
<tr>
<td>TDL</td>
<td>Tunable diode laser absorption spectroscopy</td>
</tr>
<tr>
<td>mg m⁻³</td>
<td>Milligram per cubic metre</td>
</tr>
<tr>
<td>µg m⁻³</td>
<td>Microgram per cubic metre</td>
</tr>
<tr>
<td>%</td>
<td>Percentage</td>
</tr>
<tr>
<td>v / v</td>
<td>Volume by volume</td>
</tr>
<tr>
<td>cm sec⁻¹</td>
<td>Centimetre per second</td>
</tr>
<tr>
<td>min</td>
<td>Minute</td>
</tr>
<tr>
<td>°C</td>
<td>Temperature in degree of Celsius</td>
</tr>
<tr>
<td>RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>h</td>
<td>Hours</td>
</tr>
<tr>
<td>i.d.</td>
<td>Internal diameter</td>
</tr>
<tr>
<td>ppm</td>
<td>Part per million</td>
</tr>
<tr>
<td>ppb</td>
<td>Part per billion</td>
</tr>
<tr>
<td>cm³</td>
<td>Cubic centimetre</td>
</tr>
<tr>
<td>ND</td>
<td>Not detectable</td>
</tr>
<tr>
<td>µV</td>
<td>Microvolt</td>
</tr>
<tr>
<td>W m⁻²</td>
<td>Watt per square metre</td>
</tr>
<tr>
<td>Gt yr⁻¹</td>
<td>Gigatons per year</td>
</tr>
<tr>
<td>Tg yr⁻¹</td>
<td>Teragrams per year</td>
</tr>
<tr>
<td>mg m⁻² h⁻¹</td>
<td>Milligram per square metre per hour</td>
</tr>
<tr>
<td>µs cm⁻¹</td>
<td>Microsiemen per centimetre</td>
</tr>
<tr>
<td>psi</td>
<td>Pound per square inch</td>
</tr>
<tr>
<td>tᵣ</td>
<td>Retention time</td>
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</table>
THE RELEVANCY OF THE RESEARCH WORK TO THAILAND

This work studied on the title of determination of microbial biomass gases using titration and headspace gas chromatography. The main work was focusing on the analytical techniques that were applied for determining CO₂ and CH₄ gases. Theses gases can be used as environmental key indicators to evaluate of soil fertility. Therefore, the relationship between indicator gases and soil quality can be predicted and assumed to perform further agricultural improvement in the real system.

The need of research knowledge relevance to the limited natural resource is very important. This approach can reduce natural consumption in many processes of human activity.
CHAPTER 1
INTRODUCTION

Major biomass gases are carbon dioxide (CO$_2$) and methane (CH$_4$) which are generated from respiration and/or metabolic activities of living organisms. For microorganisms, CO$_2$ and CH$_4$ are considered as microbial biomass gases in soil and sediment ecosystem. CO$_2$ gas is generated mainly from aerobic condition, respiration, while CH$_4$ is generated from anaerobic condition, methanogenesis process. Therefore, environmental condition of microorganisms can be indicated with CO$_2$ and CH$_4$ content. These carbon gas compounds have a great significant in C-cycle which can move along the different environmental system. CO$_2$ and CH$_4$ have been concerned as greenhouse gases increasing global warming effect [IPCC, 1995]. The microbial biomass gases can describe correlation between microbial activities and environmental conditions in soil, such as organic matter content, nitrogen or phosphorus transformation, metabolic intermediates, pH, average microbial numbers and change in soil conditions [Stotzky, 1965].

Measurement technique of CO$_2$ and CH$_4$ gases is critically important to give more information of their content and distribution in environmental compartment. There are several methods to determine these microbial biomass gases. Conventional method is titration techniques which can be applied to determine CO$_2$ in trapping solution using alkaline solution (KOH, NaOH etc). This technique is still be used for measuring the respiration process of soil and cultural bacteria. The instrument applied with trapping solution is called “respirometer” which has been sold commercially. Disadvantages of the titration method are large sample applying and time consuming. Modern instrumental techniques which can measure CO$_2$ and CH$_4$ gases are spectroscopy technique. These techniques measure the absorption efficiency of CO$_2$ and CH$_4$ molecule. The spectrometry techniques are infrared spectrometry (IR), fourier transform infrared spectroscopy (FTIR), infrared gas analyzers (IRGA),
infrared photoacoustic spectrometer (trace gas analyzer (TGA)) and tuneable diode laser absorption spectroscopy (TDL). These techniques used as detector are usually connected with a static chamber for measuring gas sample. They can operate continuously for a long period of time without the need for human intervention [Thomas et al., 2003]. However, they are too expensive for the measurement. Common technique applying for measuring gases is gas chromatography (GC) equipped with a suitable detector. The detectors used for gases analyses are thermal couple detector (TCD) and mass spectrometry (MS). GC technique is considered more suitable technique for gas separation using optimum column and then detection the gas with high sensitivity and low detection limit. Gas introduction system has been approved by using headspace (HS) system connected to the GC instrument [Chai et al., 2001]. HS-GC has been applied to study volatile compounds in various matrix samples. Recently, newest technique for gas measurement is using gas-sensors which have been developed for determination of microbial biomass gases and other gases. The gas-sensor has high sensitivity and selectivity and also convenient to use in filed measurement.

This work focused on CO₂ and CH₄ gases analysis using titration technique and HS-GC. The titration method was optimized for CO₂ measurement using KOH trapping solution to compare results with GC technique. In the HS-GC, CO₂ and CH₄ generation from the reaction was studied. Packed column properties such as packing material, column length, column i.d., and column stability were studied for separation of these gases. GC and HS-GC condition were optimized. The optimum condition of titration method and HS-GC were used to study microbial biomass gases in soil and sediment samples.
CHAPTER 2
OBJECTIVES

Objective of this study is to develop and optimize analytical method for determination of CO₂ and CH₄ in soil samples using headspace gas chromatography (HS-GC) and titration method. Aims of the study can be summarized as following:

1. To develop respirometer for determination of CO₂.
2. To study the optimum condition of titration method for determining CO₂.
3. To study packing materials for GC analysis.
4. To study optimum condition of HS-GC for determination of CO₂ and CH₄.
5. To determine the quantity of CO₂ and CH₄ in soil samples.
CHAPTER 3
LITERATURE REVIEW

3.1 Introduction

Microbial biomass gases (CO₂ and CH₄) are generated by microorganisms through metabolic reactions in soil. These gases can exchange carbon content in carbon cycle [Stevenson, 1982]. CO₂ is produced by microorganisms from respiration process. While CH₄ is produced by microorganisms from methanogenesis process. The quantities of CO₂ and CH₄ are used to evaluate microbial activity in soil including growth, reproduction, and metabolism [Jjemba, 2004]. The degradation of organic matter by measuring CO₂ and CH₄ is a property of all heterotrophs in microbial activity determination because it is commonly used to indicate the level of the microbial activity. [Stotzky, 1965; Nannipieri, 1984].

3.2 Carbon cycle

There are several different forms of carbon that we have to keep track for learning the carbon cycle. Major forms are inorganic-C (limestone, CO₂, CO, CaCO₃) and organic-C (organic plant material). The movement of C from one form to another form is called “carbon cycle”. Table 3.1 shows the amount of C in Gt (10¹² kg (C)) in the main reservoirs on earth. The main pathways of C are diffusion into and out off the ocean, photosynthesis, respiration, and burning of fossil fuels and biomass. All these pathways produce and/or consume CO₂.
Table 3.1 Sizes (in gigatons, or $10^{15}$ g) of the main reservoirs of carbon on earth [Seinfeld, 1997].

<table>
<thead>
<tr>
<th>Location</th>
<th>Amount ($10^{15}$ g C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rocks</td>
<td>65,000,000</td>
</tr>
<tr>
<td>Oceans</td>
<td>39,000</td>
</tr>
<tr>
<td>Soils</td>
<td>1,580</td>
</tr>
<tr>
<td>Atmosphere</td>
<td>750</td>
</tr>
<tr>
<td>Land plants</td>
<td>610</td>
</tr>
</tbody>
</table>

In general, respiration is a key to study carbon cycle that associates with photosynthesis, fermentation, decomposition, and degradation processes. Carbon dioxide that is fixed into organic compounds as a result of photoautotrophic activity is available for consumption or respiration by animal and heterotrophic microorganisms. Methane is produced in soils as an end product of anaerobic respiration, methanogenesis, as shown in Figure 3.1.

Figure 3.1 The carbon cycle of microorganisms. [Prescott et al., 1996]
3.2.1 Carbon dioxide (CO$_2$)

CO$_2$ is a colorless and odorless non-flammable gas and presents at a very small volume of the Earth's atmosphere (383 ppm), but it is a very powerful greenhouse gas having a large effect on Earth’s climate. It is also essential to photosynthesis in plants and other photoautotroph. Despite its low concentration, CO$_2$ is very important component of the Earth's atmosphere because it absorbs infrared radiation at low energy and enhances the greenhouse effect to a greater degree. CO$_2$ is a very powerful forcing, with a radiative forcing of about 1.5 W m$^{-2}$, it is twice as powerful as the major forcing greenhouse gas, methane, and relatively ten times as powerful as nitrous oxide.

Table 3.2 gives the global budget for CO$_2$. The result is a missing CO$_2$ sink of 1.4 ± 1.5 CO$_2$ Gt (C) yr$^{-1}$. Accounting for this missing is a major research problem in the CO$_2$ global cycle [IPCC, 1995].

**Table 3.2 Global CO$_2$ budget**

<table>
<thead>
<tr>
<th>Sources</th>
<th>CO$_2$ (Gt (C) yr$^{-1}$)$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forest fuel combustion and current production</td>
<td>5.5 ± 0.5</td>
</tr>
<tr>
<td>Deforestation and land use changes</td>
<td>1.6 ± 1.0</td>
</tr>
<tr>
<td>Total known sources</td>
<td>7.1 ± 1.1</td>
</tr>
</tbody>
</table>

**Sinks**

| Oceanic uptake                                    | 2.0 ± 0.8                     |
| Uptake by Northern Hemisphere forest regrowth    | 0.5 ± 0.5                     |
| Retained in atmosphere                            | 3.2 ± 0.2                     |
| Total known sinks                                  | 5.7 ± 1.0                     |

Net imbalance                                      | 1.4 ± 1.5                     |

$^a$ 1 Gt(C) = $10^{12}$ kg (C) = $10^{15}$ g (C) = 1 Pg (C)

*Source: IPCC (1995).*
3.2.2 Respiration

Respiration is the metabolism reaction of microorganisms to generate CO₂ from organic matter (i.e., cellulose, hemicellulose, and lignin) under the oxygen demand, aerobic respiration. The decomposition of organic matter is carried out by small animals and some microorganisms such as bacteria, fungi, protozoa [Maier et al., 2000].

Carbon comprises approximately 45 to 50% of dry weight of plant and tissues. When these tissues are metabolized by microorganisms, O₂ is consumed and CO₂ is liberated, in accordance with the following general reaction:

$$(\text{CH}_2\text{O})_x + \text{O}_2(\text{g}) \rightarrow \text{CO}_2(\text{g}) + \text{H}_2\text{O}(\text{l}) + \text{intermediates} + \text{cellular material} + \text{energy} \quad (3.1)$$

In this reaction, all organic carbon should eventually be released as CO₂. However, under normal aerobic conditions only 60 to 80% of the carbon is liberated as CO₂ because of incomplete oxidation and synthesis of cellular and intermediate materials. The quantities of CO₂ evolved and O₂ consumed depend upon types of substrate, environmental conditions, and microorganisms involved. Non-biological production of CO₂ or consumption of O₂ may interfere in the respiration. CO₂ may be produced by chemical decarboxylation, cell-free, heat-stable enzymes [Bunt et al., 1955], or by the action on free soil carbonate of added chemicals or organic acids produced during metabolism [Chase et al., 1957]. O₂ may be taken up during chemical oxidation of organic matter, especially in heated soils. Both CO₂ and O₂ may be absorbed on soil and water surfaces.

Soil respiration means the uptake of O₂ and/or the release of CO₂ by living in soil [Anderson, 1982]. The soil respiration of microorganisms was one of the earliest and still is one of the most frequently used index of microbial activity in soil [Stotzky, 1965]. Soil respiration can be measured either in the field or in the laboratory where experimental conditions are more controlled (Table 3.3). According to Bollag et al., 1990, the choice depends strictly on the purpose of the measurement. The information gained from field measurements is more difficult to interpret because results of a number of aspects have to be considered. Because of the complex nature of
microbial respiration, many methods have been used to collect gases and make the analyses and the difference objectives and circumstance of individual investigator, no single method can be prescribed as being the best.

**Table 3.3** Main features of respiration measurements in the field and the laboratory.

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<thead>
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<td>Determination of microbiological activity</td>
</tr>
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<td>Homogeneous soil samples</td>
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<tr>
<td>No disturbance due to sampling</td>
<td>Use of disturbed soil samples (disturbance limited with intact soil cores)</td>
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<tr>
<td>Exposure to climatic changes</td>
<td>Controlled experimental conditions</td>
</tr>
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<td>Variations of soil respiration with soil depth</td>
<td>Limited effect of soil stratification</td>
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### 3.2.3 Methane (CH\(_4\))

Methane is the most abundant hydrocarbon in the atmosphere. The global sources of CH\(_4\) are estimated at 535 Tg (CH\(_4\)) yr\(^{-1}\) (range 410 to 660) [IPCC, 1995]. Of the estimated global annual emissions, 160 Tg (CH\(_4\)) yr\(^{-1}\) is attributed to natural sources, with the most prominent contribution being emissions from wetlands. Of the estimated 375 Tg (CH\(_4\)) yr\(^{-1}\) from anthropogenic sources, 100 Tg (CH\(_4\)) yr\(^{-1}\) comes from fossil fuel combustion, and the remainder from biospheric sources. Methane is removed from the atmosphere through reaction with hydroxyl radical (OH) in the troposphere, estimated at 445 Tg (CH\(_4\)) yr\(^{-1}\), and by reaction in the stratosphere, estimated at 40 Tg (CH\(_4\)) yr\(^{-1}\). Microbial uptake in soils contributes an estimated 30 Tg (CH\(_4\)) yr\(^{-1}\) removal rate. The imbalance between the current sources and sinks of CH\(_4\) indicates that methane is accumulating in the atmosphere. Atmospheric CH\(_4\) concentrations have changed considerably over time. CH\(_4\) has increased from a preindustrial mixing ratio near 700 ppb to a present-day value of 1720 ppb [Seinfeld, 1997].
3.2.4 Methanogenesis

Methanogenesis or biomethanation is the formation of methane by microbes [Perry, 1997]. This is an important and widespread form of microbial metabolism. In most the environment, it is the final step in the decay of organic matter. During the decay process, electron acceptors (such as oxygen, ferric iron, sulfate, nitrate, and manganese) become depleted, while hydrogen (H₂) and carbon dioxide accumulate. Light organics produced by fermentation also accumulate. During advanced stages of organic decay, all electron acceptors become depleted except carbon dioxide. Carbon dioxide is a product of most catabolic processes, so it is not depleted like other potential electron acceptors. The two best described pathways involve the use of carbon dioxide and acetic acid as terminal electron acceptors:

\[
\text{CO}_2(g) + 4\text{H}_2(g) \rightarrow \text{CH}_4(g) + 2\text{H}_2\text{O}(l) \tag{3.2}
\]

\[
\text{CH}_3\text{COOH} (\text{aq}) \rightarrow \text{CH}_4(g) + \text{CO}_2(g) \tag{3.3}
\]

However, methanogenesis has been shown to use carbon from other small organic carbon compounds, such as formic acid, methanol, methylamines, dimethyl sulfide, and methanopterin.

