MODIFICATION AND EVALUATION OF A RUMINANT EMISSION MEASUREMENT SYSTEM FOR ASSESSING CATTLE METABOLISM

BY

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THESIS

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Abstract

Respiratory gas exchanges are fundamental properties in heat production calculation for metabolism and feed utilization studies. Feedstock oxidation processes produce methane, carbon dioxide, and consume oxygen associated with heat production. These gas exchanges may be used to describe the heat production in metabolism and feed utilization. An accurate measurement system is required to quantify the gas exchanges from animals. To promote the utilization of gas exchange measurement system in animal metabolism study, a methodology was documented for quantifying metabolic gas exchanges for cattle.

Ruminant Emission Measurement System (REMS) was initially constructed to measure methane emission from cattle, which is located at the Beef Cattle and Sheep Field Laboratory, the University of Illinois at Urbana-Champaign. The previous REMS needs to extend its functions with some modifications for measuring heat production in metabolism study. The evaluation will be discussed in two aspects: the integrity of REMS and the measurement variation from animals.

The modification of REMS

Upgrading plan includes some tasks to modify the initial REMS. It will execute the mission of measuring heat production. Heat production was determined using indirect calorimetry method, which measured the material exchanges of the feed oxidation processes, such as oxygen, carbon dioxide, urea nitrogen, and methane. Initial REMS had capability of measuring carbon dioxide and methane concentration. Oxygen analyzer was added in this modification and urea nitrogen will be estimated with a constant. The controlling program and electronic connections were modified to the inclusion of new gas analyzer. Previous research in the system identified that the pressure differences across the ventilation pipes contribute a great amount of uncertainty.

Therefore, the modification of REMS included replacing inclined-vertical manometers with digital pressure transducers. After construction, a series of tests and calibration were completed, such as ventilation calibration, oxygen analyzer calibration, and tests of sampling frequency and gas sampling period.

The integrity of REMS

Recovery tests were completed to assess system integrity and correct systematic errors if needed. Recovery tests simulate the gas exchange of an animal in the chamber by introducing a known amount of gas into the system and comparing to what the system measured. Among them, alcohol combustion method, constant gas injection method, and gravimetric gas injection method with varying tracer gases were considered. The analysis contains a comparison of method uncertainty, reproducibility and recovery percentages between each method to justify advantages and disadvantages of these methods and to illustrate how to use these methods to correct systematic errors. Flowrate control tracer gas method has greater reproducibility than gravimetric control tracer gas method. Alcohol combustion method is more sensitive to environmental variation than tracer gas methods. The activities, including opening doors, human movement, and respiration, will cause the fluctuation of incoming air ingredients, which has much effect on oxygen measurement in alcohol combustion method.

Eight repeated trials were completed to minimize the random errors for each chamber and each method. The results were used for evaluating and correcting systematic errors. Alcohol combustion method can general check system integrity but cannot indicate the actual issues in animal gas exchange measurement. In REMS study, the recovery percentages of respiratory quotient (the ratio of CO_2 expired to O_2 inspired) varied from 72% to 77% for six chambers without any correction. The possible errors might arise from gas concentration, incoming ventilation measurement, and exhaust air flowrate estimation. Constant gas injection method was applied to specify and correct the systematic error from ventilation through injecting low concentration SF_6 gas into the chamber. The mass recovery percentages varied from 84.29% to 101.85%. Gravimetric gas injection method directly injected high concentration CO_2 gas into the chamber. It applied to check the systematic error from carbon dioxide measurement. The mass recovery percentages of carbon dioxide varied from 82.92% to 99.46%. A more rigorous evaluation should include both alcohol combustion method and tracer gas method. The results showed the correction should be applied to the ventilation of the first chamber and third chamber and the calculation of exhaust airflow. Through gradually inverse calculating, the recovery percentages of three methods eventually amended to 100% ± 5% by the correction factors.

The application of REMS

In order to ensure that animal heat production represents the metabolism at normal condition, a small-scale animal experiment was designed to investigate whether the heat production data is influenced by the husbandry alteration and whether the current prediction models is efficient to describe heat production of modern beef cattle. Through monitoring animals' behaviors and analyzing metabolism indicators, such as heat and moisture production data, methane emission, and respiratory quotient, a two-day acclimation period is essential for steers. On the other hand, the measured heat production was compared with heat production models. The average respiratory quotient (0.91) was within the range of ruminant animals' respiratory quotient. The average total heat production of mature steers was 1.38 W/kg, and sensible heat production was 1.20 W/kg, which showed a potential bias higher than CIGR models. Since the steers used in the experiments were not at the same stages as animals in CIGR

models (fattening and breeding bulls), more experiments should be done to evaluate farm steers at different stages.

The goal of this study was to document a methodology to improve capabilities for quantifying metabolic gas exchanges for cattle in metabolism studies, which including the evaluation the system integrity and measurement variation when animals were introduced into the system. It can be applied to other studies and other systems, to evaluate the confidence of the measurement after the construction.

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CHAPTER 1: INTRODUCTION AND BACKGROUND

Gas exchanges of respiration and eructation are fundamental measurements in the research of ruminant animals. The chemical reactions in metabolism involves multiple gases exchanging, such as methane, oxygen, and carbon dioxide. Methane and carbon dioxide are known to cause global warming. U.S agriculture produced 515.7 MMT carbon dioxide, which was about 9% of total emission in 2016. Beef cattle and dairy cattle industry contributes about 1/3 greenhouse gas emission corresponded among all livestock (EPA, 2016). Therefore, determining the mechanism of greenhouse gas emission for beef cattle and dairy cattle plays a substantial role in studying and mitigating climate change. As shown in Figure 1.1, there are some energy converted to heat and fecal instead of animal production after animals consume the feed. Higher heat loss causes less energy accumulation, resulting in the lower efficiency of feed utilization for farm animals (Wittenberger, 1970). As for ruminant animals, such as beef cattle, only 20% of intake energy is deposited into tissues or animal products (Ferrell & Oltjen, 2008). The heat production commonly associates with oxygen consumption and carbon dioxide production. Studying gas exchanges can help describe the mechanism of metabolism (Brown et al., 2006).

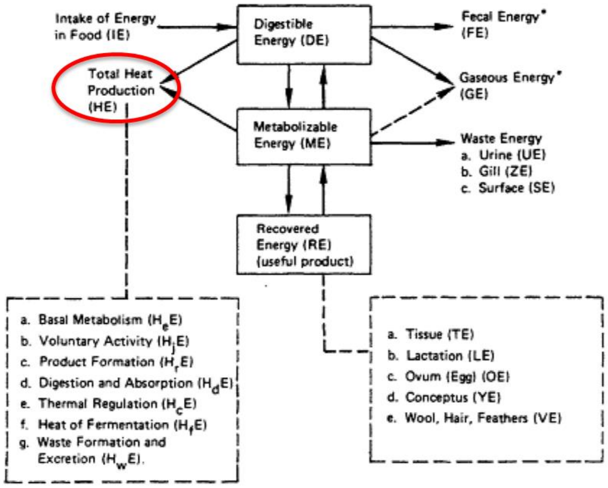

Figure 1.1. Partition of energy utilization for cattle (National Research Council, 1981).

Indirect calorimetry method measures the byproduct exchanges of metabolic processes to estimate heat production (Eq. 1.1). In this method, urinary nitrogen indicates the protein oxidation process and has been reported to 13.7g/day to 201.3g/day for cattle (Dong et al., 2014). It can be estimated in 0.032 (SD:0.01) g/l oxygen consumed (McLean, 1972a). If the nitrogen excretion rate is assumed by this relationship, the problem of heat production measurement can be converted to the problem of gas exchange measurement (McLean, 1972a).

$$\mathrm{HP} = 16.18\mathrm{O_2} + 5.02\mathrm{CO_2} - 2.17\mathrm{CH_4} - 5.99\mathrm{N} \tag{1.1}$$

where

HP = metabolic heat production rate (W).

O_2 = oxygen consumption rate (ml/s, STPD).

CO_2 = carbon dioxide consumption rate (ml/s, STPD).

CH_4 = methane consumption rate (ml/s, STPD).

N = nitrogen excretion rate (g/s).

The open-circuit chamber is a fundamental tool to determine gas exchange rates, commonly applied to animals, including cattle. There are two approaches for measuring gas exchanges: the closed-circuit chamber and the open-circuit chamber. The closed-circuit chamber is typically a well-sealed chamber without continuous fresh air input and has historically been used for small animals (Alexander, 1962). An open-circuit chamber, with continuous fresh air supply, has been widely applied to ruminant animal research (Kelly et al., 1994; Place, Pan et al., 2011).

Two vulnerabilities of measuring gas exchanges, based on the calculation shown in Equation 1.2 to 1.4, are the quantification of ventilation and gas concentrations. Typically, the ventilation rates have been measured at either the fresh air inlet or exhaust port depending on the type of ventilation. Ventilation measurement contributes lots of uncertainty, which requires calibration and correction before utilization (Calvet et al., 2013; Gates et al., 2009; Maia et al., 2015; Nienaber & Maddy, 1985). The ratio of exhaust air flowrate to incoming air flowrate may be used for estimating the exhaust air flowrate from measured incoming air flowrate based on the assumption that nitrogen is neither generated nor consumed during the process (McLean, 1972a).