The microorganisms responsible for methanogenesis are a group of obligately anaerobic archeabacteria called methanogens such as Methanococcus, Methanobacterium, and Methanosarcina. Methanogenesis occurs extensively in specialized environments including wetland soils, the digestive tracts of animals, and aquatic sediments [Crenger et al., 1990].

3.3 Analytical methods for determination of CO₂ and CH₄

3.3.1 Titration method

Titrometric analysis for CO₂ trapped in aqueous alkaline solvent [Aumus et al., 1978; Jenkinson et al., 1976] remains a popular and frequently used
method in soil gas exchange research because it is simplicity, universal adaptability, and high degree of sensitivity to soil investigation.

CO₂ evolution was monitored by static absorption of the gas under a canopy respirometer with NaOH (30 ml of 0.5 M NaOH) for a measured length of time. The CO₂ was measured by back-titration with HCl, after CO₂ was stabilized by precipitation with BaCl2. The CO₂ evolution was measured from two soils during three field seasons. A linear regression model was used to examine the relationship between CO₂ evolution and environmental factors (temperature and moisture content) which had shown to be important influencing the rate of microbial activity [Kowalenko et al., 1978].

An alkaline absorption method using 13 cm i.d. x 23 cm tall aluminium cylinders was used to measure soil respiration in tropical grassland in India [Gupta et al., 1980]. The CO₂ evolution rates for the three locations were: location 49-358, 55-378, and 55-448 mg CO₂ m⁻² h⁻¹, respectively. Moreover, the static chamber alkaline-absorption method was used to measure CO₂ flux. A glass jar filled with 20.0 mL of 1.0 M NaOH was placed with a white PVC cap. The jar was left for 24 h in the closed cylinders after which they were removed, closed with paraffin-paper sealed metal lid and transported to the laboratory for analysis. Measured CO₂ flux ranged from 0.4 to 4.2 g C m⁻² per day in a no till system in central Ohio [Duike et al., 2000].

Absorption of CO₂ through alkaline solution (KOH solution) by titration was used to measure soil respiration rates during the dry season of 1966 in Costa Rica. The CO₂ rates were between 1,000 and 2,000 mg CO₂ m⁻² h⁻¹ in gallery forest and the wet forest. Soil respiration rates were lowest in the savanna and the deciduous forest (300-400 mg CO₂ m⁻² h⁻¹) and were highest in the secondary growth vegetation (2,556 mg CO₂ m⁻² h⁻¹), respectively. The results were related to the climatic condition in these habitats. The climate limits the development of organism in the soil and the primary production in the vegetation. Comparisons of European, Canadian, and tropical soil respiration rates show the limitation of soil respiration through limitations of primary productivity [Schulze, 1967]. Rate of carbon dioxide evolution were highly correlated with highly correlated with temperature of surface soil [Kucera et al., 1971].
Cleve et al., 1978 measured CO₂ evolution for measurement of respiration in organic matter by titration method (using KOH absorption for CO₂) compared with infrared gas analysis, gas chromatography (GC), and Gilson Respirometer. At 25°C, the minimum sensitivity of the GC and Gilson were 3.8 and 3.6 µg CO₂, respectively. The minimum sensitivity of the titration method (44 µg CO₂) could have been improved with use of a micro-liter burette for titrations. The least minimum sensitivity, 0.31 µg CO₂, was attained with the infrared gas analysis.

From Nannipieri et al., 1977, changes in CO₂ evolution, phosphates and urease activity, and ATP contents were related to bacterial and fungi biomass microscopically during glucose mineralization at different concentrations of mineral nutrients. In CO₂ measuring, samples were contained in erlentmayer flask, connected to an aeration train supplied with CO₂-free air. Incubation was at room temperature and evolved CO₂ was trapped in towers containing glass beads and 20 ml of 1.0M NaOH. The results show that no one measurement of microbial biomass or activity is sufficient to interpret microbial growth in the soil system. Each of the criteria measured were sensitive to specific conditions affecting biomass and activity.

Disadvantages of the titration method are measuring large numbers of sample, time consumption, and high detection limit.

### 3.3.2 Spectroscopy methods

Spectroscopy technique is the “study of the absorption and emission of light and other radiation by matter, as related to the dependence of these processes on the wavelength of the radiation”. It is based on the Beer-Lambert absorption law, which states that the absorption of light is proportional to the concentration of the absorbing species, the line strength and the path length of the absorption. There exist different types of spectroscopic techniques, which are divided according to the radiation source used to illuminate the sample and/or the type of detection scheme, some of which shall briefly be discussed [Neftel et al., 2006].
3.3.2.1 Fourier transform infrared absorption spectroscopy

The FTIR principle involves infrared light being split into two paths by a two beam interferometer. When the beams are combined at an infrared detector, constructive and destructive interference produces a modulated signal which is a function of the optical path difference between the two beams. This so-called interferogram is converted into a spectrum by a complex fourier transform [James, 2000].

In FTIR spectroscopy the unique infrared absorption of different molecules are used to quantify their concentration. A number of gases of interest in climate change research can be uniquely and simultaneously determined, e.g. CH₄, CO₂, N₂O, CO and H₂O. With different types of mirror arrangements, long optical paths can be obtained yielding good sensitivity. The greatly improved sensitivity of FTIR permits flux measurements to be made by micrometeorological techniques [Hargreaves et al., 1996].

3.3.2.2 Infrared gas analyzers

In IRGA, the gas concentration measurement is based on the difference between absorption of infrared radiation passing through two gas sampling cells. The reference cell is filled with a gas at known concentration and the sample cell is used for the determination of the unknown sample gas concentration. Infrared radiation is alternately transmitted through each cell path and the output of the analyser is proportional to the difference in absorption between the two gas sampling cells.

Soil CO₂ concentration were investigated in a tropical monsoon forest in northern Thailand, from 1998-2000 [Shoji et al., 2004]. The CO₂ concentration in the chamber was measured with a CO₂ analyzer (Li-COR). The CO₂ concentration increased with increasing depth and exhibited a distinct seasonal trend.
3.3.2.3 Infrared photoacoustic spectrometer

The operating principle of the infrared photoacoustic spectrometer or trace gas analyzer (TGA) is as follows: a gas sample is contained in a sealed cell and irradiated with chopped IR light of selected wavelength. The wavelength is specifically absorbed by the gas to be studied and is selected using filters. The energy absorbed by the gas leads to an increase in its temperature and pressure. Since the IR light is chopped, this causes a series of pressure pulses in the cell which are detected by microphones. The voltage generated by the microphones is proportional to the gas concentration in the cell. The TGA can measure 4 gas simultaneously, e.g., N₂O, CH₄, CO₂, and NH₃.

TGA is less sensitive for N₂O (detection limit 30 ppb) than GC, but theoretically more sensitive to CH₄ (detection limit 100 ppb); however CH₄ measurements are prone to interference, probably by water vapor [Osada et al., 1998]. Disadvantage of the TGA is high cost (approximately 40,000 U.S. dollar) [James, 2000].

3.3.2.4 Tunable diode laser absorption spectroscopy

Determination of gas concentrations using tunable diode lasers (TDL) is based on the absorption of an infrared laser beam as it travels along a path through the gas sample. As in any absorption spectroscopy method, the total absorption depends on the number of absorbing molecules in the beam’s path according to Beer’s law. The sensitivity of TDL based instruments depends on the path length and the strength of the absorption line, with highest detection sensitivities for gas species having strong absorption lines in the spectral region emitted by the laser [Edwards et al., 1994]. Atmospheric gases that have been measured using TDL spectroscopy include CO, CO₂, N₂O, NO, NO₂, NH₃ and CH₄.

Due to its sensitive and fast response, TDL based instruments are ideally suited for the in situ measurement of trace gas concentrations using micrometeorological methods. Detection limits are possible in ppt range for averaging times of between 5 and 30 minutes. While TDL absorption spectroscopy has the
highest spectral resolution of any of the methods, its main limitation is the number of gas species which can be measured simultaneously with the same diode. The instrumentation is also the most expensive of those described in this report.

### 3.3.3 Gas-sensors

CO$_2$ and CH$_4$ sensor is an instrument for the measurement of carbon dioxide and methane gas. The most common principles for CO$_2$ and CH$_4$ sensors are infrared gas sensors and chemical gas sensors.

In non-dispersive infrared (NDIR) sensors, these are the simplest of spectroscopic sensors. The key components are an infrared source, a light tube, an interference (wavelength) filter, and an infrared detector. The gas is pumped or diffuses into the light tube and the electronic measures the absorption of the characteristic wavelength of light. NDIR sensors are most often used for measuring carbon dioxide [Thomas et al., 1996]. The best of these have sensitivities of 20-50 ppm. Typical NDIR sensors are still expensive and must are used for CO$_2$. New developments include using MEMS to bring down the costs of this sensor and to create smaller devices.

Chemical gas sensors with sensitive layers based on polymer- or heteropolysiloxane have more advantages for lower energy consumption and smaller size to fit into microelectronic-based systems. However, short- and long term drift effects as well as a rather low overall lifetime are major obstacles when compare with the NDIR measurement principle [Zhou et al., 1994]. Poly (dimethylsiloxane) (PDMS) hollow fiber was used as the membrane in chemical gas sensor for monitoring CO$_2$ and CH$_4$ in biogas composition. Experimental results indicated that the chemical sensors had medium precision, high reproducibility, long-term stability, reversibility and shorter response time than 1.5 minute [Rego et al., 2004].

### 3.3.4 Gas chromatography

GC method is suitable for microbial gases analysis. The popularity of GC is based on a favorable combination of very high selectivity and resolution, good
accuracy and precision, wide dynamic concentration range and high sensitivity [Santos et al., 2002].

GC can be used to determine CO$_2$ and CH$_4$ in atmosphere directly. Analyse gases and standard gases are injected into GC by sampling–pump. From Smith et al., 1973, N$_2$, O$_2$, Ar, CO$_2$, N$_2$O, and C$_1$-C$_2$ hydrocarbons in soil atmosphere were analyzed by using automatic system for injecting samples into GC. Each sample was split between three parallel columns; column A: porapack Q (60-80 mesh) and column B: Molecular sieve 5A (60-85 mesh) connected with TCD, and column C: deactivated aluminum column (100-120 mesh) connected with flame ionization detector (FID). The detection limit were as follows: CO$_2$, N$_2$O, CH$_4$ (column A): 20 ppm (v/v); O$_2$, N$_2$, Ar (column B): 75 ppm (v/v); and C$_1$-C$_4$ hydrocarbons (column C): 0.02 ppm (v/v).

Jain et al., (2005), CO$_2$, and CH$_4$ were determined by continuous gas-chromatography monitoring. GC-FID with two separate columns, Molecular sieve 13X and Hyseape D, have been used for detection of CH$_4$ and CO$_2$, respectively in the atmosphere over the surface of Maitri (Antarctica). Calibration curve of gases were obtained by using standard gases. CO$_2$ and CH$_4$ surface air concentration were 369.72 and 1.699 ppm, respectively.

Wilhite et al., 1968 used GC-TCD to analyze the Martian atmosphere (i.e. CO$_2$, CH$_4$, N$_2$, O$_2$, Ar, and N$_2$O) using porous polymer beads as packing materials, in column. Gases were injected by a JPL-designed sampling valve. This condition could be used to separate possible constituents of Martain atmosphere. Especially CH$_4$ and CO$_2$ can be separated only in 2.5 and 4.5 min, respectively.

### 3.3.5 Headspace gas chromatography

The headspace analysis appears to be one of the best techniques for solving biodegradation gases analysis, especially for volatile contaminants in soil samples because this technique has fast analysis and can decrease a problem of sample matrixes. [Solange et al., 2004].

In soil air analysis, a 400 g sample of air-dried soil was placed in a 1-liter suction flask fitted with a serum cap on the side arm. The flask was incubated at
30 °C for 68 h. Headspace gas was injected directly by 0.5 mL Hamilton gas syringe into GC-TCD. Porapak Q (6.35 mm x 366 cm) and Molecular sieve 5A (6.35 mm x 61 cm) were used to separate gas samples. The GC method described is sensitive in the ppm range for nitrogenous gases CO₂ and CH₄. It is very rapid, requiring only 12 min for one completed analysis [Bailey et al., 1973].

In the determination of CH₄ and CO₂ emission from landfill, gas samples were collected using a homemade closed acrylic chamber. The chambers were placed on the soil surface with 5 cm inserted into soil 10 min. 35 mL gas samples were collected from the headspace of the chamber and transferred to 12.6 ml closed serum bottle before injected to GC by gas tight syringe. CH₄ concentration was analyzed by a Shimadzu 14A gas chromatograph with a glass Porapak Q (80/100 mesh) column (0.26 mm x 2.0 m) and FID [Hegde et al., 2003]. For CO₂ analysis, TCD was used. In this experiment, average CH₄ emission rate was 13.17, 65.27 and 0.99 mg m⁻² h⁻¹ in 1–2, 2–3 and 5 year-old landfills, respectively. Similarly, average CO₂ emission rate was 93.70, 314.60 and 48.46 mg m⁻² h⁻¹, respectively.