As shown in Equation 1.5, if any of these variables were not accurate, the gas exchange rate and heat production rate would be unreliable. To be specific, incoming oxygen concentration significantly influences the accuracy in the calculation of heat production and is commonly modified during the utilization (Nienaber & Maddy, 1985).

$$\dot{V}_{O2} = Q^{Air}_{In.STPD}(C^{In}_{O2} - (E/I)\times C^{Ch}_{O2})\times 10^{-6} \tag{1.2}$$

$$\dot{V}_{CO2} = Q^{Air}_{In.STPD}((E/I)\times C^{Ch}_{CO2} - C^{In}_{CO2})\times 10^{-6} \tag{1.3}$$

$$\dot{V}_{CH4} = Q^{Air}_{In.STPD}((E/I)\times C^{Ch}_{CH4} - C^{In}_{CH4})\times 10^{-6} \tag{1.4}$$

$$E/I = \frac{Q^{Air}_{Ex.STPD}}{Q^{Air}_{In.STPD}} = \frac{1-(C^{In}_{CO2}+C^{In}_{O2}+C^{In}_{CH4})\times 10^{-6}}{1-(C^{Ch}_{CO2}+C^{Ch}_{O2}+C^{Ch}_{CH4})\times 10^{-6}} \tag{1.5}$$

where

C^{In}_{CO2}, C^{In}_{O2}, C^{In}_{CH4} = carbon dioxide, oxygen, methane gas concentration at incoming air, respectively (ppm$_v$).

C^{Ch}_{CO2}, C^{Ch}_{O2}, C^{Ch}_{CH4} = carbon dioxide, oxygen, methane gas concentration at chamber air, respectively (ppm$_v$).

$Q^{Air}_{In.STPD}$, $Q^{Air}_{Ex.STPD}$ = incoming/exhaust air flowrate at dry basis (273.15 K and 101325 Pa) (m^3 s^{-1}).

E/I = ratio of exhaust air flowrate to incoming air flowrate of nitrogen content balance.

Applying recovery tests on the measurement system before the animal experiment can evaluate and ensure the measured result from the measurement system is valid. The accuracy of the system measured value depends on the performance of each measuring device and can be substantiated by validation, calibration, and correction (McLean & Tobin., 1987). The principle of recovery tests is to simulate gas exchanges of animals through injecting or consuming known quantities (theoretical mass) of gases, while monitoring the gas flux with the measurement system. The ratio of measured value to theoretical value reflects the integrity of a measurement system.

Two widely applied recovery test approaches involves tracer gases method or alcohol combustion. Tracer gas methods simulate gas emission of animals. Selection of gas is one consideration of applying tracer gases. Methane and carbon dioxide are commonly applied tracer gases (A. L.Hellwing et al., 2012; Klein & Wright, 2006; Murray et al., 1999). Inert gas, such as sulfur hexafluoride, has also been applied and can be advantageous because it typically has no background influence and low toxicities (Goopy et al., 2011). The injection controller is another consideration of applying tracer gases. Constant injection method uses a mass flow controller to inject tracer gas constantly into the chamber (A. L. Hellwing et al., 2012). The gravimetric method measures weight change of the gas cylinder during the injection (Cooper et al., 1991). Alcohol combustion method mimics both the gas consumption and gas production of respiration (Cooper et al., 1991; Lin et al., 2001). The theoretical ratio of carbon dioxide production to oxygen consumption for ethanol combustion is about 0.67. This number is within the range of the ruminant animal respiratory quotient (RQ). This approach cannot accurately estimate the gas recovered percentage due to the unpredictable ethanol evaporation process. So RQ is commonly selected to represent the results instead of the recovery percentages (McLean & Tobin, 1987).

The maintenance of a gas measurement system should contain plans for testing system integrity. Evaluating the integrity and correcting systematic errors if needed is essential to ensure the reliability of measurement system. Although much research uses the open-circuit chamber to measure gas exchanges, no single source was found to summarize a complete process to evaluate the system and correct systematic errors after evaluation. Additionally, not a single source discusses how to choose integrity based on the research purposes and laboratory conditions.

After the construction and evaluation of a gas measurement system, it is necessary to investigate the measurement variation after animals were introduced to the system. Any change to the husbandry results in a challenge to homeostasis and requires a period to return to the baseline condition (Elton, 2001). Acclimation period varied from 2 days to 17 days based on different animals and measurement systems (Brown-Brandl et al., 2003; Brown-Brandl et al., 2005; Brown-Brandl et al., 2014; Webster et al., 1976). Although the initial design of open-circuit chamber must consider the animals’ size and physiological response, it is still necessary to verify if the heat production and any other indicators measured represent the results of animals under normal condition. Applying small-scale tests to check the acclimation of the animal before conducting formal experiments can indicate confidence of the measured values.

In the case of ruminant emission measurement system (REMS), a ventilation hood-type open-circuit chamber was constructed to capture methane emission in eructation and respiration of beef cattle along with a series of parameters, such as temperature, humidity and ventilation rate at the University of Illinois, Urbana and Champaign (Maia et al., 2015). The previous REMS did it have functions to conduct metabolism study. There is a critical need to improve capabilities for quantifying metabolic gas exchanges for cattle.

1.1 Objectives

The purpose of this thesis is to document a methodology to improve capabilities for quantifying metabolic gas exchanges for cattle in metabolism studies. To explain the details of this method, three objectives were explored:

A. Design and complete the upgrading plan of REMS to measure heat production for metabolism studies including the modification of digital manometers, an oxygen analyzer, electric circuits, data collection systems and calculation approaches.
B. Compare three recovery methods to evaluate system integrity and discuss how to use these methods to correct systematic errors based on the results of repeated tests.
C. Determining appropriate acclimation period through the analysis of behavior and metabolism indicators, and discuss the representativeness of current heat production data with prediction models.

1.2 Organization of the Thesis

Chapter 1 gives a general idea about the research topic and the background information about understanding objectives of this research. Chapter 2 provides an overview of previous REMS design, modification plan and the evaluation of ventilation estimation methods for metabolism study. Chapter 3 presents system integrity tests description and correction analysis for new REMS. Chapter 4 outlines the experiments about acclimation and the comparison between measured heat production data and current heat production prediction models. Chapter 5 describes the conclusion of this research and potential future work. There are also very detailed descriptions about integrity tests and heat production tests including procedures, data analysis codes and results in appendices.

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CHAPTER 2: MODIFICATIONS TO IMPROVE RUMINANT EMISSION MEASUREMENT SYSTEM: FLOW, GASES AND CONTROLS, DATA ACQUISITION AND CALCULATIONS

2.1 Background and Introduction

Ruminant Emission Measurement System (REMS) is an open-circuit respiratory chamber initially designed to capture eructated methane emissions from beef cattle, and is a part of animal metabolism laboratory, at the Beef Cattle and Sheep Field Laboratory, University of Illinois, at Urbana-Champaign. REMS consists of six subsystems: gas sampling, fresh air supply and measurement, thermal environmental controller, instrument control, gas analysis and ventilated hood chambers (Maia et al., 2015). Animals can stand up, lie down, and see outside the chambers through transparent polycarbonate panels. Fresh air is supplied through ventilation pipes, fresh water is provided in a bowl-type drinker, and feed is placed into a feed bin in a chamber before starting data collection.

Methane emissions calculations requires parameters, which are measured by REMS: air temperature, relative humidity, methane concentration, and the differential pressure across the ventilation orifice meter (Maia et al., 2015). The calculation of methane emission was initially designed using the following equation (Eq. 2.1). The exhaust air flowrate was derived from incoming air flowrate under the assumption of ignoring the moisture generation. Since animal’s heat production is partially comprised moisture production, the assumption mentioned above cannot be applied to the gas exchange calculation of indirect calorimetry.

$$ER = V_{in}\left(\frac{\rho_{in}}{\rho_{ch}}\frac{C_{ch}}{T_{ch}} - \frac{C_{in}}{T_{in}}\right)\cdot 10^{-6}\,\frac{M\cdot P_b}{R} \qquad (2.1)$$

where

ER = generated gas mass flow (g s^{-1}).
C_{ch} = chamber gas concentration (ppm_v).
C_{in} = incoming background gas concentration (ppm_v).
T_{ch} = chamber dry-bulb temperature (K).
T_{in} = incoming background dry-bulb temperature (K).
M = molecular mass of gas (g mol^{-1}).
P_b = molecular mass of gas (g mol^{-1}).
R = universal ideal gas constant (8.314; m^3 Pa K^{-1} mol^{-1}).
V_{in} = incoming air flowrate (m^3 s^{-1}).
ρ_{in} , ρ_{ch} = incoming and chamber air density ($kg.m^{-3}$).

The original REMS consisted of five gas concentration measurements and psychrometric property measurements. In order to conduct animal metabolism study with REMS, an oxygen analyzer was required and the electronic connections would need to be modified to accommodate another gas analyzer. Previous studies conducted with REMS identified measuring the pressure differences across the ventilation orifice contributed a great amount of uncertainty to the emission measurement. Therefore, it is necessary to improve the ventilation measurement.

REMS needs to be modified and upgraded to satisfy the research of animal metabolism. Animal metabolism study requires expanding gas measurement of REMS with multiple gases: oxygen, carbon dioxide, and methane. Digital pressure transducers, used to measure ventilation rate, replaced inclined-vertical oil manometers to capture the variability throughout the measurement. Electric circuit and data acquisition system were reformed for animal metabolism study (Appendix E).

The objective of this chapter was to improve measurement precision and expand capabilities to include indirect calorimetry in REMS. The specific tasks are:

A. Replace vertical oil manometer with digital manometer; calibrate fresh air measurement subsystem.
B. Add oxygen analyzer into the system, calibrate and evaluate the oxygen measurement
C. Modify the instrument control program and document the operation procedure.

D. Discuss the assumptions and equations between methane emission and indirect calorimetry calculations.

2.2 Digital Manometer Selection and Calibration in Fresh Air Measurement Subsystem

A stable and accurate ventilation measurement can improve measurement accuracy. REMS uses orifice meters to measure the ventilation rate of each chamber. The pressure difference across orifice meter is related to the ventilation rate based on the Bernoulli’s principle. In the original REMS, operators should manually record the pressure difference from inclined-vertical oil manometers before each experiment. The number measured represented an average value for the whole test. The previous study stated that this ventilation measurement contributes the most uncertainty in the calculation of gas recovered (Maia et al., 2015). Besides, this operation does not describe any pressure changes during the process. Therefore, a more advanced measurement plan was necessary to solve this problem.

A digital differential pressure transducer (Model 260, MS2, Setra Systems, Inc) replaced the inclined-vertical manometer. The pressures across the orifice meters varied from 0.7”-1.5” water column. The digital pressure transducers can give range up to 10 inches water column with the output of 0-5 VDC signal. As shown in Figure 2.1, digital pressure transducers were installed above ventilation pipes. Then, it connected to a 15 V power supply that was located at the instrument control box. LabVIEW received the digital signals from pressure transducer through data acquisition board and calculated the ventilation rate from the pressure difference simultaneously.

Figure 2.1. The location of a differential pressure transducer in ventilation supply subsystem.

The calibration of orifice meters and digital manometers followed Chamber-Nozzle Airflow System Calibration Reference, which was described in the previous study (Ramirez, 2014). As shown in Figure 2.2, a centrifugal blower provided a stable airflow. The inclined-vertical oil manometer measured the pressure differences across the nozzle inside the wind chamber. The readings of oil manometer compared with the readings of a digital manometer, which gave a relationship between reference flow and orifice meter theoretical flow.

Figure 2.2. The calibration of orifice meters using Chamber-Nozzle Airflow System.

Each orifice meter was calibrated in 21 different flowrates, which were analyzed with a linear regression model. As shown in Table 2.1, the regression coefficients varied from 0.98 to 1.11. Pressure differences across the orifice meter were converted into the volumetric flowrate based on the mathematical model (Ramirez, 2014). The standard uncertainty of digital pressure transducer is 6.23 pascal based on manufacturer's traceable reports. The overall standard uncertainty of volumetric flowrate was calculated based on moisture air density, regression standard error and slope standard error from orifice meter calibration (Ramirez, 2014).

Table 2.1. Summary of orifice-meter calibration results (x: Differential pressure in digital manometer (in.wc)).

Orifice Meter	Slope	Standard Error	Regression Standard Error	Volumetric flow rate (m^3/s)
1	1.0766	6.17E-04	1.016877206	$0.0070303x^{0.5}$
2	1.01766	8.32E-04	1.524684079	$0.0074375x^{0.5}$
3	1.04692	5.76E-04	1.00935124	$0.0072295x^{0.5}$
4	0.97586	8.15E-04	1.57040969	$0.0077561x^{0.5}$
5	1.06193	5.98E-04	1.023720961	$0.0071274x^{0.5}$
6	1.1092	5.53E-04	0.877803823	$0.0068237x^{0.5}$

2.3 Expansion Gas Analysis System with Oxygen Measurement in Gas Analysis Subsystem

The heat production measurement requires measuring three gases: oxygen, carbon dioxide, and methane. REMS uses Infrared Photoacoustic Spectroscopy Muti-Gas Analyzer (INNOVA 1412i) to measure gas concentration including methane, carbon dioxide, sulfur hexafluoride, ammonia, and nitrous oxide. The oxygen analyzer (Paramagnetic oxygen analyzer, 600P, California Analytical Instrument, Inc) was added into REMS to satisfy the requirement of heat production studies. New gas analysis subsystem has two gas analyzers that share the same sampling pipe. The flow rates of sample gas were adjusted to fulfill the requirement of two gas analyzers. Moreover, the electric circuit in the multiplexer box was also changed and shown in Appendix E.

Understanding the performance of a gas analyzer is significant before formally using in measurement. Oxygen analyzer needs warm up and calibration before each time using. The operation of calibration and data collection can be executed through LabVIEW. The pins of the remote-control function and auto-calibration function of oxygen analyzer connected to the relay board. Oxygen concentration signals are converted to voltage change signals that are received through data acquisition board.

Oxygen analyzer always has a drift after a certain amount of time. As shown in Figure 2.3, span gas (20.9%) were continuously injected for 72 hours. After each 24 hours, the average values of last 20 samples and last 10 samples were extracted as the mean of gas concentration after each 24 hours. Although the vibration of voltage signals is observable, the means of last 10 samples and last 20 samples were still very close for all three periods. The measured oxygen concentration went down during 72 hours. Considering animals need to be fed daily, calibrating oxygen analyzer every 24 hours can ensure the results are reliable.

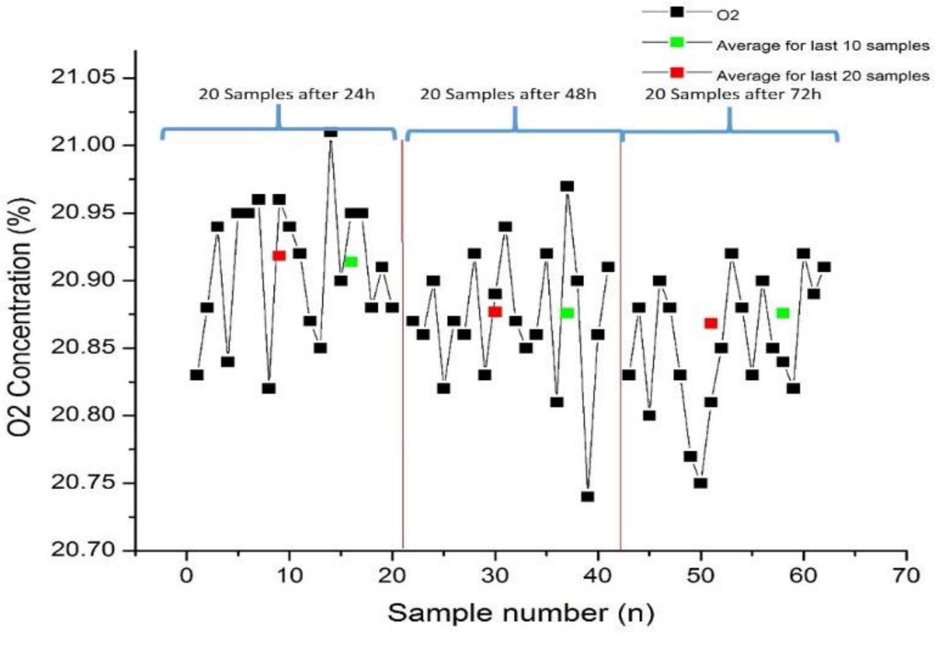


Figure 2.3. The measured results of oxygen concentration in 72 hours' experiment using 20.9% span gas.

The standard uncertainty of oxygen concentration measurement will be used in uncertainty analysis. Based on the specification of paramagnetic oxygen analyzer, the standard uncertainty was calculated from resolution, repeatability, calibration reference standard error and manufacturer’s accuracy (Maia et al., 2015). As shown in Equation 2.3, the uncertainty of oxygen analyzer consists of five parts. A normal error distribution (divisor = $\sqrt{3}$)and rectangular error distribution (divisor = 1) are applied to each part of the equation based on the confidence interval of 95% and data limits (Taylor & Kuyatt, 1993).

$$\Delta C_{O2} = \sqrt{\left(\frac{SDPC}{1}\right)^2 + \left(\frac{REPI}{\sqrt{3}}\right)^2 + \left(\frac{PCT \cdot AC}{\sqrt{3}}\right)^2 + \left(\frac{RD}{\sqrt{3}}\right)^2 + \left(\frac{RES}{\sqrt{3}}\right)^2} \tag{2.2}$$

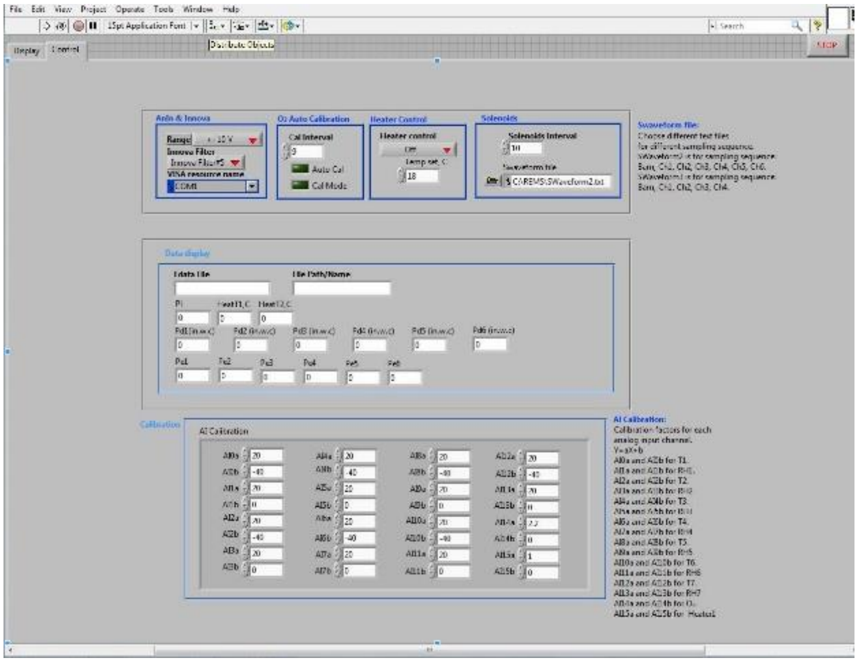
where

ΔC_{O2} = gas concentration combined standard uncertainty (%).
SDPC = standard deviation (n = 13) of post-calibration repeatability (±1%, %, normal distribution).
REPI = instrument repeatability from manufacturer (±1%, full scale, rectangular distribution).
PCT = primary certified tolerance (±2% of AC, %, rectangular distribution).
AC = actual concentration from manufacturer of primary certified tank (20.85%).
RD = range drift for measured gas concentration (±1% for 24 hours, ppmv, rectangular distribution).
RES = instrument resolution (0.1% for full scale).