Ekaberg, et al., (2004) used a headspace technique coupled with automatic sampler for determination of the climate gases (CH₄, CO₂ and N₂O) in air samples and soil atmosphere. 1 g fresh weight of samples was incubated at 15 °C for 16 h before gas sampling by autosampler injection. The climate gases were analyzed by gas chromatography mass spectroscopy (GC-MS). The gases were determined with high sensitive and high sample throughput (18 samples h⁻¹). The detection limit (3σ) for the gases was 0.10 μL L⁻¹ for CH₄, 20 μL L⁻¹ for CO₂, and 0.02 μL L⁻¹ for N₂O, respectively.

Herbert et al., 1972 used a simple GC system for the separation and quantitative estimation of some gases evolved or utilized by micro-organisms: O₂, N₂, NO/CO, CO₂, N₂O, CH₄, C₂H₂, C₂H₄, and C₂H₆. Silica gel effectively resolved CH₄, C₂H₆, NO, or CO, N₂O and CO₂ but did not separate O₂ and N₂. CO₂ and N₂O were irreversibly adsorbed on the molecular sieve 5A column but in no way did they interfere with the analysis. Detection limit of CH₄ and CO₂ were 8.00x10⁻⁶ and 3.54x10⁻⁵ μg, respectively.

The major problems appear to limit the application of gas chromatography to the analysis of the soil air [Bunting et al., 1975]. The initial
problem is the lack of an adequate and compact method of sampling and transporting field samples. And an equally important problem is the lack of an adequate system of storing samples without change of composition.

3.4 Review of methods used

3.4.1 Titration method

Titration method is simple method to determine CO\textsubscript{2}. The principles of titration method as following: CO\textsubscript{2} (generated from soils or a reaction of Na\textsubscript{2}CO\textsubscript{3} and HCl standard solutions) is trapped in alkaline solution such as KOH, NaOH. Then, it is determined the total quantity of CO\textsubscript{2} collected by titration method with standardized HCl. Before the titration started, excess barium chloride (BaCl\textsubscript{2}) was added to precipitate in carbonate form and the residue OH\textsuperscript{-} was titrated following below reaction:

Generated CO\textsubscript{2} in standard solution;

\[ \text{Na}_2\text{CO}_3\text{(aq)} + 2\text{HCl}(aq) \rightarrow \text{CO}_2(g) + 2\text{NaCl}(aq) + \text{H}_2\text{O}(l) \]  \hspace{1cm} (3.4)

Added excess alkaline solution;

\[ 2\text{KOH}(aq) + \text{CO}_2(aq) \rightarrow \text{K}_2\text{CO}_3(aq) + \text{H}_2\text{O}(l) \]  \hspace{1cm} (3.5)

Added barium chloride;

\[ \text{K}_2\text{CO}_3(aq) + \text{BaCl}_2(aq) \rightarrow \text{BaCO}_3(s) + 2\text{KCl}(aq) \]  \hspace{1cm} (3.6)

Titrate the remaining of KOH with standardized HCl

\[ \text{KOH}_{\text{(residue)}} + \text{HCl}(aq) \rightarrow \text{KCl}(aq) + \text{H}_2\text{O}(l) \]  \hspace{1cm} (3.7)
The amount of CO$_2$ evolution is calculated from the volume of HCl in titration as following equation (3.8) [Stotzky, 1965]

$$\text{Weight CO}_2 = \frac{44M(B-S)}{2000}$$

when $B$ is the volume (milliliters) of acid needed to titrate the KOH solution from the blank solution to the end point, $S$ is the volume (milliliters) of acid needed to titrate the KOH solution from the standard solution to the end point, and $M$ is molarity of the acid.

In titration method, there are several characteristics of respirometer using for determination of CO$_2$. Isermeyer used closed jars to estimate soil respiration. 50 g of sieved soil was weighted and placed in the bottle of a 1 L jar. 25 mL of NaOH 0.05 M was pipetted into the jar and immediately closed using a rubber ring and tow crossing pegs. Jaggi used closed bottle to estimate the soil respiration. 20 g of soil samples was place in a plastic or glass tube. The tube was put into a Duran bottle. The CO$_2$ evolution was trapped by NaOH solution under the tube [Alef et al., 1995]. Rowell (1994) used a 250 mL conical flask with a rubber bung for making a simple respirometer. Into the bung screw a small hook and suspend a vial (2 cm diameter, 8 cm long) from the hook with thread. NaOH solution was added into the flask before the bung was brought to close.

### 3.4.2 Headspace gas chromatography

Headspace techniques are used for the analysis of volatile components in liquid and solid samples [Kellner et al., 2004; Drozd et al., 1979]. The sample is placed in a glass vial of appropriate size and closed with a teflon-lined silicone septum. The vial is carefully thermostated until equilibrium is established. The gas phase is sampled by syringe for manual procedures or with an electropneumatic dosing system in automatic headspace analyzer (i.e. headspace sampler). The original analyte concentration (in Henry’s law region) is then given by equation (3.9)
\[ C_i^o = \frac{C_g (K V_l + V_g)}{V_i} \]  

(3.9)

where \( C_i^o \) is the initial analyte concentration in the liquid phase, \( C_g \) is the concentration of analyte in the gas phase, \( K \) is the gas-liquid partition coefficient for the analyte at the analysis temperature, \( V_l \) is the volume of liquid phase, and \( V_g \) is the volume of gas phase [Yentongchai, 1992]. From equation (3.9) it can be seen that the concentration of the analyte in the headspace above a liquid in equilibrium with a vapor phase will depend on the volume ratio of the gas and liquid phases and the compound-specific partition coefficient which, in turn, is matrix dependent. The sensitivity of the headspace sampling method can be increased in some instances by adjusting the pH, salting out or raising the temperature of the sample.

The headspace sampling methods are used predominantly for the determination of trace concentration (in high ppb level [Nicholas et al., 2002]) of volatile substances in samples which are difficult to handle by conventional chromatographic means. Example include dilute solutions where the matrix would obscure the components of interest, damage the column or require excessively long separation times due to the presence of late eluting peaks, inorganic or high molecular weight polymers which cannot be volatilized or solubilized under normal conditions, and inhomogeneous mixtures, such as blood [Charles et al., 1980], sewage, colloids, etc., which require extensive sample clean up prior to analysis. In the above situations, the advantages of the headspace technique are economy of effort and the attainment of a sample which relatively free from its matrix and the problems associated with the chromatographic properties of the matrix. The main disadvantage of quantitative headspace analysis is the need for careful calibration [Kolb, 1999].

It is a common practice in analytical chemistry to directly determine an unknown product through the measurements of the reactant of chemical reactions [Guzoski et al., 2003]. Headspace gas chromatography of gas generation from the chemical reaction is based on a conversion of a fixed percentage (or a constant rate), including complete conversion, of an unknown product from a solution phase in a sample. The term “fixed percentage” here means that the conversion can be
incomplete but the final solution phase conversion rate is a constant; therefore, quantitative analysis of the analyte in a condensed phase can be achieved through calibration. One-step reaction for producing analyte gas can summed for the chemical reaction process:

$$rR + bB_{\text{(condensed)}} \rightarrow pP + qQ_{\text{(gas)}}$$  \hspace{1cm} (3.10)

where Q is an analyte gaseous species, B is the reactant solution, and R is a reagent. From the relationship, the molar concentration of the product gas in the headspace at the completion of the reaction (C_Q) is determined in equation (3.11) [Chai et al., 2001; Zhu et al., 2005].

$$C_B = \frac{1}{\alpha} \cdot \frac{b}{q} \cdot \frac{V_T - V_L}{V_S} \cdot C_Q$$  \hspace{1cm} (3.11)

where C_B is the molar concentration of the condensed reactant B in the original sample solution, α is a correlation coefficient of linear (≤ 1), b/q is the stoichiometric ratio of analyte B and the gas product Q in the reaction, V_T is volume of sample vial, V_S is volume of analyte, and V_L is final total volume of all the condensed phase species in the reactor.
CHAPTER 4
MATERIAL AND MATHODS

In this chapter, instrument and equipment details, operating condition used, chemicals and preparation procedures of all solutions are given. Procedures of the experiment studied involve with CO₂ and CH₄ titration method for trapping CO₂, packed materials studied for optimizing GC packed column, and headspace GC for gases analysis.

4.1 Instrument and equipments

4.1.1 Gas chromatography

Gas chromatography in this work was performed by using an Agilent model 6890, USA equipped with thermal conductivity detector (TCD) which was operated by using Chemstation software. Operating condition was described as shown below:

- Column : Packed column
- Carrier gas : He
- Fuel gas : None
- Oxidant gas : None
- Make up gas : None
- Injection mode : Headspace injection system
- Injection temperature : 150 °C
- Detector temperature : 250 °C
- Temperature program : Isothermal running
4.1.2 Headspace system

Headspace (HS) system was performed by using a HP-7694E automatic headspace sampler (Agilent Technologies, USA) which was connected directly to GC inlet via transfer line.

4.1.3 pH/Conductivity meter

A Model 220 pH/Conductivity Meter (Denver Instrument Company, USA) was used for measuring pH and conductivity values in solution.

4.1.4 Analytical balance

Analytical balance (Sartorius AG GOTTINGGEN, Germany) was used to weigh the sample or reagent in the preparation step.

4.1.5 Automatic pipette

Automatic pipette (PROPETTE, USA) was used to transfer the solution.

4.1.6 Nitrogen gas for sample preparation

Nitrogen gas purity 99.99% (TIG, Thailand) was used to flush gas in a square bottle of studying solution.

4.1.7 Tubing

Tygon tubing (Cole-palmer instrument CO, USA) was used to transfer gas.
4.1.8 Plastic screw-cap and silicone rubber

Plastic screw-cap with aperture GL45 and silicone rubber scaling for GL45 (SCHOTT, Germany) was connected to the square bottle for preventing gas leak.

4.1.9 Glass tube

Glass tube (SCHOTT, Germany) was used to make packed column with different size.

4.1.10 Vial and their accessories

Vial (Agilent Technologies, USA) of 21.5 mL which was tightly sealed with 20 mm headspace septa (National Scientific Company, USA) and 20 mm unlined aluminium cap (Agilent Technologies, USA) was used to contain studying solution or gas samples.

4.1.11 Gas-tight syringe

Gas-tight syringe (Agilent Technologies, USA) was used for injection of gas sample.

4.1.12 Manual hand operated crimper

Manual hand operated crimper (Agilent Technologies, USA) was used to crimp a vial.
4.2 Chemical and reagents

All chemical reagents used in this work are listed in Table 4.1.

Table 4.1 Chemical and reagents

<table>
<thead>
<tr>
<th>Chemical reagent and material</th>
<th>Suppliers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium hydroxide (AR grade)</td>
<td>Riedel-de Haën (Germany)</td>
</tr>
<tr>
<td>Sodium carbonate anhydrous (Reagent grade)</td>
<td></td>
</tr>
<tr>
<td>Methyl iodide</td>
<td></td>
</tr>
<tr>
<td>Barium chloride dehydrate (AR grade)</td>
<td>Merck (Germany)</td>
</tr>
<tr>
<td>Silica gel 60A</td>
<td></td>
</tr>
<tr>
<td>Hydrochloric acid (AR grade)</td>
<td>Lab-scan (Thailand)</td>
</tr>
<tr>
<td>Dietyl ester (AR grade)</td>
<td></td>
</tr>
<tr>
<td>Phenolphthalein indicator (Ω)</td>
<td>Fisher (USA)</td>
</tr>
<tr>
<td>Molecular sieve 5A (100/120)</td>
<td>Altech (USA)</td>
</tr>
<tr>
<td>Chromosorb W</td>
<td>Hewlett Packard (USA)</td>
</tr>
</tbody>
</table>

4.3 Preparation of solutions

Preparation procedures for all solutions were prepared by using de-ionized water (18 mΩ), Milli-Q system (Milford, Massachusetts, USA).

4.3.1 Potassium hydroxide solution

0.44 M Potassium hydroxide (KOH) was prepared by dissolving 56.11 g of KOH in deionized-distilled water and then made up volume to 1000 mL in a volumetric flask which was further standardized by 0.01 M potassium hydrogen phthalate (KHP).
4.3.2 **Hydrochloric acid solution**

1 M hydrochloric acid (HCl) was prepared by pipetting 83.5 mL of concentrated HCl (37% v/v) and made volume up to 1000 mL in a volumetric flask which was further standardized by 0.2 M Na₂CO₃.

4.3.3 **Sodium carbonate solution**

0.2 M Sodium carbonate (Na₂CO₃) was prepared by dissolving 10.5990 g of Na₂CO₃ in deionized-distilled water and then made up to 1000 mL in a volumetric flask.

4.3.4 **Barium chloride solution**

1.0 M Barium chloride (BaCl₂) was prepared by dissolving 122.1150 g of barium chloride in deionized-distilled water and then made up to 1000 mL in a volumetric flask.

4.3.5 **Phenolphthalein indicator**

 Phenolphthalein of 0.100 g was dissolved in 50.0 mL methanol and made up volume to 100.0 mL in a volumetric flask with deionized-water.

4.3.6 **Glassware cleaning**

All glassware, pipette tips, vials and other materials were carefully cleaned by washing with liquid detergent to remove dust or particulate remaining and then soaking in 10% HNO₃ solution at least 12 h. After that, glasswares were rinsed with DI water and dried in an oven at 60 °C at least 24 h before use.
4.4 Procedure and Methods

4.4.1 A simple respirometer

A simple respirometer was created in the lab for determination of CO₂ as shown in Figure 4.1. It consists of gas generation chamber, which is a Duran bottle with tightly screw cap containing Na₂CO₃ solution and trapping solution bottle, which is an erlenmeyer flask containing KOH solution for trapping CO₂. The system has a three (T)-way valve for adding of reagents and controlling gas flow through the bottle.