2.4 Instrument Control Subsystem Modification (LabVIEW)

LabVIEW software is the platform for collecting data, controlling devices, and calculating. The data, such as temperature, humidity, differential pressure, and gas concentration, were collected through data acquisition board at a constant frequency, and then calculated and stored in “.csv” files. This software can also control relay board to switch gas sampling and sensors between each chamber. The modification of LabVIEW program was a part of REMS upgrading plan.

Main program consists of two panels: the control panel and the display panel. As shown in Figure 2.4, the control panel contains the major controlling functions. The new feature is the oxygen calibration box for controlling oxygen analyzer calibration. It was used for setting up the time to execute calibration. If the “Cal Interval” was set up, oxygen analyzer will execute calibration during the barn gas sampling after a certain time. In addition, “solenoids box” was modified for changing sample sequences though each chamber and the number of samples in each cycle of each chamber.



Range: +/-10 V
Innova Filter: Innova Filter#5
VISA resource name: COM1
Cal Interval: 9
Heater control: Off
Temp set, C: 18
Solenoids Interval: 10
Swaveform file: C:\AREMS\SWaveform2.txt

Swaveform file:
Choose different text files
for different sampling sequence.
SWaveform2 is for sampling sequence:
Bam, Ch1, Ch2, Ch3, Ch4, Ch5, Ch6.
SWaveform1 is for sampling sequence:
Bam, Ch1, Ch2, Ch3, Ch4.

Edata file:
File Path/Name:
Pi: 0
Heat T1, C: 0
Heat T2, C: 0
Pd1 (in.w.c): 0
Pd2 (in.w.c): 0
Pd3 (in.w.c): 0
Pd4 (in.w.c): 0
Pd5 (in.w.c): 0
Pd6 (in.w.c): 0
Pe1: 0
Pe2: 0
Pe3: 0
Pe4: 0
Pe5: 0
Pe6: 0

AI0a: 20
AI0b: -40
AI1a: 20
AI1b: 0
AI2a: 20
AI2b: -40
AI3a: 20
AI3b: 0
AI4a: 20
AI4b: -40
AI5a: 20
AI5b: 0
AI6a: 20
AI6b: -40
AI7a: 20
AI7b: 0
AI8a: 20
AI8b: -40
AI9a: 20
AI9b: 0
AI10a: 20
AI10b: -40
AI11a: 20
AI11b: 0
AI12a: 20
AI12b: -40
AI13a: 20
AI13b: 0
AI14a: 2.2
AI14b: 0
AI15a: 1
AI15b: 0

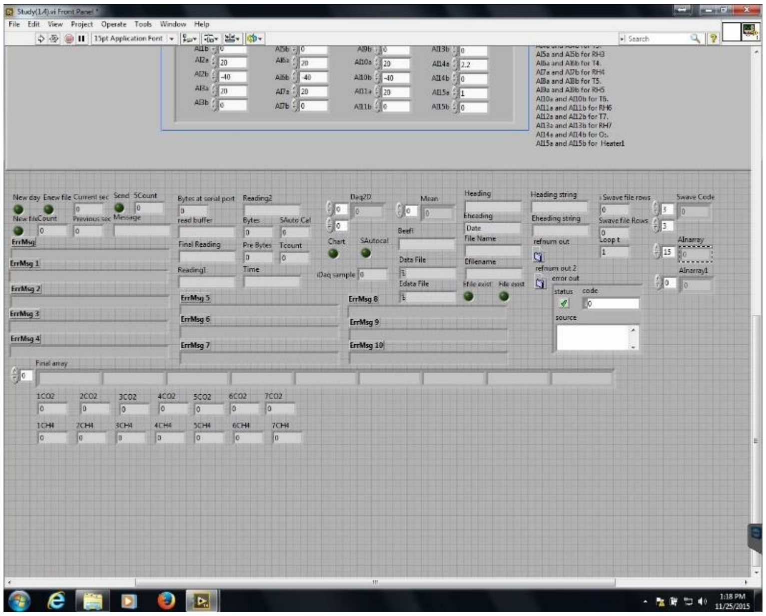
AI Calibration:
Calibration factors for each
analog input channel.
Y=aX+b
AI0a and AI0b for T1.
AI1a and AI1b for RH1.
AI2a and AI2b for T2.
AI3a and AI3b for RH2.
AI4a and AI4b for T3.
AI5a and AI5b for RH3.
AI6a and AI6b for T4.
AI7a and AI7b for RH4.
AI8a and AI8b for T5.
AI9a and AI9b for RH5.
AI10a and AI10b for T6.
AI11a and AI11b for RH6.
AI12a and AI12b for T7.
AI13a and AI13b for RH7.
AI14a and AI14b for O2.
AI15a and AI15b for Heater1

Figure 2.4. Control panel of "Study (1.3). VI".

Another new feature in control panel was the "error message box" (Figure 2.5). New LabVIEW program could identify communication problems between software and hardware if the data displayed improperly. As shown in Table 2.2, each message box indicates one possible error source due to the communication problem between software and hardware. If any of these boxes showed the occurrence of error messages, operators could inversely check the hardware and identify the possible reason to the problem.

Table 2.2. The meanings of error messages in each error message box

Error message	Meaning	Error message	Meaning
ErrMsg	Relay #1 low Communication	ErrMsg6	Solenoid 6,7 output
ErrMsg1	Relay #1 high Communication	ErrMsg7	DQ #2 pressure transducer sensor
ErrMsg2	Solenoid 0-5	ErrMsg8	Error from DQ#2 pin
ErrMsg3	Solenoid 0-5 output	ErrMsg9	For O2 calibration control
ErrMsg4	DQ #1 Temperature/Humidity sensor	ErrMsg10	For O2 calibration control output
ErrMsg5	Solenoid 6,7		



AI1b 0
AI2a 20
AI2b -40
AI3a 20
AI3b 0
AI5b 0
AI6a 20
AI6b -40
AI7a 20
AI7b 0
AI9b 0
AI10a 20
AI10b -40
AI11a 20
AI11b 0
AI13b 0
AI14a 2.2
AI14b 0
AI15a 1
AI15b 0

AI5a and AI5b for RH3
AI6a and AI6b for T4.
AI7a and AI7b for RH4
AI8a and AI8b for T5.
AI9a and AI9b for RH5
AI10a and AI10b for T6.
AI11a and AI11b for RH6
AI12a and AI12b for T7.
AI13a and AI13b for RH7
AI14a and AI14b for O₂.
AI15a and AI15b for Heater1

Current sec 0
SCount 0
Count 0
Previous sec 0
Message
Bytes at serial port 0
read buffer
Reading2
Bytes 0
SAuto Cal 0
Final Reading
Pre Bytes 0
Tcount 0
Reading1
Time
Daq2D 0
iDaq sample 0
Mean 0
Beefi
Data File 1
Edata File 1
Heading
Eheading
Date
File Name
Efilename
Heading string
Eheading string
i Swave file rows 0
Swave file Rows 0
Loop t 1
Swave Code 0
AInarray 0
AInarray1 0
code 0
ErrMsg
ErrMsg 1
ErrMsg 2
ErrMsg 3
ErrMsg 4
ErrMsg 5
ErrMsg 6
ErrMsg 7
ErrMsg 8
ErrMsg 9
ErrMsg 10
Final array
1CO2 0
2CO2 0
3CO2 0
4CO2 0
5CO2 0
6CO2 0
7CO2 0
1CH4 0
2CH4 0
3CH4 0
4CH4 0
5CH4 0
6CH4 0
7CH4 0

Figure 2.5. Error message boxes of "Study (1.3). VI".

Standard operation procedure for animal testing

A> Open "Study (1.4).vi" file.

B> Select "COM1" in "VISA resource name"; Select calibration period in "Cal Interval"; Select number of samples in "Solenoids interval" and sampling sequence in "Swaveform file".

C> In display panel, type in animal's label and contact email for emergency situation.

D> Click run button to start program.

E> To stop program, click red stop button on up right corner. The files are saved in "data" folder and "Edata" folder. Data in "data" folder is the original file for each sampling. Data in "Edata" folder is the average number of last five samples for each chamber at each cycle.

2.5 Comparison of Moisture Air Mass Balance and Nitrogen Content Balance in the Calculation of Fresh Air Supply

2.5.1 Introduction

The open-circuit chamber is preferred to measure the gas exchange of animals. Compared to the sealed closed-circuit chamber, the open-circuit chamber has continuous fresh air supply (Alexander, 1962). Positive pressure ventilation (PSV) forces ambient air into the chamber, and the exhaust air flowrate is calculated from the measured incoming air flowrate. Negative pressure ventilation (NSV) pulls chamber air outside the chamber, and the incoming air flowrate is calculated from measured exhaust air flowrate (Zhang, 1994). Since the ventilation rates were only measured either at fresh air inlet or exhaust port depending on the type of ventilation, the estimation of another flowrate always established from the mathematical equations and assumptions.

The mathematical relationship between incoming air flowrate and exhaust air flowrate is typically made under some assumption. Two common assumptions are: nitrogen content balance (NCB) and moisture air mass balance (MAMB). Nitrogen content balance is to assume animal does not consume or produce nitrogen, and the nitrogen content is constant between incoming air and exhaust air (Marks et al., 1987; Nienaber et al., 2009). When the gas exchange process of the animal was considered only involving the process of respiratory and rumen fermentation (ruminant animals), the nitrogen concentration can be determined by eliminating the concentration of carbon dioxide, oxygen, and methane (Eq. 2.4). Nitrogen content balance relies on the gas concentration measurement. If the uncertainty of one gas concentration measurement is significant, this relationship might be wrong.

$$\frac{V_{Ex}}{V_{In}} = \frac{\rho_{In}}{\rho_{Ch}} \tag{2.3}$$

$$E/I = \frac{Q^{Air}_{Ex.STPD}}{Q^{Air}_{In.STPD}} = \frac{1-(C^{In}_{CO2} + C^{In}_{O2} + C^{In}_{CH4}) \times 10^{-6}}{1-(C^{Ch}_{CO2} + C^{Ch}_{O2} + C^{Ch}_{CH4}) \times 10^{-6}} \tag{2.4}$$

where

V_{In}, V_{Ex} = incoming air flowrate and exhaust air flowrate (m^3 s^{-1}).
ρ_{In}, ρ_{Ch} = incoming and chamber air density (kg.m^{-3}).
E/I = ratio of exhaust flowrate to incoming air flowrate in nitrogen content balance.
$Q^{Air}_{In.STPD}$, $Q^{Air}_{Ex.STPD}$ = incoming/exhaust air flowrate at dry basis (273.15 K and 101325 Pa) (m^3 s^{-1}).
$C^{In}_{CO2}, C^{In}_{O2}, C^{In}_{CH4}$ = carbon dioxide, oxygen, methane gas concentration at incoming air, respectively (ppm_v).
$C^{Ch}_{CO2}, C^{Ch}_{O2}, C^{Ch}_{CH4}$ = carbon dioxide, oxygen, methane gas concentration at chamber air, respectively (ppm_v).

Moisture air mass balance is to assume the overall incoming mass flow equals to the sum of mass generation and mass exhausted. As shown in Equation 2.3, when the mass generation is not significant, it could be omitted, and the incoming mass equals to the exhausted mass (Gates et al., 2009; Maia et al., 2015). The animal moisture generation is a portion of total heat production. This assumption is invalid to use in this case.

There is a critical need to discuss the constraint of each assumption during the application. A 1% error in ventilation estimation would cause at least 21% error in calculated O_2 consumption (Arch et al., 2006). However, there is not direct comparisons illustrating how to choose ventilation assumptions and what should be considered when applying it to the calculation. A case study including ethanol combustion method used REMS on the Beef Cattle and Sheep Field Laboratory, University of Illinois at Urbana-Champaign. The calculation involved in this chapter were processed into MATLAB and described in Appendix A. The objectives of this study were to:

A. Illustrate the condition of using two assumptions by comparing the significance of each variable in the equations.
B. Illustrate the constraint of two assumptions by discussing the possible issues during the measurement.

2.5.2 Materials and Methods

The Ruminant Emission Measurement System (REMS) consists of a positive pressure ventilation subsystem. The incoming air flowrate can be determined by a precision orifice meter (Ramirez, 2014). Eight replicated ethanol combustion test were applied for simulating gas exchange of animals through consuming or producing a known amount of gas in the measurement system (McLean & Tobin, 1987). Fresh air was supplied before each trial. A scale (TS4kD, OHAUS CORPORATION, USA) with polycarbonate plate was put into the open-circuit chamber on a table. Three alcohol lamps with 200 proof ethanol (1 gallon, Ethyl Alcohol, Decon Laboratories Inc) were placed on the plate and weighed before the start of the gas sampling system. For each trial, data collection consisted of 10 background samples before lamps lit, 40 chamber samples after lamps, followed by another 10 background samples. The weight and time were recorded when the ethanol lamps were extinguished.

2.5.3 Calculation

2.5.3.1 Mass injected or consumed (Theoretical result)

The known amount of ethanol combusted in the chamber, produced carbon dioxide, and consumed oxygen. The gas exchange was calculated from the chemical equation, and theoretical gas exchange was determined by the Equation 2.5 and 2.6.

$$\dot{V}_{O2}^{Theo} = \frac{(\Delta\dot{m}_{Ethanol} \times C_{Ethanol} \times 3 \times 22.414)}{46.0694 \times 1000} \tag{2.5}$$

$$\dot{V}_{CO2}^{Theo} = \frac{(\Delta\dot{m}_{Ethanol} \times C_{Ethanol} \times 2 \times 22.414)}{46.0694 \times 1000} \tag{2.6}$$

where

$\dot{V}_{O2}^{Theo}$ = estimate O_2 generation rate at dry basis (273.15 K and 101325 Pa) (m^3 s^{-1}).
$\dot{V}_{CO2}^{Theo}$ = estimate CO_2 consumption rate at dry basis (273.15 K and 101325 Pa) (m^3 s^{-1}).
$\Delta\dot{m}_{Ethanol}$ = ethanol consumption rate (g/s).
$C_{Ethanol}$ = ethanol concentration (0.99).

2.5.3.2 Mass recovered using MAMB (Measured result)

The calculation of gas exchange using MAMB was to establish the mass balance between incoming and exhaust airflow (Eq. 2.7). During the animal experiment, the mass consumption or production term of animal were unknown (Eq. 2.3). However, this portion is a known value in recovery test since sum of mass exchanges are equivalent to the ethanol exchange rate (Eq. 2.8).

$$\dot{m}_{In}^{ma} + \Delta m = \dot{m}_{Ex}^{ma} \tag{2.7}$$

$$V_{In} \times \rho_{In} + \Delta\dot{m}_{Ethanol} = V_{Ex} \times \rho_{Ch} \tag{2.8}$$

where

$\dot{m}_{In}^{ma}$, $\dot{m}_{Ex}^{ma}$ = incoming/exhaust moist air mass flow rate (g s^{-1}).
Δm = mass generation rate (g s^{-1}).

As shown in Equation 2.9 to 2.13, gas generation or consumption equal to the volumetric difference between incoming gas flowrate and exhaust gas flowrate. With considering of the relationships between exhaust and incoming air flowrate, the volumetric oxygen consumption rate and carbon dioxide generation can be calculated:

$$V_{exchange.M} = \left| V_{In}^{gas} - V_{Ex}^{gas} \right| \tag{2.9}$$

$$V_{In}^{gas} = V_{In} \times C_{In} \times \frac{22.414P_a}{RT_{In}} \times 10^{-6} \tag{2.10}$$

$$V_{Ex}^{gas} = \frac{V_{In} \times \rho_{In} + \Delta\dot{m}_{Ethanol}}{\rho_{Ch}} \times C_{Ch} \times \frac{22.414P_a}{RT_{Ch}} \times 10^{-6} \tag{2.11}$$

$$\dot{V}_{O2.M} = (\frac{V_{In} \times C_{O2}^{In}}{T_{In}} - \frac{V_{In} \times \rho_{In} + \Delta\dot{m}_{Ethanol}}{\rho_{Ch}} \times \frac{C_{O2}^{Ch}}{T_{Ch}}) \times \frac{22.414P_a}{R} \times 10^{-6} \tag{2.12}$$

$$\dot{V}_{CO2.M} = (\frac{V_{In} \times \rho_{In} + \Delta\dot{m}_{Ethanol}}{\rho_{Ch}} \times \frac{C_{CO2}^{Ch}}{T_{Ch}} - \frac{V_{In} \times C_{CO2}^{In}}{T_{In}}) \times \frac{22.414P_a}{R} \times 10^{-6} \tag{2.13}$$

where

$V_{exchange.M}$ = recovered gas exchange rate (273.15 K and 101325 Pa) (m^3 s^{-1}).
V_{In}^{gas} = incoming gas volumetric flow rate (m^3 s^{-1}).
V_{Ch}^{gas} = exhaust gas volumetric flow rate (m^3 s^{-1}).
T_{In}, T_{Ch} =incoming/chamber air temperature (K).
$\dot{V}_{O2.M}$ = oxygen consumption volumetric rate at dry basis using MAMB (273.15 K and 101325 Pa) (m^3 s^{-1}).
$\dot{V}_{CO2.M}$ = carbon dioxide generation volumetric rate at dry basis using MAMB (273.15 K and 101325 Pa) (m^3 s^{-1}).