![Diagram of respirometer](image)

**Figure 4.1** An apparatus of respirometer was studied: (a) respirometer without N₂ flow, (b) respirometer with N₂ flow

4.4.2 CO₂ generation and titration method

Na₂CO₃ solution 20.0 mL was transferred into a square bottle and then connected to the glass tube and closed with a screw cap and rubber scaling, as shown in Figure 4.1 (b). N₂ gas was flushed into the bottle through an erlenmeyer flask that contained KOH solution for removing excess air. Next, 10.0 mL of HCl was injected through T-way valve to Na₂CO₃ solution and mixed thoroughly. CO₂ gas was generated from the reaction between Na₂CO₃ and HCl and then moved through the tube to trapping solution of KOH. The reaction was completed after 5 minute. Then,
N₂ gas was flushed into the reaction bottle to remove all CO₂ gas from the reaction chamber. Finally, KOH trapping solution flask was further carried out by adding an excess solution of BaCl₂ to precipitate BaCO₃ and then titrated with standard HCl solution. Potentiometric and conductometric titration were carried out comparable with phenolphthalein indicator to measure the end-point of the titration. Blank solution was also performed to justify the value of CO₂ concentration.

Study parameters of this section were divided into CO₂ generation conditions (HCl concentration and reaction time) and titration condition (KOH concentration and addition of BaCl₂ concentration)

**4.4.2.1 HCl concentration**

HCl concentration (10 mL) was varied in the range of 0.001, 1.0, 3.0 and 5.0 M for reacting with Na₂CO₃ in the square bottle (Figure 4.1 (b)) by adding and mixing thoroughly with 20.0 mL of 0.2 M Na₂CO₃. The reaction was completed after 5 minute. Concentration of KOH 0.44 M, 40.0 mL and 1.0 M BaCl₂, 10.0 mL were used for trapping CO₂ and further titration step. The titration was carried out within the KOH solution flasks as described above.

**4.4.2.2 Reaction time**

Reaction time of CO₂ generating in this reaction was studied by varying time between 1, 3, 5 and 8 minute before flushing gas with N₂. The trapping solution of KOH was fixed at 0.44 M, 40.0 mL. Then, the trapping solution was taken for titration by adding 1.0 M BaCl₂, 10.0 mL and phenolphthalein indicator.

**4.4.2.3 KOH concentration**

KOH concentration was used as trapping solution for CO₂ in the flask. The varying KOH concentrations were in the range of 0.0085 to 0.4444 M by using volume of 20.0 mL. Fixed parameters were 20.0 mL of 0.2 M Na₂CO₃, 10 mL of 3.0 M HCl concentration and 5 min of reaction time, respectively. Then, titration for
remaining KOH was performed with standard HCl solution by adding an excess BaCl₂ 1.0 M, 10.0 mL and phenolphthalein indicator.

### 4.4.2.4 BaCl₂ concentration

BaCl₂ solution was used to terminate carbonate equilibrium by precipitation to BaCO₃. Study concentrations of BaCl₂ were in the range 0.05 to 1.0 M by adding before titrated with HCl solution. Fix parameters were 20.0 mL of 0.2 M Na₂CO₃, 10 mL of 3.0 M HCl concentration, 5 min of reaction time, and 40.0 mL of 0.44 M KOH, respectively.

### 4.4.3 HS-GC for CO₂ and CH₄ analysis

In this study, CO₂ and CH₄ were generated from the reaction in a closed vial. Parameters that are concern directly in the generation of CO₂ and CH₄ were studied. Pack column properties, GC condition, and headspace system were optimized for determination of CO₂ and CH₄ from the reaction.

#### 4.4.3.1 CO₂ and CH₄ gases generation

CO₂ was generated from the reaction between Na₂CO₃ and HCl as described in section 4.4.2. CH₄ was generated by reacting CH₃I solution and HCl solution using Zn powder as reducing agent. In a vial, the solution of 0.2 M Na₂CO₃ 10 µL and 45.2 x 10⁻³ mg L⁻¹ CH₃I 5 µL were transferred into a vial and then Zn powder 0.1 g was added. The vial was closed tightly and then 5 mL of 1.0 M HCl was added into the solution. CO₂ and CH₄ gases were generated from those reactions and kept in the vial space upper the solution. This vial gases sample can inject directly by headspace system into the study columns.

Gases generation factors were optimized such as concentrated HCl (0.01, 0.05, 0.10, 1.00, and 2.00 M) and an amount of Zn (0.05, 0.10, 0.30, and 0.50 g). GC condition was used as following; inlet temperature 150 °C, oven temperature at 110 °C, detector temperature at 250 °C, flow rate of carrier gas at 2 mL
min\(^{-1}\). HS-GC conditions were including vial temperature (80 °C), vial equilibration time (2 min), vial pressure (14.7 psi), loop and transfer line (85 °C), pressurization time (0.13 min), loop fill time (0.15 min), loop equilibrium time (1.00 min), and injection time (1.5 min).

**4.4.3.2 Packed column studied**

Packing materials, column size (length and internal diameter (i.d.)) and column stability were studied. Preparation steps of each column were described in more detail in Appendix A.

**4.4.3.2.1 Packing materials**

Packing materials selected in this study were Chromosorb W, molecular sieve, and silica gel. These materials were packed into a glass column (4.0 mm x 101 cm) with approximately 6.5 g.

**4.4.3.2.2 Column length**

This parameter was studied on silica packed column by varying the length of the column for 45, 101 and 155 cm with fixing i.d. of 4.0 mm.

**4.4.3.2.3 Column internal diameter (i.d.)**

This parameter was studied on Silica packed column by varying the i.d. of the column for 3.1 and 4.0 mm with fixing length of 101 cm.

**4.4.3.2.4 Column stability**

Column stability was performed by injecting 1 µL of methanol by a syringe into the column to observe the response of the signal comparing with used time.
4.4.3.3 GC optimization

Parameters that concerned in GC separation were optimum such as inlet temperature (110, 150, and 180 °C), oven temperature (90, 100, 110, and 130 °C), detector temperature (160, 200, and 250 °C), and average velocity of carrier gas (28, 45, 57, and 77 cm sec⁻¹).

CO₂ and CH₄ standard gases were generated following as section 4.4.3.1 and separated by silica gel pack column (3.1 mm x 101 cm, 6.5 g) under HS conditions in section 4.4.3.1.

4.4.3.4 Headspace optimization

The HS-GC parameters were optimized such as vial temperature (45, 60, 80, and 100 °C), vial equivalent time (1, 2, 5, and 10 min), vial pressurization (4, 14, 24, and 34 psi), loop and transfer line temperature (85, 90, and 100 °C), pressurization time (0.05, 0.13, 0.30, and 0.50 min), loop fill time (0.02, 0.08, 0.15, and 0.30 min), loop equilibration time (0.10, 0.50, 1.0, and 2.0 min), and injection time (0.1, 0.5, 1.0, and 2.0 min).

CO₂ and CH₄ standard gases were generated following as section 4.4.3.1 and separated by silica gel pack column (3.1 mm x 101 cm, 6.5 g) under GC conditions in section 4.4.3.1.

4.4.4 Analytical performance

4.4.4.1 Titration performance

Analytical performance was investigated in term of detection limit, linearity range, and precision. The optimum titration conditions in Section 4.4.2 were used.
4.4.4.1.1 Calibration curve

20 mL of various Na₂CO₃ concentrations (0.05, 0.10, 0.15, and 0.20 M) was used for making a calibration curve. Fixed parameters were 40.0 mL of 0.44 M KOH, 10 mL of 3.0 M HCl concentration and 5 min of reaction time. Then, titration for remaining KOH was performed with standard HCl solution by adding an excess BaCl₂ 1.0 M, 10.0 mL and phenolphthalein indicator.

4.4.4.1.2 Detection limit (DL)

The detection limit was determined by using Na₂CO₃ solution at lowest concentration (in calibration curve) reacted with HCl solution in 5 time. The 3 times of standard deviation was converted to the concentration to give the instrumental detection limit.

4.4.4.1.3 Linear range

Linear range of the analyte was performed by preparing the various Na₂CO₃ standard solutions at 4 concentration ranges (0.05 – 0.20 M)

4.4.4.1.4 Precision

Various Na₂CO₃ concentrations (0.05, 0.10, 0.15, and 0.20 M) were repeatedly performed. Relative standard deviations (%RSD) were determined (n = 3). The obtained %RSD was used to indicate the precision of analysis.

4.4.4.2 HS-GC performance

HS-GC performance was investigated in term of detection limit, linearity range, and precision. The optimum HS-GC conditions in Section 4.4.3.4 were used.
4.4.4.2.1 Detection limit

Detection limit was calculated by comparing signal-to-noise ratio value equal at 3 to 1, referring to the lowest concentration of the analyte could be detected in the studied condition.

4.4.4.2.2 Linear range

Linear range of the analyte was performed by preparing 0.2 M Na$_2$CO$_3$ standard solution (2, 4, 6, and 8 µL) and 42.5 x 10$^{-2}$ mg L$^{-1}$ CH$_3$I (2, 4, 6, and 8 µL)

4.4.4.2.3 Precision

Various Na$_2$CO$_3$ and CH$_3$I concentrations (in calibration curve) were repeatedly performed. Relative standard deviations (%RSD) were determined (n=3).

4.4.5 Comparison between direct injection and HS injection

In HS injection, 10 µL of 0.2 M Na$_2$CO$_3$ (93.861 µg m$^{-3}$ CO$_2$), 20 µL dilution of CH$_3$I (69.783 µg m$^{-3}$ CH$_4$) and Zn powder (0.10 g) was added into a headspace vial. The vial was closed by capped tool. 5.0 mL of 1.0 M HCl solution was injected into the vial. The vial was shaken and brought to analyze by headspace technique. In direct injection, vial that contained all reagents was used to heat at 80 °C about 2 min and injected to GC by gas tight syringe (250 µL). Gases were separated under the optimum GC condition in Section 4.4.3.3 and the optimum HS conditions in Section 4.4.3.4.
4.4.6 Comparison between titration method and HS-GC

Titration method was compared with HS-GC for determination of CO₂ from the reaction. In titration method, 500 µL of 2.0 M Na₂CO₃ solution was added to react with 5 mL of 1.0 M HCl within 21.5 mL of closed headspace vial. The reaction vial was placed in headspace sampler. The headspace system was operated under the optimum condition in Section 4.4.3.4 by using Helium (He) as a carrier gas. CO₂ occurred in gas phase was injected into a closed headspace vial of 0.44 M KOH solution. The CO₂ was trapped in 10 mL of KOH solution. 1.0 M BaCl₂ (10.0 mL) were used for precipitating carbonate. The KOH remaining was titrated with 1.0 M HCl standard solution for determining CO₂ evolution. In HS-GC method, 2.0 M Na₂CO₃ solution (500 µL) was added to react with 1.0 M HCl (5 mL) within a closed headspace vial (21.5 mL). The closed vial was placed in headspace sampler. The headspace system was operated under the optimum condition in Section 4.4.3.4. CO₂ in gas phases was injected into GC and detected signals by detector. The quantity of CO₂ obtained from two methods was compared.

4.4.7 Real sample analysis

In titration method, soil sample of 100.0 g was weighed into a square bottle. Food for coliform and H₂O was added. The contained samples were incubated in 7 days at 25 °C. CO₂ released was trapped by 40.0 mL of 0.44 M KOH. Then, titration for remaining KOH was performed with HCl 1.0 M by adding an excess BaCl₂ 1.0 M, 10.0 mL and phenolphthalein indicator.

In HS-GC measurement, soil and sediment samples were weighed 10.0 g into headspace vials. Food for coliform and H₂O was added. The contained samples were incubated in 7 days at 25 °C. The quality of CO₂ and CH₄ were determined by the optimum HS-GC condition in Section 4.4.3.4.
CHAPTER 5
RESULTS AND DISCUSSION

Results obtained from the experimental study are described in this chapter i.e., respirometer design, CO₂ determination using titration method, packed column studied, optimized GC using pack column, optimized HS-GC, comparison between direct injection and HS-GC system, comparison between titration and HS-GC, and applying to real sample analyses.

5.1 Respirometer design

In this study, a simple respirometer was designed for determination of CO₂ by titration method. It is based on simple operation and inexpensive equipment. The feature of the respirometer has been shown in Figure 4.1, section 4.4.1. Comparison between flushing with N₂ gas and non-flushing with N₂ gas in the system was studied to observe the efficiency of removing gas from the empty space in the bottle. When the reaction between Na₂CO₂ and HCl was occurred, CO₂ gas was generated and moved suddenly to the upper layer of the bottle above reaction solution. Then, CO₂ gas moved directly to the trapping solution of KOH in the other flaks which was connected to the reaction bottle by a transfer tube. The efficiency of trapping solution in the close system was considered an important factor to study CO₂ content this manner because the concentration of CO₂ in the study condition might be very low and it could not be detected or trapped by the KOH solution. In addition, CO₂ content in the atmosphere is about 383 ppm [Seinfeld, 1997], which will react directly to the KOH solution in the open environment. Therefore, the respirometer was designed in a close system and it required flushing system to remove atmosphere CO₂ out off the system and then completely remove CO₂ from generating reaction to react with KOH solution. CO₂ concentration can be calculated by Appendix B.
Comparison between flow and non-flow \( \text{N}_2 \) into the respirometer is shown in Table 5.1. The flowing system showed higher value of \( \text{CO}_2 \) trapping more than no-flowing system. Thus, the respirometer system was conducted with flowing \( \text{N}_2 \) gas to remove all atmospheric \( \text{CO}_2 \) and pushing \( \text{CO}_2 \) after the reaction was completed.

<table>
<thead>
<tr>
<th>system</th>
<th>( \text{mg m}^{-3} \text{ CO}_2 \text{ cal} ) mean</th>
<th>( \text{mg m}^{-3} \text{ CO}_2 \text{ trapped} ) mean±SD</th>
<th>% Efficiency* mean±SD</th>
<th>%RSD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) without ( \text{N}_2 ) flow</td>
<td>280.74</td>
<td>41.17±3.67</td>
<td>14.66±1.16</td>
<td>8.92</td>
</tr>
<tr>
<td>(b) with ( \text{N}_2 ) flow</td>
<td>280.74</td>
<td>170.24±4.23</td>
<td>60.64±1.33</td>
<td>2.48</td>
</tr>
</tbody>
</table>

* % Efficiency = \( \frac{\text{mol of CO}_2 \text{ trapped} \times 100}{\text{mol of CO}_2 \text{ calculation}} \)

### 5.2 \( \text{CO}_2 \) generation and titration method

Preliminary results showed that the respirometer with flow \( \text{N}_2 \) was used to determine \( \text{CO}_2 \). In this study, parameters for \( \text{CO}_2 \) generation (HCl concentration and reaction time), KOH concentration and BaCl\(_2\) concentration were optimized. The titration performance was investigated.

#### 5.2.1 Effect of HCl concentration

\( \text{CO}_2 \) standard gas was generated from the reaction in a closed bottle. Concentration of HCl affects directly to generate \( \text{CO}_2 \) gas as shown in equation (5.1).

\[
\text{Na}_2\text{CO}_3(\text{aq}) + 2\text{HCl}(\text{aq}) \leftrightarrow 2\text{NaCl}(\text{aq}) + \text{CO}_2(\text{g}) + \text{H}_2\text{O}(\text{l}) \quad (5.1)
\]

An excess amount of acid can guarantee a complete conversion of carbonate into carbon dioxide [Chai, 1999]. Optimum concentration of HCl was investigated in this study to ensure the complete reaction with \( \text{Na}_2\text{CO}_3 \) 0.2 M, 20.0 mL
in the respirometer study. The HCl concentrations (10 mL) were in the range of 0.1 to 5.0 M.

Titration curve of this system is shown in Figure 5.1. There showed significantly distinguished 2 end points which were pH 8.0 and pH 4.0, respectively. Reason of these occurring reactions was divided into two reactions. First, remained KOH solution reacted with standard HCl solution, as shown in equation 5.2. This equilibrium point (A) is neutralization between strong acid and strong base condition. This end point value associated with the amount of CO₂ trapped by KOH solution. Second, precipitated of BaCO₃ in the solution is dissolved in the titration step using HCl solution and undergoes as equation 5.3. This point (B) was not involved in the calculation of CO₂ content.

\[
\text{KOH} \text{(aq)} + \text{HCl} \text{(aq)} \rightleftharpoons \text{KCl} \text{(aq)} + \text{H}_2\text{O(ℓ)} \quad (5.2)
\]
\[
\text{BaCO}_3 \text{(s)} + 2\text{HCl} \text{(aq)} \rightleftharpoons \text{BaCl}_2 \text{(aq)} + \text{CO}_2 \text{(g)} + \text{H}_2\text{O(ℓ)} \quad (5.3)
\]

**Figure 5.1** Titration curve between standard 1.0 M HCl and remained KOH (adding BaCl₂ before titration).

Titration curve for varying of HCl concentration reacted with Na₂CO₃ in square bottle are shown in Figure 5.2. Effects of HCl concentrations to perform reaction with Na₂CO₃ are shown in Figure 5.3. The result shows that CO₂ concentration was significantly increased when higher HCl concentration was
increasingly added in the reaction. However, for more than 1.0 M of HCl concentration showed significantly constant value of CO₂ content. Thus, the excess HCl concentration of the performing experiment was 3.0 M.

**Figure 5.2** Titration curve for varying of HCl concentration. (Remaining KOH solution was added with BaCl₂ before titration): symbols; blank (○); 0.1 M (▲); 1.0 M (■), 3.0 M (x); 5.0 M (●)

**Figure 5.3** Effect of HCl concentrations to perform reaction with Na₂CO₃

### 5.2.2 Reaction time

In this study, optimum reaction time was studied by varying time in the range of 1, 3, 5 and 8 minute, respectively. Results are shown in Figure 5.5. Increasing reaction time showed increasing value of CO₂ trapping. However, for more than 5
minute of reaction time showed significantly constant value of CO₂ content. Thus, the optimum time for performing experiment was 5 minute.

![Figure 5.4 Titration curve for varying reaction time between Na₂CO₃ and 1.0 M HCl. (Remaining KOH solution was added with BaCl₂ before titration): symbols; blank (◊); 1 min (▲); 3 min (x), 5 min (■); 8 min (●)](image)

![Figure 5.5 Effect of reaction time during the reaction between Na₂CO₃ and HCl in square bottle](image)

5.2.3 KOH concentration

KOH reacts with CO₂ in the solution as K₂CO₃ form, as shown in equation 5.4. An excess use of KOH can guarantee the complete reaction with the CO₂. In the system study had been observed the effect of KOH concentration for trapping CO₂, as shown in Figure 5.6.
There showed that higher KOH concentration increased absorbing efficiency of CO$_2$. CO$_2$ content was constantly at 0.25 M KOH and higher concentration value. Thus, the optimum KOH concentration for performing experiment was 0.44 M.

\[
2\text{KOH}(aq) + \text{CO}_2(g) \rightleftharpoons \text{K}_2\text{CO}_3(aq) + \text{H}_2\text{O}(l)
\]  (5.4)

**Figure 5.6** Effect of KOH concentration for trapping CO$_2$

### 5.2.4 Effect of BaCl$_2$ concentration

BaCl$_2$ solution is added to precipitate the carbonate in reaction (5.5) and to prevent the reaction between K$_2$CO$_3$ and HCl solution in the next procedure [Rowell, 1994].

\[
\text{K}_2\text{CO}_3(aq) + \text{BaCl}_2(aq) \rightleftharpoons \text{BaCO}_3(aq) + 2\text{KCl}(aq)
\]  (5.5)

In this study, optimum concentration of BaCl$_2$ will be studied. Various concentration of BaCl$_2$ (0.05, 0.10, 0.50, and 1.0 M) is performed. The result is shown in Figure 5.8.
Figure 5.7 Titration curve for varying of BaCl$_2$ concentration. (Remaining KOH solution was added with BaCl$_2$ before titration): symbols; blank (◊); 0.01 M (▲); 0.1 M (■), 0.5 M (x); 1.0 M (•)

Figure 5.8 Effect of BaCl$_2$ concentration for precipitating the carbonate

BaCl$_2$ concentration up to 0.5 M can precipitate the carbonate completely because of the obtained CO$_2$ concentration without different value. BaCl$_2$ concentration in this range had high efficiency to precipitate carbonate completely. For this reason, an excess BaCl$_2$ concentration of 1.0 M was chosen.
5.2.5 Titration performance

End point of the titration was observed by using potentiometric and conductometric titration compared with pH indicator to study the changes. The end points obtained from potentiometric and conductometric measurement are easily distinguished because the results can be calculated by performing first and second derivative of the data. Adding BaCl₂ into the solution increased the amount of precipitation and, therefore, the end point color change was difficult to observe. This problem was approved by using pH-meter and conductance probe to measure the change of pH and conductance values of the titrated solution.

CO₂ was generated from various concentration of Na₂CO₃; 0.05, 0.10, 0.15 and 0.20 M using the optimum condition. The observations of indicator, pH value and conductance values were obtained from the titration between HCl 1.0 M and KOH remaining in the trapping solution; beginning with KOH 0.44 M, 40.0 mL. Comparisons of each end point performances are shown in Figure 5.9. Table 5.2 shows values of analytical performance of the titration. The experiment was carried out in three replicates. Amount of HCl used in the titration was used to calculate the amount of KOH remaining in the solution and then the amount of CO₂ concentration was calculated from the different values between KOH beginning and KOH remaining.

Titration method can determine CO₂ at detection limit equal to 14.12 mg m⁻³ CO₂, linear range equal to 50-180 mg m⁻³ CO₂, % recovery equal to 61.71 ± 4.31 %, and precision (%RSD) equal to 3.98 - 9.63 %, respectively. Statistical test between indicator, pH values and conductance values was performed using analysis of variance (ANOVA) with student’s F-test, 95% testing confidence level. No significant difference was observed between the end point of titration method by indicator, pH values and conductance values at 95% confidential (Fcalculated = 0.0056, Fcritical = 4.2565)
Figure 5.9 Comparison of titration curves from various Na₂CO₃ concentrations (remaining KOH, 0.44 M, 40.0 mL titrated with HCl 0.1 M); (a) First-derivative values and (b) Conductance values: symbols; blank (○); 0.05 M (▲); 0.10 M (x), 0.15 M (■); 0.20 M (•)

Table 5.2 Content values of CO₂ obtained from different end point indication (indicator, pH value and conductance value).

<table>
<thead>
<tr>
<th>Na₂CO₃ [M]</th>
<th>CO₂ Cal. (mg m⁻³)</th>
<th>CO₂ trapped (mg m⁻³)</th>
<th>Indicator</th>
<th>pH Value</th>
<th>Conductance Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>79.18</td>
<td>49.75±4.98</td>
<td>51.34±4.36</td>
<td>49.75±4.98</td>
<td></td>
</tr>
<tr>
<td>0.10</td>
<td>158.36</td>
<td>94.62±12.24</td>
<td>100.83±0.31</td>
<td>94.62±12.24</td>
<td></td>
</tr>
<tr>
<td>0.15</td>
<td>237.55</td>
<td>150.05±5.94</td>
<td>152.95±6.19</td>
<td>150.05±5.94</td>
<td></td>
</tr>
<tr>
<td>0.20</td>
<td>316.73</td>
<td>178.42±8.24</td>
<td>182.65±9.83</td>
<td>178.42±8.24</td>
<td></td>
</tr>
</tbody>
</table>

Regression line

- y = 923x + 1.1877
- y = 907x + 11.283
- y = 923x + 1.187

R²

- R² = 0.9834
- R² = 0.9710
- R² = 0.9834

Data obtained from 3 replicates (n = 3)
Table 5.3 Percentage recovery of CO2 by comparing with CO2 total generation from the reaction which obtained from different end point indication (indicator, pH value and conductance value).

<table>
<thead>
<tr>
<th>Na2CO3 [M]</th>
<th>CO2 Cal. (mg m⁻³)</th>
<th>Indicator value</th>
<th>%RSD</th>
<th>pH value</th>
<th>%RSD</th>
<th>Conductance value</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>79.18</td>
<td>62.83±8.29</td>
<td>10.01</td>
<td>64.83±5.51</td>
<td>8.49</td>
<td>62.83±8.29</td>
<td>10.01</td>
</tr>
<tr>
<td>0.10</td>
<td>158.36</td>
<td>59.75±5.00</td>
<td>8.37</td>
<td>63.67±7.73</td>
<td>12.14</td>
<td>59.75±5.00</td>
<td>8.37</td>
</tr>
<tr>
<td>0.15</td>
<td>237.55</td>
<td>63.17±2.50</td>
<td>3.96</td>
<td>64.39±2.61</td>
<td>4.05</td>
<td>63.17±2.50</td>
<td>3.96</td>
</tr>
<tr>
<td>0.20</td>
<td>316.73</td>
<td>56.33±2.60</td>
<td>4.62</td>
<td>57.68±3.10</td>
<td>5.38</td>
<td>56.33±2.60</td>
<td>4.62</td>
</tr>
</tbody>
</table>

Data obtained from 3 replicates (n = 3)

5.3 HS-GC for CO2 and CH4 analysis

HS-GC is a powerful technique for the analysis of volatile species in corrosive and complex matrix sample. This method involves the thermodynamic equilibrium of volatile substances within the aqueous and the gas phase of the simple closed thermostated vial. The gas phase containing the volatile substances is injected into the GC column and analyzed without the influence of the matrix.

In this study, parameters affecting the reaction of CO2 and CH4 generation were studied such as HCl concentration and amount of catalyst. Moreover, packed column properties, GC parameters, and headspace system were optimized for determination of CO2 and CH4, respectively.

5.3.1 CO2 and CH4 gases for HS-GC

Studied gases of CO2 and CH4 were generated by reaction (5.6) and (5.7) in a closed vial, and then placed in the headspace system for continuously injection. These gases were separated on a pack column of silica gel (3.1 mm x 101 cm) and detected with thermal conductivity detector (TCD). CO2 and CH4 concentration can be calculated shown in Appendix B.
Na₂CO₃ (aq) + 2HCl (aq) → CO₂ (g) + 2NaCl (aq) + H₂O (l) (5.6)

2CH₃I (aq) + 2H⁺ (aq) + Zn (s) \xrightarrow{\Delta} 2CH₄ (g) + ZnI₂ (aq) (5.7)

5.3.1.1 Effect of HCl concentration

The HCl concentration directly effects gas generation. An excess amount of acid can guarantee a complete conversion of carbonate into carbon dioxide. However, using a higher concentration of acid will increase the risk of the corrosion problem in the headspace sampler [Chai et al., 2001] and degrade nature of reagent. In this study, effect of HCl concentration will be studied. Various HCl concentrations within the range of 0.001, 0.05, 0.10, 1.00, and 2.00 M were employed. The results are shown in Figure 5.10.

![Figure 5.10 Effect of HCl concentration to generate CO₂ and CH₄ (93.861 µg m⁻³ CO₂ and 69.783 µg m⁻³ CH₄): symbols; CO₂ (■); CH₄ (□)](image_url)

It can be noticed that peak area of CO₂ and CH₄ were increased when HCl concentration was added in the reaction. The optimum HCl concentration that was an excess concentration was 0.10 M which gave the highest peak area. High HCl concentration can cause decrease of peak area. This may be because of degrading nature of methyl iodide reagent.
5.3.1.2 Effect of the amount of Zn

Zinc (Zn), a reducing agent, is added to react with CH₃I to generate CH₄. In this study, the Zn weights used in the reaction was varied, where other parameters were kept constant. The Zn weight ranges selected were 0.05, 0.10, 0.30, and 0.50 g. Figure 5.11 shows that peak area will be increased when Zn was added in the reaction. An amount of increased Zn will accelerate the reaction to generate CH₄. However; an amount of increased Zn may be improved to produce H₂ from an acid. So that peak area of CH₄ was decreased when the Zn weight was added to increase. For the reason, the optimum Zn weights (0.10 g) were chosen for further analysis.

![Figure 5.11](image)

**Figure 5.11** Effect of the amount of Zn to produce CO₂ and CH₄ (93.861 µg m⁻³ CO₂ and 69.783 µg m⁻³ CH₄): symbols; CO₂ (■); CH₄ (□)

5.3.2 Packed column studied

Column is important for chromatographic analysis. This work had been attempted to study packed column to give the high sensitivity and also reproducible data. Packed column properties were optimized i.e. types of packing material, column length, column i.d., and column stability.
5.3.2.1 Packing materials

Solid sorbents which were used in this optimization process were Chromosorb, molecular sieve and silica gel. The separation mechanism depends on distribution coefficient of gas on a solid sorbent. In gas solid chromatography (GSC), general sorbents which have been used are molecular sieve, porous polymer, silica gel, and carbon black [Meloan, 1999].