2.5.3.3 Mass recovered using NCB (Measured result)

Nitrogen content balance estimates exhaust air flowrate from measured incoming air flowrate (Eq. 2.5). Through assuming that nitrogen content kept constant during burning process, the recovered gas volumetric flowrate could be calculated in Equation 2.14 and 2.15.

$$\dot{V}_{O2} = Q^{Air}_{In.STPD}(C^{In}_{O2} - (E/I)\times C^{Ch}_{O2})\times 10^{-6} \tag{2.14}$$

$$\dot{V}_{CO2} = Q^{Air}_{In.STPD}((E/I)\times C^{Ch}_{CO2} - C^{In}_{CO2})\times 10^{-6} \tag{2.15}$$

where

$\dot{V}_{O2}$ = oxygen consumption volumetric rate at dry basis (273.15 K and 101325 Pa) ($m^3\ s^{-1}$).
$\dot{V}_{CO2}$ = carbon dioxide generation volumetric rate at dry basis (273.15 K and 101325 Pa) ($m^3\ s^{-1}$).

As shown in Equation 2.16 and 2.17, air flowrate measurement by REMS converted to dry basis under standard temperature and pressure (273.15 K and 101325 Pa) using psychrometric property relastionships (Albright, 1990).

$$P_W = 610.78e^{(\frac{17.269T_{db}}{T_{db}+237.30})} \tag{2.16}$$

$$Q^{Air}_{In.STPD} = V_{In}\times(P_a - P_w)[\frac{273.16}{101325\times(T_{db}+273.16)}] \tag{2.17}$$

where

P_w = vapor pressure of moisture in the air (Pa).
P_a = Barometric pressure (Pa).
T_{db} = dry-bulb temperature (^oC).

2.5.3.4 Respiratory Quotient and Recovery Percentage

Respiratory quotient (RQ) was another important factor to study animal energetics (Eq. 2.18). It is presented to check both gases (McLean & Tobin, 1987). Recovery percentage (RP) represents how much gas recovered with respect to the known amount of gas (Eq. 2.19).

$$RQ = \frac{\dot{V}_{CO2.j}}{\dot{V}_{O2.j}} \tag{2.18}$$

$$RP = \frac{\dot{V}_{i,j}}{\dot{V}^{Theo}_{j}}\times 100\% \tag{2.19}$$

where

i = CO_2, O_2.
j = MAMB (M), NCB (N).

2.5.3.5 Uncertainty analysis of measured gas exchange using MAMB

A well-documented uncertainty analysis of each component in REMS has been published, which described the calculation methods using MAMB, and the analysis for this study followed a similar process (Maia et al., 2015).

2.5.3.6 Uncertainty analysis of measured gas exchange using NCB

The standard uncertainty of measured gas exchange is calculated following Equation 2.20.

$$\Delta V_{gas} = \sqrt{\left(\frac{\partial_{gas}}{\partial Q_{In.STPD}^{Air}}\Delta Q_{In.STPD}^{Air}\right)^2 + \left(\frac{\partial V_{gas}}{\partial Q_{Ex.STPD}^{Air}}\Delta VCF\right)^2 + \left(\frac{\partial V_{gas}}{\partial C_i^{In}}\Delta C_{gas}^{In}\right)^2 + \left(\frac{\partial V_{gas}}{\partial C_i^{Ch}}\Delta C_{gas}^{Ch}\right)^2} \tag{2.20}$$

Based on the Equation 2.17, the standard uncertainty of incoming air flow rate under standard condition was shown in Equation 2.21.

$$\Delta Q_{In.STPD}^{Air} = \sqrt{\left(\frac{\partial Q_{In.STPD}^{Air}}{\partial T_{db}}\Delta T_{db}\right)^2 + \left(\frac{\partial Q_{In.STPD}^{Air}}{\partial Pa}\Delta P_a\right)^2 + \left(\frac{\partial Q_{In.STPD}^{Air}}{\partial V_{In}}\Delta V_{In}\right)^2} \tag{2.21}$$

Ventilation correction factor was calculated based on oxygen concentration, carbon dioxide concentration, and incoming air flowrate. Therefore, the standard uncertainty of E/I could be estimated as shown in Equation 2.22:

$$\Delta(E/I) = \sqrt{\left(\frac{\partial(E/I)}{\partial C_{O2}^{In}}\Delta C_{O2}^{In}\right)^2 + \left(\frac{\partial(E/I)}{\partial C_{CO2}^{In}}\Delta C_{CO2}^{In}\right)^2 + \left(\frac{\partial(E/I)}{\partial C_{CH4}^{In}}\Delta C_{CH4}^{In}\right)^2 + \left(\frac{\partial(E/I)}{\partial C_{O2}^{Ch}}\Delta C_{O2}^{Ch}\right)^2 + \left(\frac{\partial(E/I)}{\partial C_{CO2}^{Ch}}\Delta C_{CO2}^{Ch}\right)^2 + \left(\frac{\partial(E/I)}{\partial C_{CH4}^{Ch}}\Delta C_{CH4}^{Ch}\right)^2} \tag{2.22}$$

The standard uncertainty of oxygen concentration, methane and carbon dioxide concentration can be determined due to manufacturer's specification (Maia et al., 2015). Since the gas analyzers used for oxygen and carbon dioxide are different, the standard uncertainty was different (Eq. 2.23).

$$\Delta C_{gas} = \sqrt{\left(\frac{SDPC}{1}\right)^2 + \left(\frac{REPI}{\sqrt{3}}\right)^2 + \left(\frac{PCT \cdot AC}{\sqrt{3}}\right)^2 + \left(\frac{RD}{\sqrt{3}}\right)^2 + \left(\frac{RES}{\sqrt{3}}\right)^2} \tag{2.23}$$

where

ΔC_{gas} = gas concentration combined standard uncertainty (% for O_2, ppm$_v$ for CH_4 and CO_2).

SDPC = standard deviation (*n* = 13) of post-calibration repeatability (±1%, % for O_2, ppm$_v$ for CH_4 and CO_2).

REPI = instrument repeatability from manufacturer (±1%, full scale, % for O_2, ppm$_v$ for CH_4 and CO_2).

PCT = primary certified tolerance (±2% of AC, % for O_2, ±1% ppm$_v$ for CH_4 and CO_2).

AC = actual concentration from manufacturer of primary certified tank (20.85% for O_2, 499.9 ppm$_v$ for CH_4 and CO_2).

RD = range drifts for measured gas concentration (±1% for 24 hours O_2, ±2.5% ppm$_v$ for three month CH_4 and CO_2).

RES = instrument resolution (0.1% for full scale O_2, 2 ppm$_v$ for CH_4 and CO_2).

2.5.4 Results and Discussion

Eight replicated experiments showed the average uncertainty of recovered mass using NCB were 62 to 70 times larger than the uncertainty of recovered mass using MAMB. The gas consumption or gas production using NCB expressed as taking difference of "concentration × flowrate" between incoming air and chamber air (Eq. 2.14 and 2.15). Exhaust air flowrate is calculated from gas concentration, which makes gas exchange measurement heavily dependent on the gas concentration measurement. In the REMS study, carbon dioxide concentration measurement has an uncertainty about 71 ppm_v that is almost 1.5% of ΔCO_2 in ethanol combustion test. The uncertainty of oxygen concentration measurement in REMS is 3000ppm_v, which is almost 42% of ΔO_2 in ethanol combustion test. As shown in Figure 2.6, the oxygen recovery percentages varied from 127.28% to 151.25% in NCB. Additional assessment and correction should be applied if using NCB in the gas exchanges calculation (Table 2.3).

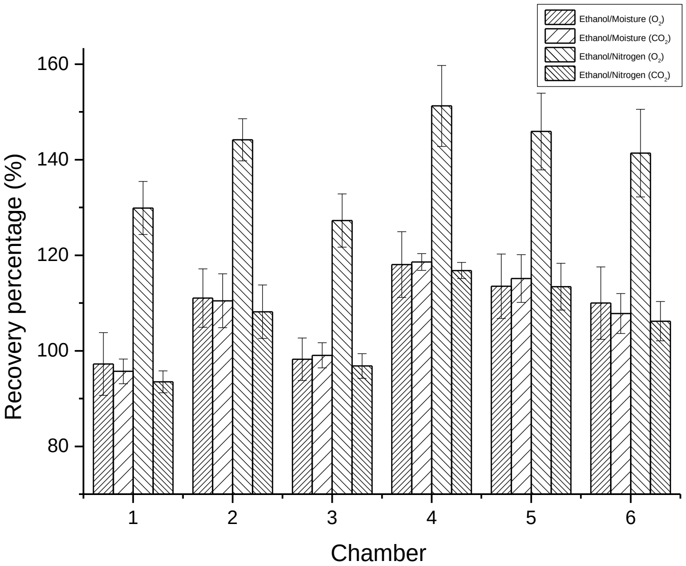


Figure 2.6. Mean and standard deviation of recovery percentages for comparing nitrogen content balance and moisture air mass balance in ventilation estimation during ethanol combustion test.

Considering the laboratory condition and the precision of gas analyzers, moisture mass balance is better than nitrogen content balance. Nitrogen content balance involves more measurement devices than NCB which leaded to more uncertainty in measurement. In addition, for some gas emission studies, such as methane emission, the measurement system does not always have oxygen sensor and carbon dioxide sensor, which limits the application of NCB. Moisture air mass balance uses air density to establish the relationship between exhaust air flowrate and incoming air flowrate (Eq. 2.8). Air density calculations only involve temperature and relative humidity measurement. These two parameters are also essential in NCB to standardize the ventilation rate (Eq. 2.17). Therefore, the equipment requirement in MAMB is less than NCB, which makes MAMB more feasible for utilization.

Table 2.3. Comparisons of advantages and disadvantages for between MAMB and NCB.