Various packing materials (Chromosorb W, molecular sieve 5A and Silica gel) were employed. Column was packed in a glass column with internal diameter 4.0 mm and 101 cm length. Chromatogram of gases separation is shown in Figure 5.12. The results showed that Chromosorb W was not suitable to use for this purpose. Chromosorb W packing material can adsorb all analysis gases onto the material which is shown by no identification peak on the chromatogram. Thus, Chromosorb W can not separate CO₂ and CH₄. Molecular sieve 5A showed CH₄ peak at the retention time of 2.28 minute but CO₂ peak was not shown in this chromatogram and it could be concluded that CO₂ gas was strongly adsorb onto molecular sieve material. Detection limit of CH₄ on molecular sieve column was 1.03 µg m⁻³. Silica gel material was more effective material to resolve CO₂ and CH₄ peak with the retention of 1.57 and 19.51 minute, respectively. Therefore, silica gel was a perfect choice for CO₂ and CH₄ analysis packing column. Detection limits of CO₂ and CH₄ using silica gel column were 1.43 and 1.88 µg m⁻³, respectively.
Figure 5.12 Chromatograms of CO$_2$ and CH$_4$ separated on a glass column (i.d. 4.0 mm x 101 cm) packed with various packing materials: (a) Chromosorb W; (b) molecular sieve 5A; (c) silica gel; under GC condition: inlet temperature 150 $^\circ$C, column temperature 110 $^\circ$C, detector temperature (TCD) = 250 $^\circ$C, and average velocity of carrier gas (He) = 45 cm sec$^{-1}$.

5.3.2.2 Column length

The column length which affected directly to the retention time and peak area of the analyte gas was investigated to ensure the optimum separation condition of CO$_2$ and CH$_4$. The glass column used which was packed with silica gel was varied in the length of 45, 101, and 155 cm. The performing column was connected into the GC and gas sample was introduced by headspace system. Results are shown in Table 5.4. A shorter column applied (45 cm and 101 cm) was introduced with CO$_2$ and CH$_4$ gases at the concentration of 5.866 and 4.361 µg m$^{-3}$, respectively. A longer column of 155 cm, the amount of CO$_2$ and CH$_4$ introduced for studying the column were 211.186 and 17.446 µg m$^{-3}$, respectively. Detection limit was performed by using a concentration range over the study concentration range of the introduction. We found that detection limit of shorter column was lower than detection limit of
longer column. Considering sensitivity of separation from slope in regression line, shorter column especially 101 cm had high sensitivity (high slope) in CO₂ and CH₄ analysis. Thus, column length in 101 cm was chosen for further study.

**Table 5.4** Comparison data for different column length used (i.d. 4.0 mm) with silica gel packing materials for CO₂ and CH₄ gases analysis with headspace system

<table>
<thead>
<tr>
<th>Column Length (cm)</th>
<th>tₐ (min)</th>
<th>DL (µg m⁻³)*</th>
<th>CO₂ Regression line &amp; R²</th>
<th>CH₄ Regression line &amp; R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>0.46</td>
<td>4.67</td>
<td>y=0.0098x+1.338</td>
<td>y= 0.0475x+2.496</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R² = 0.9991</td>
<td>R² = 0.9906</td>
</tr>
<tr>
<td>101</td>
<td>1.57</td>
<td>19.51</td>
<td>y=0.2019x+33.511</td>
<td>y= 0.1307x+13.678</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R² = 0.9838</td>
<td>R² = 0.9940</td>
</tr>
<tr>
<td>155</td>
<td>3.45</td>
<td>28.03</td>
<td>y=0.0886x+24.029</td>
<td>y= 0.1453x+18.517</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R² = 0.9990</td>
<td>R² = 0.9932</td>
</tr>
</tbody>
</table>

Data obtained from 3 replicates (n = 3)

* Appendix C

**Figure 5.13** Calibration curve comparing between column length used (i.d. = 4.0 mm) for CO₂ and CH₄ gases analysis by headspace system: symbols; 45 cm (■); 101 cm (▲); 155 cm (♦)
5.3.2.3 Column internal diameter (i.d.)

Various i.d. columns (3.1 and 4.0 mm) were investigated for comparison the efficiency of the CO₂ and CH₄ gases analyses. The glass column used which was packed with silica gel was fixed in column length (101 cm). For studying the column, a wide column applied (4.0 mm) was introduced with CO₂ and CH₄ gases at the concentration of 5.866 and 4.361 µg m⁻³, respectively. A narrow column applied (3.1 mm) was introduced with CO₂ and CH₄ were 23.465 and 4.361 µg m⁻³, respectively. Results are shown in Table 5.5. The wide column gave low detection limit in CO₂ and CH₄ analysis. However, the narrow column had high sensitivity and rapid analysis time in separation. Thus, the narrow column (3.1 mm) was chosen for further study.

Table 5.5 Comparison data for different column i.d. used (length = 101 cm) with silica gel packing materials for CO₂ and CH₄ gases analysis with headspace system.

<table>
<thead>
<tr>
<th>Column i.d.(mm)</th>
<th>t_R (min)</th>
<th>DL (µg m⁻³)</th>
<th>Regression line &amp; R²</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CO₂</td>
<td>CH₄</td>
<td>CO₂</td>
<td>CH₄</td>
</tr>
<tr>
<td>3.1</td>
<td>1.57</td>
<td>12.22</td>
<td>2.23</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.0</td>
<td>1.58</td>
<td>19.51</td>
<td>1.37</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data obtained from 3 replicates (n = 3)
Figure 5.14 Calibration curve comparing between column i.d. used (length = 101 cm) for CO₂ and CH₄ gases analysis by headspace system: symbols; 3.1 mm (▲); 4.0 mm (■)

5.3.2.4 Column stability

The column stability can consider from stable peak of solvent. Methanol (MeOH) was used as a solvent to check the stable peak when time of usage column was varied. Figure 5.15 shows that no significant effects are observed on peak area.

Figure 5.15 Column stability studied by injecting methanol for several hours of column operation.
5.3.3 GC optimization

In this study, the optimum packed column used for CO$_2$ and CH$_4$ determination was silica gel that packed in a glass column with i.d. of 3.1 mm and length of 101 cm. 93.861 µg m$^{-3}$ CO$_2$ and 69.783 µg m$^{-3}$ CH$_4$ of headspace gases were analyzed. Criteria used for selecting the optimum condition of GC were high sensitivity and reproducibility. Study parameters which had been concerned were inlet temperature, column temperature, detector temperature, and flow rate of carrier gas.

Figure 5.16 shows results of inlet temperature, column temperature, detector temperature, and flow rate of carrier gas. The best results were obtained with the system of using inlet temperature at 150 °C, column temperature at 110 °C, detector temperature at 250 °C, and average velocity of carrier gas at 45 cm sec$^{-1}$ (= 2 mL min$^{-1}$).

![Figure 5.16 Optimized GC using packed column; (a) inlet temperature; (b) column temperature; (c) detector temperature; (d) average velocity of carrier gas: symbols; CO$_2$ (■); CH$_4$ (▲)](image-url)
5.3.4 Headspace optimization

Parameters in headspace (HS) system were optimized such as vial temperature, vial equilibration time, vial pressure, pressurization time, loop and transfer line temperature, loop fill time, loop equilibration time, and injection time.

5.3.4.1 Vial temperature

In equation (5.6), the carbonate can be instantly converted into CO$_2$ that has a solubility (1.45 kg m$^{-3}$) in water at room temperature. A higher temperature can accelerate the decomposition of Na$_2$CO$_3$ into CO$_2$ to completely remove the CO$_2$ in the liquid phase into the vapor phase [Chai et al., 2001]. Moreover, a higher temperature can accelerate the reduction reaction of CH$_3$I into CH$_4$ following equation (5.7).

In this case, the variation of vial temperature was studied under vial equilibration time (2.0 min), vial pressure (14.7 psi), pressurization time (0.13 min), loop and transfer line (85 °C), loop fill time (0.15 min), loop equilibrium time (1.00 min), and injection time (1.5 min). The results are shown in Figure 5.17 (a). Various vial temperature within this range showed no significant effect on peak area of CO$_2$. Vial temperature (95 °C) had an effect to peak area of CH$_4$. This may be due to the degradation of methyl iodide that is the starting reagent to generate CH$_4$. The optimum vial temperature (80 °C) was chosen.

5.3.4.2 Vial equilibration time

Vial equilibration (eq.) time is time that vial spends in the vial heating area. The length of time is determined by the type of sample (solid, liquid), the amount of sample, and the partition coefficient of the analyses. Various vial eq. time within the range of 1, 2, 5, and 10 min were performed under vial temperature (80 °C), vial pressure (14.7 psi), pressurization time (0.13 min), loop and transfer line (85 °C), loop fill time (0.15 min), loop equilibrium time (1.00 min), and injection time (1.5 min). The results are shown in Figure 5.17 (b). Various vial eq. time with in this range
had no effect on peak area of CO$_2$ and CH$_4$. For decreasing analysis time, vial eq. time of 2 min was chosen for further study.

5.3.4.3 Vial pressure

With many liquid (e.g. aqueous) samples, the pressure developed in the vial may be sufficient to ensure filling the vial loop without additional pressure. In general, 1.5-2 atmospheres pressure in the vial will be appropriate for filling the valve loop. It may be possible to make a pressure gauge fitted with a needle that could be used to determine the actual vial pressure developed under the time and temperature conditions [Operating and Service Manual, 1996].

In this experiment, the vial pressurization of carrier gas (He) was optimized under vial temperature (80 $^\circ$C), vial equilibration time (2.0 min), pressurization time (0.13 min), loop and transfer line (85 $^\circ$C), loop fill time (0.15 min), loop equilibrium time (1.00 min), and injection time (1.5 min). Data of peak area obtained with vial pressure 4, 14, 24, and 34 psi are presented in Figure 5.17 (c). The results showed that various vial pressure within this range had no effect on peak area of CO$_2$ and CH$_4$. The objective of this step was to give the pressure that may be sufficient to ensure filling the vial loop. Then the optimum vial pressure was 14 psi.

5.3.4.4 Pressurization time

Pressurization time is time during which the vial pressurization gas is introduced into the sample vial. Pressure gas (He gas) can dilute gas phase in sample vial. A long time of pressurization can increase the pressure gas in the vial but can cause the exposure. A short time of pressurization can decrease sensitivity of sample gas because the sample gas was introduced into GC decreasingly.

The variations of pressurization time (0.05, 0.13, 0.30, and 0.50 min) were performed under vial temperature (80 $^\circ$C), vial equilibration time (2.0 min), vial pressure (14.0 psi), loop and transfer line (85 $^\circ$C), loop fill time (0.15 min), loop equilibrium time (1.00 min), and injection time (1.5 min). The results are shown in Figure 5.17 (d). Various pressurization times within this range had no effect on peak
area of CO$_2$ and CH$_4$. For decreasing analysis time, the optimum pressurization time was 0.13 min.

![Figure 5.17](image)

**Figure 5.17** Effect of parameters in headspace sampler: (a) vial temperature, (b) vial equilibration time, (c) vial pressure, and (d) pressurization time: symbols; CO$_2$ (■); CH$_4$ (▲)

### 5.3.4.5 Loop and transfer line temperature

As a general rule, the temperature for the loop and transfer line should be set 5 °C higher than the vial temperature to avoid condensation. Loop and transfer line (TR.line) at various temperatures (85, 90, and 100 °C) were optimized under vial temperature (80 °C), vial equilibration time (2.0 min), vial pressure (14.0 psi), pressurization time (0.13 min), loop fill time (0.15 min), loop equilibrium time (1.00 min), and injection time (1.5 min). The results shows that signal of peak area will be decreased when the temperature of loop and TR.line was increased (in Figure 5.18 (a)). This may be because of the destruction of CO$_2$ and CH$_4$ by higher temperature during flow. Thus the optimum temperature of loop and TR.line was 85 °C.
5.3.4.6 Loop fill time

Loop fill time is time in which the headspace/vial pressurization gas mixture passes through the sample loop to vent. A very short loop-fill time may help increase sensitivity in headspace analysis. In this study, the loop fill time was varied under conditions: vial temperature (80°C), vial equilibration time (2.0 min), vial pressure (14.0 psi), pressurization time (0.13 min), loop and transfer line (85°C), loop equilibrium time (1.00 min), and injection time (1.5 min). The results are showed in Figure 5.18 (b). Various loop fill time within this range had no effect on peak area of CO₂ and CH₄. Consequently the optimum loop fill time was 0.08 min.

5.3.4.7 Loop equilibration time

Loop equilibration (eq.) time is time to allow the analyses in the sample loop to equilibrate to the higher loop temperature and to allow pressure and flow in the loop to stabilize. Optimum loop eq. time was determined under conditions: vial temperature (80°C), vial equilibration time (2.0 min), vial pressure (14.0 psi), pressurization time (0.13 min), loop and transfer line (85°C), loop fill time (0.08 min), and injection time (1.5 min). The results are shown in Figure 5.18 (c). Peak area of CO₂ and CH₄ was significant decreased when loop eq. time was increased in headspace sampler. This may be because of properties of gas which can be destroyed in long time. Thus the optimum time of loop eq. time (0.10 min) was chosen.

5.3.4.8 Injection time

Injection time is time in which the gases in the sample loop are injected into the GC. This time must be sufficient for complete sample transfer. If the time is too short, sensitivity is decreased because not all the sample will be transferred. It is not a problem if the time is longer than necessary. Various injection times within the range of 0.1, 0.5, 1.0, and 2.0 min were performed under conditions: vial temperature (80°C), vial equilibration time (2.0 min), vial pressure (14.0 psi), pressurization time (0.13 min), loop and transfer line (85°C), loop fill time (0.08 min),
and loop equilibration time (0.10 min). The results are shown in Figure 5.18 (d). Various injection times within this range have an effect on peak area of CO₂ and CH₄. The objective of this step was to obtain the time that has an efficient time for complete sample transfer. Therefore, the optimum time of injection was 1.00 min.

![Figure 5.18](image)

**Figure 5.18** Effect of parameters in headspace sample: (a) loop and TR.line temperature, (b) loop fill time, (c) loop equilibration time, (d) injection time: symbols; CO₂ (■); CH₄ (▲)

### 5.3.5 HS-GC performance

From the above results, the optimum conditions of pack column studied, GC, and headspace sampler can conclude in Table 5.6 and 5.7, respectively.
Table 5.6 The optimum condition in GC

<table>
<thead>
<tr>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
</tr>
<tr>
<td>: Silica gel pack column (3.1 mm x 101 cm, 6.5 g)</td>
</tr>
<tr>
<td>Temperature</td>
</tr>
<tr>
<td>: inlet temperature = 150 °C</td>
</tr>
<tr>
<td>: column temperature = 110 °C</td>
</tr>
<tr>
<td>: detector temperature = 250 °C</td>
</tr>
<tr>
<td>Detector</td>
</tr>
<tr>
<td>: Thermal conductivity detector (TCD)</td>
</tr>
<tr>
<td>Carrier gas (He)</td>
</tr>
<tr>
<td>: 45 cm sec⁻¹</td>
</tr>
</tbody>
</table>

Table 5.7 The optimum condition in headspace sampler

<table>
<thead>
<tr>
<th>Headspace parameters</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vial temperature</td>
<td>80 °C</td>
</tr>
<tr>
<td>Loop and TR. line temperature</td>
<td>85 °C</td>
</tr>
<tr>
<td>Vial equilibrium time</td>
<td>2.00 min</td>
</tr>
<tr>
<td>Pressurize time</td>
<td>0.13 min</td>
</tr>
<tr>
<td>Loop fill time</td>
<td>0.08 min</td>
</tr>
<tr>
<td>Loop equilibrium time</td>
<td>0.10 min</td>
</tr>
<tr>
<td>Injection time</td>
<td>1.00 min</td>
</tr>
<tr>
<td>Vial pressure</td>
<td>14.0 psi</td>
</tr>
</tbody>
</table>

The studied gases, CO₂ and CH₄, from the reaction can be determined by HS-GC. The result is shown in Table 5.8.

The linear ranges were established by Na₂CO₃ and CH₃I standard solutions with different concentration. Peak areas were determined in triplicate. A good correlation was obtained between peak area and concentration of CO₂ and CH₄ (in Table 5.8).

The detection limit was determined by injecting the standard solution at low concentration (signal to noise = 3) 5 times. Standard deviation was calculated from the data. The 3 times of standard deviation was converted to the concentration to
give the instrumental detection limit. The values of detection limit are summarized in Table 5.8.

Precision is the degree of agreement among individual test results (closeness of result). Relative Standard Deviation (RSD) is used to evaluate the precision. The precision (RSD) was determined by preparing three replicate standard and determining peak area in triplicate as described in Section 4.4.4.2. The values of %RSD are summarized in Table 5.8.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Liner equation</th>
<th>$R^2$</th>
<th>Linear range (µg m$^{-3}$)</th>
<th>%RSD</th>
<th>DL (µg m$^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO$_2$</td>
<td>$y=4.3292x+95.463$</td>
<td>0.9979</td>
<td>3.0-50.0</td>
<td>2.92-9.23</td>
<td>2.29</td>
</tr>
<tr>
<td>CH$_4$</td>
<td>$y=27.094x+8.3546$</td>
<td>0.9978</td>
<td>0.10-0.70</td>
<td>8.61-10.84</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Data obtained from 3 replicates ($n = 3$)

### 5.4 Comparison between direct injection and HS-GC system

In this experiment, we compared the result between GC injection and HS-GC injection. 93.861 µg m$^{-3}$ CO$_2$ and 69.783 µg m$^{-3}$ CH$_4$ that was generated from the reaction in closed vial was used to study the quantity of CO$_2$ and CH$_4$ while other parameters were fixed. The results are shown in Table 5.9. It was considered that peak area by HS-GC injection (1,000 µL) was higher significantly than GC injection (normalization = 1,000 µL) almost three fold. It may be because HS-GC injection can control gas-generating temperature in all experiment. Therefore, volatile gases cannot leak during injection. But GC injection cannot control gas-generating temperature during transportating the volatile gases from a headspace vial. The volatile gases may be leak. Then, peak area by direct injection was also decrease. For the reason, HS-GS injection can improve the sensitivity more than GC injection.
Table 5.9 The result data from GC and HS-GC injection.

<table>
<thead>
<tr>
<th></th>
<th>Peak area of CO₂</th>
<th></th>
<th>Peak area of CH₄</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean±SD % RSD</td>
<td>mean±SD %RSD</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 250 µL</td>
<td>37.23±3.76 10.97</td>
<td>10.7±0.67</td>
<td>3.32</td>
<td></td>
</tr>
<tr>
<td>- normalization (1,000 µL)</td>
<td>148.92±3.76 10.97</td>
<td>42.80±0.67</td>
<td>3.32</td>
<td></td>
</tr>
<tr>
<td>HS-GC injection</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- 1,000 µL</td>
<td>425.27±14.2 6.20</td>
<td>118.23±9.64</td>
<td>8.16</td>
<td></td>
</tr>
</tbody>
</table>

Data obtained from 3 replicates (n = 3)

5.5 Comparison between titration and HS-GC

Titration method is the simplest method for CO₂ analysis [Stotzky, 1965]. In general, the titration method provides excellent measurement precision in the quantification of acidic or basic species due to a sharp pH change at the end point. Unfortunately, very dilute weak acid or bases samples, or extremely low concentrations of strong acid or bases, cannot be accurately measured by titration method because they lack a distinct endpoint. The titration method is usually performed in a beaker, where the titrated species is dissolved in an aqueous solution with a fairly large volume. If the sample size is very small, a dilute sample solution will be prepared for the titration. In such a case, even strong acidic or basic species cannot provide a distinct change at the end-point, thus obtaining poor measurement precision and accuracy [Chai et al., 2005].

In this experiment, titration method was compared with HS-GC for determination of CO₂ from the reaction. For titration method, 500 µL of 2.0 M Na₂CO₃ solution was added to react with 5 mL of 1.0 M HCl within 21.5 mL of closed headspace vial. The reaction vial was placed in headspace sampler before the headspace system was operated by using Helium (He) as a carrier gas. CO₂ was injected into a closed headspace vial of 0.44 M KOH solution (10 mL). 1.0 M BaCl₂ (10.0 mL) were used for precipitating carbonate. The KOH remaining was titrated with 1.0 M HCl standard solution for determining CO₂ evolution. For HS-GC method,
2.0 M Na₂CO₃ solution (500 µL) was added to react with 1.0 M HCl (5 mL) within a closed headspace vial (21.5 mL) as same as titration method. But the closed vial was placed in headspace sampler before CO₂ in gas phases was injected into GC and detected signals by detector.

In Table 5.10, the results show that it was not difference CO₂ values at the same CO₂ concentration obtained from titration method and HS-GC. Statistical test between titration and HS-GC was performed using analysis of variance (ANOVA) with student’s t-test, 95% testing confidence level. No significant difference was observed between the CO₂ values of titration method and HS-GC at 95% confidential (t calculated = 0.3412, t critical = 4.3027)

When the data of CO₂ concentration between titration method that used square bottle (500 mL) for containing the reagent solution, flushing volatile gases by N₂ flow, and trapping CO₂ with 0.44 M KOH solution and HS-GC method that used headspace vial (21.5 mL) for containing the reagent solution, flushing volatile gases by He flow in headspace sampler, and detecting CO₂ with TCD were compared. At the same concentration of CO₂, titration method gave higher CO₂ concentration more than HS-GC almost 10 folds. It may be because N₂ flow can push CO₂ residued into KOH solution. Then, KOH solution can trap a lot of CO₂. The results are shown in Table 5.10.

Figure of merits from titration method and HS-GC can summarize in Table 5.11. HS-GC gave low detection limit and high precision more than titration method.
Table 5.10 Comparison data of titration and HS-GC method

<table>
<thead>
<tr>
<th>Method</th>
<th>Reaction container</th>
<th>Flow characteristic</th>
<th>Measuring CO₂</th>
<th>mg m⁻³ CO₂ mean±SD</th>
<th>%RSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titration</td>
<td>square bottle ¹</td>
<td>N₂ flow</td>
<td>KOH solution ³</td>
<td>51.34 ± 4.36</td>
<td>8.49</td>
</tr>
<tr>
<td>vial ²</td>
<td>He flow ⁴</td>
<td>KOH solution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HS-GC</td>
<td>vial</td>
<td>He flow</td>
<td>TCD ⁵</td>
<td>5.48 ± 0.23</td>
<td>4.22</td>
</tr>
</tbody>
</table>

¹ volume of square bottle = 500 mL
² volume of headspace bottle = 21.5 mL
³ 0.44 M KOH
⁴ Using headspace sampler
⁵ Thermal conductivity detector
⁶ Data obtained from 3 replicates (n = 3)

Table 5.11 Figure of merits by titration and HS-GC method

<table>
<thead>
<tr>
<th></th>
<th>CO₂</th>
<th>CH₄</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Titration</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DL</td>
<td>14.12 mg m⁻³</td>
<td>ND</td>
</tr>
<tr>
<td>- Linear range</td>
<td>50-180 mg m⁻³</td>
<td>ND</td>
</tr>
<tr>
<td>- %RSD</td>
<td>3.98-9.63</td>
<td>ND</td>
</tr>
<tr>
<td><strong>HS-GC</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- DL</td>
<td>2.29 µg m⁻³</td>
<td>0.02 µg m⁻³</td>
</tr>
<tr>
<td>- Linear range</td>
<td>3-50 µg m⁻³</td>
<td>0.1-0.7 µg m⁻³</td>
</tr>
<tr>
<td>- %RSD</td>
<td>2.92-9.23</td>
<td>8.61-10.84</td>
</tr>
</tbody>
</table>
5.6 Real sample analysis

The determination of CO$_2$ and CH$_4$, microbial biomass gases, in soil is important for description of microbial activity in soil such as organic matter content, nitrogen or phosphorus transformation, metabolic intermediates, pH, average microbial numbers and change in soil conditions. For the reason, if we know about the amount of CO$_2$ and CH$_4$, we will understand a wider view of environmental effect.

For soil samples, the study site was installed at the back of Vegetable Garden in Salaya Campus of Mahidol University, Nakhonphathom. The soil is a loose soil that is covered with glasses and leaves. Mean temperature of the air was 32 °C. For sediment samples, the study site was located at the canal of Vegetable Garden in Salaya campus and the canal at Phayathai campus in Bangkok. Location for sampling soil and sediment samples are shown in Appendix D.

In sampling steps, soil samples were collected in the range of 1-10 cm under soil surfaces as same as the sediment sampling. The matrices such as glasses, leaves, tree roots, and earthworms were moved out by hand. These samples were collected in strong-closed bags.

In the measurement of CO$_2$ and CH$_4$ in soil and sediment, the collected samples were weighted and contained within 24 h into a square bottle (500 mL) or headspace vial (21.5 mL) depending on characteristics of measurement. Food for coliform and H$_2$O was added into the bottle to activate metabolism of microorganisms. The contained samples were incubated at 25 °C in control time.

Soil and sediment samples were used to determine microbial biomass gases (CO$_2$ and CH$_4$) by titration and HS-GC method. In titration method, the samples were weighed about 100 g into square bottle. Food for coliform and H$_2$O was added into the bottle to activate metabolism of microorganisms. The CO$_2$ released was trapped in 0.44 M KOH (40.0 mL) and measured by titration with 1.0 M HCl standard solution. In HS-GC method, the samples were weighted about 10 g into 21.5 mL of headspace vial. Food for coliform and H$_2$O was added into the vial. The vial was placed in headspace tray to measure by headspace sampler and GC. The characteristic of soil and sediment samples are shown in Table 5.12.
Table 5.12 Some characteristic of soil used.

<table>
<thead>
<tr>
<th>Location</th>
<th>pH</th>
<th>Water content (%)</th>
<th>% C</th>
<th>% H</th>
<th>% N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable Garden Soil (Salaya)</td>
<td>6.83</td>
<td>22.82</td>
<td>4.54</td>
<td>0.70</td>
<td>0.06</td>
</tr>
<tr>
<td>Sediment</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- the canal of Vegetable Garden (Salaya)</td>
<td>8.45</td>
<td>59.67</td>
<td>5.24</td>
<td>0.53</td>
<td>0.12</td>
</tr>
<tr>
<td>- the canal at Phayathai (Bangkok)</td>
<td>8.03</td>
<td>64.44</td>
<td>5.45</td>
<td>1.15</td>
<td>0.40</td>
</tr>
</tbody>
</table>

In soil samples from vegetable garden (Salaya), CO₂ evolution was detected at 3.12 g CO₂ m⁻² d⁻¹ by titration method and at 7.51 g CO₂ m⁻² d⁻¹ by HS-GC as shown in Table 5.13. The difference values of CO₂ from two methods can cause by various distributions of microorganisms in soil samples. These samples should be collected from a lot of difference places in the same location for identifying this environment. In sediment samples from the canal at Phayathai campus (Bangkok), CO₂ evolution was detected at 8.01 g CO₂ m⁻² d⁻¹ by HS-GC method. We cannot find CH₄ in all soil and sediment samples.
Table 5.13 CO₂ and CH₄ in real samples by HS-GC

<table>
<thead>
<tr>
<th>Location</th>
<th>Titration</th>
<th>HS-GC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>CO₂ (g m⁻² d⁻¹)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mean±SD</td>
</tr>
<tr>
<td>Vegetable Garden Soil (Salaya)</td>
<td>3.12±0.50</td>
<td>16.00</td>
</tr>
<tr>
<td>Sediment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- the canal of Vegetable Garden (Salaya)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>- the canal at Phayathai (Bangkok)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

a Data obtained from 3 replicates (n = 3)
b gram per square meter per day
c ND = Not detectable
CHAPTER 6
CONCLUSIONS

Biomass gases analyses can be performed to determine amount of gases in soil samples for studying the correlation with their environmental conditions. The analytical methods in this work were a simple titration method for CO$_2$ with a design respirometer system and HS-GC technique for CO$_2$ and CH$_4$. The amount of both gases tells us about microbial activity and their environmental impacts. Higher amount of CO$_2$ content showed that there was aerobic condition occurring and more degradation process was happening. High level of CH$_4$ indicated the anaerobic condition and there was methenogens taken place and also lacked of oxygen in the system. In addition, the content of CO$_2$ and CH$_4$ can be correlated with pollutants loaded and environmental conditions. It is not an easy to interpret the situation with these gases concentration, but it also requires many parameters to assess the effect of the soil system. In this study, microbial biomass gases (CO$_2$ and CH$_4$) were determined in selected soil samples by HS-GC for CO$_2$ and CH$_4$ gases and by titration method for CO$_2$.