	Moisture air mass balance	**Nitrogen content balance**
Application	Gas emission (CH_4) Initial REMS	Indirect calorimetry (O_2 & CO_2 & CH_4) Upgraded REMS
Equation	Equation 2.7	Equation 2.4
Assumption	Mass generation is zero	Nitrogen is not consumed and produced during the experiment.
Advantage	Require less equipment	Without gas limitation
Disadvantage	Assumption is not valid for some application including indirect calorimetry.	Rely heavily on gas exchange measurement
Recommendation for REMS	Can be applied to gas emission measurement like CH_4 or NH_3 or CO_2	Additional correction needed before applying it to heat production measurement

In animal gas exchange measurement, the mass generation term (Δm) inside the chamber is commonly assumed negligible using MAMB (Table 2.3). It is important to justify if the assumption is a valid. This term represents the overall mass generation including the moisture production, gas generation, and gas consumption. It could be simply acquired in mass recovery test because the chemical equation of ethanol combustion process is identified. In Figure 2.7, the mass generation term was corrected with a multiplier (α) before applying it to Equation 2.8 in mass recovery test to simulate the results. When the mass of combusted ethanol was ignored (α=0), the RP of oxygen was increased from 103% to 117%, and the RQ was decreased from

0.65 to 0.57. The gas generation is not negligible in the calculation of oxygen exchange. However, ignoring the mass generation term had less influence on carbon dioxide than oxygen. As shown in Equation 2.12 and 2.13, $(\Delta \dot{m}_{Ethanol})$ was always multiplied by the chamber gas concentration. Typically, oxygen concentration (19.8%) were almost 40 times higher than the carbon dioxide concentration (5000ppm_v), which makes $(\Delta \dot{m}_{Ethanol} \times C^{Ch})$ have higher weight for oxygen than carbon dioxide. Therefore, mass generation term can be ignored for the measurement of gas whose concentration is relative low in ambient air, such as methane and carbon dioxide.

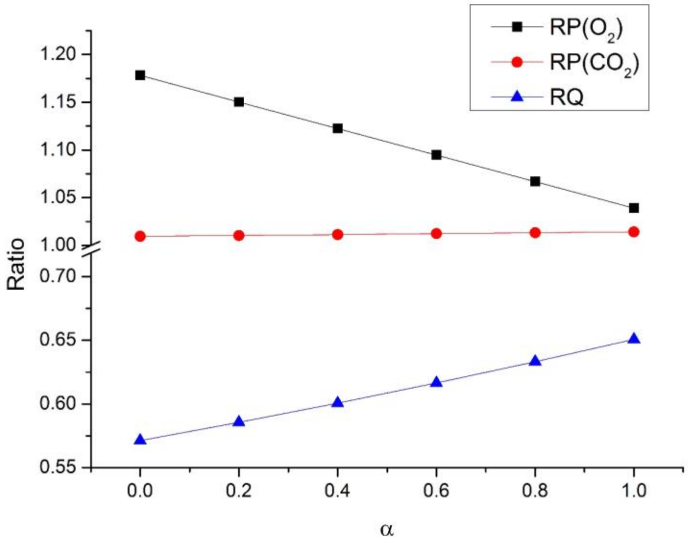


Figure 2.7. The effect of the correction factor (α) to RQ and RP for using MAMB to calculate the ventilation estimation during alcohol combustion tests on a single chamber.

2.5.5 Conclusion

Two mathematical relationships between incoming air flowrate and exhaust air flowrate are discussed in this study: MAMB and NCB. Moisture air mass balance has previously been used in estimating methane emission of beef cattle in some studies. Nitrogen content balance has previously been applied for heat production calculation. Based on the discussion in this study, these two assumptions cannot be applied interchangebly.

There are some concerns in using these two assumptions. Moisture air mass balance os not appropriate for indirect calorimetry since moisture production is a portion of heat production. As for gas exchange measurement, it is better than NCB. In animal experiments, the mass generation term cannot be ignored for gas with relative high concentration in ambient air, like oxygen. Therefore, moisture air mass balance can apply to carbon dioxide, ammonia and methane emission calculation. Nitrogen content balance has much systematic error if using low precision gas analyzers. Additional assessment and correction should be applied if using NCB in the gas exchanges calculation.

2.6 References

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CHAPTER 3: INTEGRITY ASSESSMENT OF OPEN-CIRCUIT RESPIRATION CHAMBERS FOR RUMINANT ANIMAL INDIRECT CALORIMETRY

3.1 Abstract

Open-circuit chambers have been widely applied to gas exchange measurement for indirect calorimetry and greenhouse gas emission. The system reliability is crucial and needs to be assessed. Recovery tests can evaluate the system integrity without testing the accuracy of each component. These tests simulate gas exchanges of animals through injecting or consuming known quantities (theoretical mass) of gases, while monitoring the gas flux with the measurement system. The difference between theoretical mass and measured mass by the system indicates the performance of the system. Alcohol combustion method (ACM) burns a known amount of pure ethanol to simulate both consumption (O_2) and production (CO_2). Gravimetric gas injection method (GRAV) directly measures the weight change of a compressed carbon dioxide gas cylinder during the injection process. Constant gas injection method (CGIM) constantly injects sulfur hexafluoride gas into the chamber. An experiment was conducted that included eight repeated trials for each of three recovery methods and each chamber with a total of 144 tests to Ruminant Emission Measurement System on the Beef Cattle and Sheep Field Laboratory at University of Illinois, Urbana-Champaign. The recovery percentages of respiratory quotient for six chambers in ACM varied from 72.58% to 77.76%, which indicated the errors occurred in system. CGIM and GRAV were used for identifying the error from ventilation measurement and the calculation of exhaust air flowrate. The correction factors were generated from the results and proved effective as recovery percentages were improved to an acceptable

level (100% ± 5%) for all chambers. This well-documented approach can apply to other studies and other systems to assist in selecting appropriate integrity tests and correction methods.

3.2 Introduction

Animal gas exchange measurement is a fundamental tool in animal heat production and agricultural greenhouse gas emissions studies (Bellarby et al., 2013; Brown et al., 2006). These studies are interested in measuring methane, carbon dioxide, and oxygen gas exchanges. Methane is released during ruminal fermentation whitch consumed 2% to 12% gross energy (Johnson & Johnson, 1995). Carbon dioxide production and oxygen consumption reflect the feed oxidation process in metabolism (Brown et al., 2006). The relationship of these two gases partially describes the ruminant digestion and energy utilization of animals (Ferrell & Oltjen, 2008).

There are many methods to measure gas exchange rates, such as open-circuit chamber technology, closed-circuit chamber technology, the respiratory facemask method, and isotropic tracers' method (Alexander, 1962; Hegarty et al., 2007; McLean & Tobin, 1987). The open-circuit chamber is a steady state measurement system with stable fresh air supply. The closed-circuit chamber is typically a well-sealed chamber without continuous fresh air input, and has historically been used for small animals. It is less practical for cattle. For large animals, respiratory facemask technology needs to train animals to adapt the foreign object and restraint, and usually cost much time and lots of damage to the equipment. Isotropic tracer method may require additional surgery that increases risks of infection and discomfort, and the variability of the data is significant due to the open field environment. Therefore, an open-circuit chamber, with continuous fresh air supply, is widely applied to ruminant animal research (Kelly et al., 1994; Place et al., 2011).

When applying the open-circuit chamber to the heat production measurement, the reliability always needs assessment prior to conducting tests with animals. Indirect calorimetry is one method to establish the relationship between animal heat production and gas exchange measurement. There are four required variables for indirect calorimetry: urea nitrogen extraction rate and gas exchange rates of methane, carbon dioxide and oxygen (McLean, 1972). The calculation approach of estimating gas exchange is to take the difference between the gas compositions of incoming air and exhaust air, which requires not only the gas concentration but also the ventilation. The calculation of ventilation involves some environment parameters, such as temperature, relative humidity, and differential pressure (Figure 3.1). Each parameter's measurement contributes some error to the final emission result. Ventilation measurement associated with environment parameters measurement contributes lots of uncertainty in gas emission measurement and typically requires calibration and correction before utilization (Calvet et al., 2013; Ramirez, 2014). Incoming O_2 concentration significantly influences the accuracy in the calculation of heat production (Nienaber & Maddy, 1985). Since chamber oxygen concentration cannot drop more than 1% to ambient air for maintaining the same respiration of animals, a 1% error in ventilation estimation could potential cause at least 21% error in calculated O_2 consumption (Arch et al., 2006).

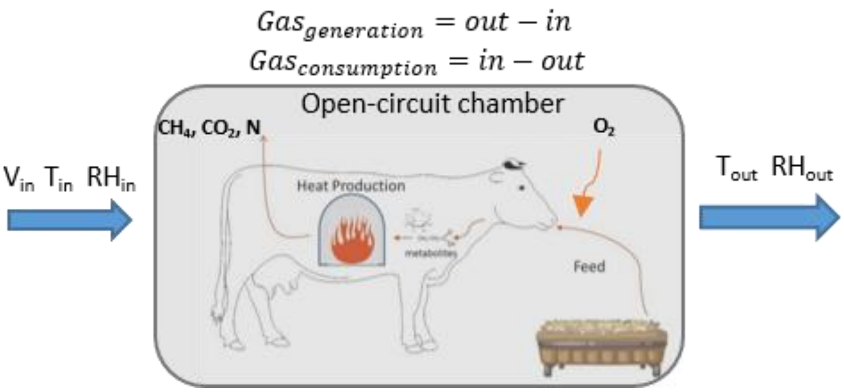


Figure 3.1. Overview of indirect calorimetry approach.