Study gas of CO$_2$ and CH$_4$ was performed by using a simple chemical reaction to generate gas. The reactions are shown as bellowing:

$$\text{Na}_2\text{CO}_3 \text{ (aq)} + 2\text{HCl (aq)} \rightarrow \text{CO}_2 \text{ (g)} + 2\text{NaCl (aq)} + \text{H}_2\text{O (l)} \quad (6.1)$$

$$2\text{CH}_3\text{I (aq)} + 2\text{H}^+ \text{ (aq)} + \text{Zn (s)} \xrightarrow{\Delta} 2\text{CH}_4 \text{ (g)} + \text{ZnI}_2 \text{ (aq)} \quad (6.2)$$

The titration method using respirometer flowing with N$_2$ was used to determine CO$_2$. There showed more potential to measure CO$_2$ by this method because of easy to handle and in-expensive apparatus. The analytical performance showed that the values of detection limit, linearity range, and %RSD in titration method were
14.12 mg m\(^{-3}\)CO\(_2\), 50-180 mg m\(^{-3}\)CO\(_2\), and 3.98 - 9.63 %, respectively. The percentage efficiency of trapping CO\(_2\) was in range 61.41 ± 4.26 %.

HS-GC was a powerful technique to separate CO\(_2\) and CH\(_4\) gases and also simultaneously determination in the sample. Packed column of silica gel (3.1 mm x 101 cm, 6.5 g) was used for separating CO\(_2\) and CH\(_4\) under the optimum GC condition. The technique gave high sensitivity and low detection limit which were 2.29 µg m\(^{-3}\) for CO\(_2\) and 0.02 µg m\(^{-3}\) for CH\(_4\), respectively.

Soil samples were collected from vegetable garden in Salaya campus of Mahidol university. Sediment samples were obtained from the canal of vegetable garden in Salaya Campus and the canal at Phayathai in Bangkok. The quantities of CO\(_2\) in vegetable garden soil using titration method and HS-GC were 3.12 g CO\(_2\) m\(^{-2}\) d\(^{-1}\) (%RSD=16.00, n=3) and 7.51 g CO\(_2\) m\(^{-2}\) d\(^{-1}\) (%RSD=10.91, n=3), respectively. The difference values of CO\(_2\) can cause by various distributions of microorganisms in soil samples. These samples should be collected from a lot of difference places in the same location for identifying this environment. In sediment of the canal at Phayathai using HS-GC was 8.01 g CO\(_2\) m\(^{-2}\) d\(^{-1}\) (%RSD=15.12, n=3). We cannot find CH\(_4\) in all soil and sediment samples.

Conclusionly from the experiment results of titration and HS-GC methods to determine microbial biomass gases, the titration method using respirometer flowing with N\(_2\) was suitable for determination of CO\(_2\) because this method was simple operation, inexpensive equipments and high efficiency in analysis. In case of low concentration of CO\(_2\) in samples, HS-GC was chosen to investigate CO\(_2\) evolution because it gave low detection limit and high precision in experiments.
Suggestion for further study:

Although HS-GC is a powerful method for the analysis of microbial biomass gases, this method still has some drawbacks. The main drawback is high %RSD in the experiment. In order to eliminate this draw back for HS-GC in the future, it is suggested to use internal standard gas in the experiment. Moreover, the study in the effect of partial pressures of H\textsubscript{2} generated from acid reacted with reducing agent (Zn) and in the solvent effect from HCl concentration will give more details in understanding the headspace phase’s effect.

In microorganism respiration study, soil moisture, temperature, the availability of nutrients and soil structure should be studied. Air-drying reduces the soil respiration significantly. However, remoistened soils show very high initial activities, probably as a result of the release of concentrations of easily degradable organic compounds such as amino and organic acids caused by chemical and physical process at the moistening of dry soils [Alef et al., 1995]. The study in the affects causing to habitat microorganisms will describe changes of microorganisms in this environment.
REFERENCES


Thomas C, Juliette NR, Robert RG, Mark EH. (2003). An inversion algorithm for the detection of methane over the wetlands by open-path FTIR in various atmospheric conditions. Department of Environmental, Earth, and Atmospheric Sciences, University of Massachusetts Lowell, America.


APPENDIX
APPENDIX A

Preparation of pack column

1. Preparation column for containing packing material: glass column was washed by 10% HNO₃ and acetone, respectively and dry at 105 °C in oven overnight.
2. Containing packing material into column: packing material was contained into glass column closely and distributionally by sonication about 3 h.
3. Glass wools were closed at the tip of column for preventing packing material into detector.

Conditioning the column

1. Connect only column with injector port (not connect with detector)
2. Flow carrier gas about 30 min following:
   - 25 mL min⁻¹ for 1/8 metal or 2 mm i.d.glass
   - 50 mL min⁻¹ for 1/4 metal or 4 mm i.d.glass
3. Set oven temperature more than analysis temperature at 25 °C or less than maximum column temperature at 25 °C about 4-48 h.
4. Flow carrier gas to column for cooling column.
5. Connect column with detector
6. Flow carrier gas through column to detector at 30 min
7. Flow carrier gas through column about 1-2 h before analysis
APPENDIX B

Calculation of CO₂ & CH₄ concentration

1. Titration method

From Stotzky, 1965, The amount of CO₂ evolved can be calculated following the formula (B1)

\[
\text{Weight CO}_2 = \frac{44M(B-S)}{2000}
\]  

(B1)

Where B is volume (mL) of acid needed to titrate the KOH in the bottom from the blank solution to the end point. S is volume (mL) of acid needed to titrate the KOH in the bottom from the standard solution to the end point. And M is molarity of the acid.

2. HS-GC method

From Chai et al., 2001, CO₂ & CH₄ concentration was calculated from equation (B2) and (B3)

\[
rR + bB_{\text{(condensed)}} \rightarrow pP + qQ_{\text{(gas)}}
\]  

(B2)

Where B is the analyte to be determined in sample, Q is a gaseous species, and R is a reagent. From the relationship, the molar concentration of the condensed analyte B in the original sample solution (\(C_B\)) is determined in equation (B3)
where $C_Q$ is molar concentration of the product gas in the headspace at the completion of the reaction, $\alpha$ is a correlation coefficient of linear ($\leq 1$), $b/q$ is the stoichiometric ratio analyte B and the gas product Q in the reaction, $V_T$ is volume of sample vial, $V_s$ is volume of analyte, and $V_L$ is final total volume of all the condensed phase species in the reactor.

1.1 CO$_2$ calculation

We can generate CO$_2$ from equation

$$\text{Na}_2\text{CO}_3(\text{aq}) + 2\text{HCl}(\text{aq}) \rightarrow 2\text{NaCl}(\text{aq}) + \text{CO}_2(\text{g}) + \text{H}_2\text{O}(\text{l}) \quad \text{(B4)}$$

For example, we added 0.2 M Na$_2$CO$_3$ (2.5 µL) and 5 mL of 0.1 M HCl into a 21.5 mL of headspace vial at 80°C.

From equation (B3):

$$C_B = \frac{1}{\alpha} \cdot \frac{b}{q} \cdot \frac{V_T - V_L}{V_s} \cdot C_Q$$

when $C_B = 0.2$ M Na$_2$CO$_3$, $\alpha = 1$, $b = 1$, $q = 1$, $V_T = 21.5$ mL, $V_L = 16.5$ mL ($21.5 - 5$ mL), $V_s = 2.5$ µL. So that,

$$(\text{conc.CO}_2) C_Q = 0.1 \text{ mM}$$

Then

$$\text{Conc.CO}_2 = 4.4 \text{ mg.L}^{-1}$$

Because temperature was 80°C, the pressure of CO$_2$ was different. ($P_{CO2} = \text{unknown value}$). We can calculate $P_{CO2}$ from ideal gas law ($PV = nRT$)
Because of;

\[ n_{\text{CO}_2} = n_{\text{Na}_2\text{CO}_3} \]

\[ n_{\text{Na}_2\text{CO}_3} = 5 \times 10^{-4} \text{ mmol} \] (calculated from 0.2 M Na\(_2\)CO\(_3\) (2.5 µL))

Therefore;

\[
P_{\text{CO}_2} = \frac{nRT}{V} \\
\frac{P_{\text{CO}_2}}{16.5\text{ml}} = \left(5 \times 10^{-4} \text{ mmol}\right) \left(0.0821\text{ atm.L.mol}^{-1}\text{.K}^{-1}\right) (353\text{K})
\]

\[
P_{\text{CO}_2} = 8.78 \times 10^{-4} \text{ atm} \quad (= 0.6674 \text{ mmHg})
\]

From equation (C4) in Appendix C;

\[
\text{mg m}^{-3} \text{ CO}_2 = \frac{0.6674 \times 44 \times 4.4}{62.4 \times (273.2 + 80)}
\]

when \( P = 0.6674 \text{ mmHg}, MW \text{ of CO}_2 = 44 \text{ g mol}^{-1}, \text{conc.} \text{CO}_2 \text{ in ppm} = 4.4, \)

\text{temperature of reaction} = 80^\circ\text{C},

so that,

\[
\text{mg.m}^{-3} \text{ CO}_2 = 5.865 \times 10^{-3} \text{ mg m}^{-3} \\
= 5.865 \text{ µg m}^{-3}
\]

### 1.2 CH\(_4\) calculation

We can generate CH\(_4\) from equation

\[
2\text{CH}_3\text{I (aq)} + 2\text{H}^+ + \text{Zn(s)} \xrightarrow{\Delta} 2\text{CH}_4 (g) + \text{ZnI}_2(\text{aq})
\]  \hspace{1cm} (B5)

For example, we added 5 µL of 11.3 \times 10^3 \text{ ppm CH}_3\text{I}, 5 \text{ ml} of 0.1 \text{ M HCl}, and 1.0 \text{ g of}

\text{Zn powder into a 21.5 mL of headspace vial at 80}^\circ\text{C}.

From equation (B3);
\[ 11.3 \times 10^{-3} \text{ ppm} = \frac{1}{2} \frac{2}{5} (21.5 - 16.5) \text{mL} C_Q \]

when \( C_B = 11.3 \times 10^{-3} \text{ CH}_3\text{I}, \alpha = 1, b = 2, q = 2, V_T = 21.5 \text{ mL}, V_L = 16.5 \text{ mL} (21.5 - 5 \text{ mL}), V_s = 5 \mu\text{L} \). So that,

\[(\text{conc. CH}_4) C_Q = 11.3 \text{ ppm} \]

Because temperature was 80 °C, the pressure of CH\(_4\) was different (\( P_{CH_4} \) = unknown value). We can calculate \( P_{CH_4} \) from ideal gas law (PV = nRT). Because of;

\[ n_{CH_4} = n_{CH_3I} \]

\( n_{CH_3I} = 0.3981 \times 10^{-6} \text{ mol} \) (calculated from 11.3 \( \times 10^{-3} \text{ CH}_3\text{I} \) (5 \( \mu\text{L} \)). Therefore;

\[ P_{CH_4} = \frac{nRT}{V} \]

\[ P_{CH_4} = \frac{(0.39081 \times 10^{-6}) (0.0821 \text{ atm.L.mol}^{-1}.\text{K}^{-1})(353\text{K})}{16.5 \text{mL}} \]

\[ P_{CH_4} = 0.6991 \times 10^{-3} \text{ atm} (= 0.5314 \text{ mmHg}) \]

From equation (C4) in Appendix C;

\[ \text{mg.m}^{-3} \text{ CH}_4 = \frac{0.5314 \times 16 \times 11.3}{62.4 \times (273.2 + 80)} \]

when \( P = 0.6674 \text{ mmHg}, MW \text{ of CH}_4 = 16 \text{ g mol}^{-1} \), \( \text{conc.} \text{CH}_4 \text{ in ppm} = 11.3 \), temperature of reaction \( = 80^\circ \text{C} \). So that,

\[ \text{mg m}^{-3} \text{ CH}_4 = 4.3614 \times 10^{-3} \text{ mg m}^{-3} \]

\[ = 4.3614 \mu\text{g m}^{-3} \]
APPENDIX C

Conversion from ppm (mg L⁻¹) to mg m⁻³

From Seinfeld et al., 1997, atmospheric species concentrations are sometimes expressed in terms of mass per volume, most frequently as μg m⁻³, the molar concentration of species \( i \), in mol m⁻³, is

\[
c_i = \frac{10^{-6} m_i}{M_i}
\]

where \( M_i \) is the molecular weight of species \( i \).

Noting that the total molar concentration of air at pressure \( p \) and temperature \( T \) is \( c = p/RT \), then

\[
\text{Concentration of } i \text{ in ppm} = \frac{RT}{p M_i} \times \text{Concentration of } i \text{ in } \mu g \text{ m}^{-3}
\]

(C2)

If \( T \) is in K (K = 273 +°C), \( p \) in Pa (1 atm = 101.325 x 10³ Pa), and \( R \) (the value of the molar gas constant) in 8.314 J mol⁻¹ K⁻¹

\[
\text{Concentration of } i \text{ in ppm} = \frac{8.314 T}{p M_i} \times \text{Concentration of } i \text{ in } \mu g \text{ m}^{-3}
\]

At 25° C and 760 mmHg (Canada’s National Occupational Health& Safety Resource (CCOHS))

we can calculate “mg m⁻³” in simple equation:

\[
\text{Concentration of } i \text{ in mg m}^{-3} = \frac{M_i}{24.45} \times \text{Concentration of } i \text{ in ppm}
\]

(C3)
At different temperature and pressures:

Concentration of $i$ in mg m$^{-3} = \frac{P \text{ (in mmHg)} \times M_i \times \text{Concentration of } i \text{ in ppm}}{62.4 \times (273.2 + T^0\text{C})}$ (C4)

where $V = \frac{RT}{P}$; $V = \text{gas volume (Liter; L)}$, $R = 0.0821 \text{ atm L mol}^{-1} \text{ K}^{-1}$
APPENDIX D

Location of sampling soil and sediment samples in Thailand

Figure D1 Location for sampling soil and sediment samples: (a) soil and (b) sediment in Salaya campus, Nakornphatom, (c) sediment at Phayathai campus, Bangkok.
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