The recovery tests serve to validate the integrity of the whole system without considering each measurement component individually (McLean & Tobin, 1987). Recovery percentage (RP) relates the measured quantity to the known quantity. If the measurement system was perfect, and all components were adequately accurate and precise, the theoretical mass (injected or consumed) would equal to the mass recovered (RP=100%).

The alcohol combustion test is a combined method of gas consumption and gas production processes achieved by comparing the gas exchange of O_2 and CO_2. It is commonly used for simulating animal respiratory processes (Cooper et al., 1991). Typically, respiratory quotient (RQ), the ratio of CO_2 expired to O_2 inspired, is compared to the theoretical ethanol combustion stoichiometry ratio（0.67) for cross checking the recovered results from oxygen and carbon dioxide measurement (Cooper et al., 1991; Lin et al., 2001).

The gravimetric gas injection method is another recovery test (Cooper et al., 1991). The gas is injected into the system, and the weight of gas cylinder is recorded before and after testing. This method is more practical since scales are common equipment in the laboratory. However, a longer duration is typically necessary to get enough weight change for reducing uncertainty from weight measurement. Another drawback is the precision of the scale. Since measuring a heavy gas cylinder needs a large scale capacity, it often sacrifices the resolution of a scale (A. L. F. Hellwing et al., 2012).

The constant gas injection method uses a mass flow controller to provide a specific and constant injection rate. This approach requires a highly accurate mass flow controller to estimate the mass injected, which is specifically selected based on the gas so that the flow controller has the correct gas correction factor. Many researchers are interested in methane and carbon dioxide, and these two gases are commonly used for recovery tests. However, these gases exist in the ambient air, which are affected by the variation of background air (incoming air) composition (A. L. Hellwing et al., 2012; Klein & Wright, 2006; Murray et al., 1999). Sulfur hexafluoride (SF_6) tracer gas may be used instead. It does not occur naturally in the animal environment and has low toxicity (Goopy et al., 2011).

Uncertainty can illustrate how much error will be potentially introduced using different equipment and operations in each test. It can also reflect the expected variation between measured values and the true value due to random effects and imperfect correction for system effects in the statistical side (type A evaluation) and non-statistical side (type B evaluation) (International Organization for & International Electrotechnical, 2008). Applying uncertainty analysis to each method can summarize and quantify expected uncertainty sources (Maia et al., 2015).

After expanding the REMS with the heat production calculation (Chapter 2), the whole system assessment was necessary to evaluate the integrity of measurement system for all six open-circuit chambers. The uncertainty of gas measurement comes from instruments, the assumption used in the calculation, and the variability of the measurement process (Calvet et al., 2013). By comparing three methods from these aspects, a well-documented approach was established for evaluating and correcting the measurement system results based on laboratory conditions. The calculation involved in this chapter were processed into MATLAB and described in Appendix A to Appendix C. The objectives of this study were to:

A. Evaluate three recovery tests with respect to equipment and operating procedures.
B. Evaluate three recovery tests with respect to mathematical assumptions and systematic errors.
C. Utilize recovery tests to correct systematic errors.

3.3 Materials and methods

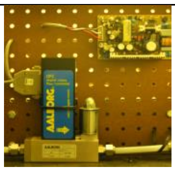
3.3.1 System Overview

The Ruminant Emission Measurement System (REMS) can measure variables required to calculate gas exchange rates, including gas concentration and environment parameters, such as temperature, humidity, and incoming ventilation rate (Maia et al., 2015). It is located at the Beef Cattle and Sheep Field Laboratory at the University of Illinois, Urbana-Champaign. This system is a positive pressure open-circuit respiratory system that secures cattle at their shoulders and measures the gas exchanges from respiration and eructation. REMS can achieve functions of data collection, system control, simple calculation and data recording. The primary gas measurement devices are a paramagnetic oxygen analyzer (600P, California Analytical Instrument Inc) and a photoacoustic multi-gas monitor (INNOVA 1412, LumaSense Technologies Inc), which can measure oxygen, carbon dioxide, ammonia, nitrous oxide, methane, sulfur hexafluoride and vapor concentration. The system includes an air conditioning unit and measurable ventilation supply ranging from 479 lpm to 525 lpm.

3.3.2 Recovery methods

As described in Table 3.1, three recovery methods were implemented for comparison and each utilized different equipment and different gases. The carbon dioxide gas cylinder selected in GRAV was about 4 kg due to the capacity of the mass balance. The gas analyzers' accuracy was confirmed using span gas (CO_2: 5000ppm (± 50ppm), SF_6: 4000ppm (± 40ppm), O_2: 20.85% (± 0.05%)).

Table 3.1. The equipment and implementation specifications of three recovery methods.

	Alcohol combustion method	Gravimetric gas injection method	Constant gas injection method
			
Equipment for controlling mass	Mass balance (OHAUS TS4KD)	Mass Balance (OHAUS NVT10001)	Flow controller (Aalborg DFC36)
Tracer gas source	200 proof ethanol (O_2, CO_2)	CO_2: 99.99% (± 0.01%)	SF_6: 4000ppm$_v$ (± 40ppm$_v$)
Operation	Measuring weight; lighting ethanol lamp; recording time	Measuring weight; recording time	Computer based gas flow controlling (4 lpm)

3.3.3 Experiment procedures

Eight replicate trials were tested for each method and each chamber for the total of 144 tests. As shown in Figure 3.2, the measurement system started with 10 background samples at the barn (approx. 10 mins), followed by 40 chamber samples (approx. 30 mins) and 10 background samples at the end (approx. 10 mins). The gas injection and ethanol combustion were started at the beginning of the chamber gas sampling. The last five barn samples at both the beginning and the end were used to estimate average background concentration. The last 12 chamber samples represent the gas concentration at steady state.

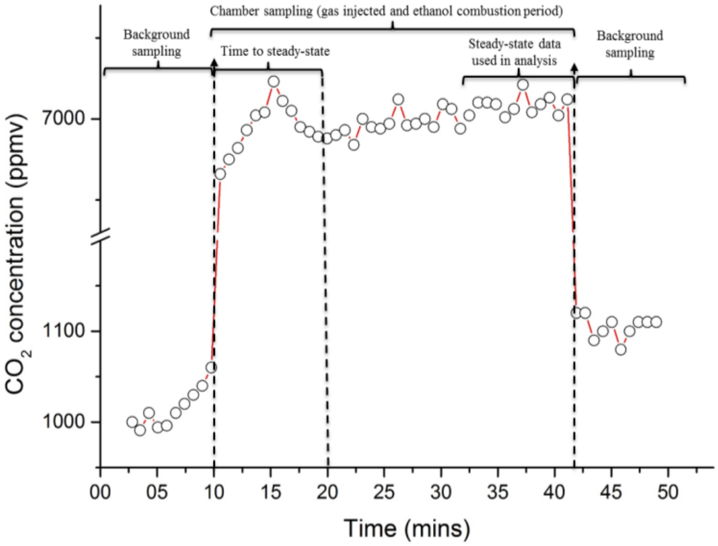


Figure 3.2. Recovery test procedure measured background (barn) first, then chamber gas concentration, and finished with background (barn).

3.3.4 Calculation Analysis

3.3.4.1 Gas flow balance

The theoretical gas exchanges (mass injected or consumed) were compared with the measured gas exchange rates from the system. Recovery percentage represents how much gas recovered with respect to the known amount of gas (Eq. 3.1). It is also adjusted to evaluate RQ by dividing the calculated value to the theoretical ratio for ethanol combustion (0.667).

$$RP = \frac{V^{Rec}}{V^{Theo}} \times 100\% == \frac{RQ}{0.667} \times 100\% \qquad (3.1)$$

Where

RP = recovery percentage (%).

V^{Theo} = theoretical gas exchange rate at dry basis (273.15 K and 101325 Pa) ($l\ s^{-1}$).

V^{Mea} = measured gas exchange rate at dry basis (273.15 K and 101325 Pa) ($l\ s^{-1}$).

RQ = respiratory quotient (the volumetric ratio of CO_2 expired to O_2 inspired).

The theoretical gas exchanges based on the known quantities of gas released or consumed were calculated following the equations in Table. 3.2.

Table 3.2. Calculation approaches of theoretical gas exchanges based on gas injection or mass consumption.

Method	Theoretical gas exchanges	Variables and parameters
Alcohol combustion method	$V_{O2}^{Theo} = \frac{m_{ethanol} \times C_{ethanol} \times 3 \times 22.414}{46.0694}$ (3.2) $V_{CO2}^{Theo} = \frac{m_{ethanol} \times C_{ethanol} \times 2 \times 22.414}{46.0694}$ (3.3)	V_{O2}^{Theo} , V_{CO2}^{Theo} = theoretical O_2 consumption and CO_2 generation rate ($l\ s^{-1}$) $m_{ethanol}$ = ethanol consumption rate ($g\ s^{-1}$) $C_{ethanol}$ = ethanol concentration (99%)
Gravimetric gas injection method	$V_{gra}^{Theo} = \frac{22.414 \times m_{gas} \times C_{CO2}^{cyl}}{44.01}$ (3.4)	V_{gra}^{Theo} = CO_2 mass consumption rate ($l\ s^{-1}$) m_{gas} = mass different for CO_2 cylinder (g) $C_{CO2}^{cyl} = \frac{y_{CO2} \times 44.01}{y_{CO2} \times 44.01 + (1 - y_{CO2}) \times 28.01}$ y_{CO2} = CO_2 concentration in gas cylinder (99.99%)
Constant gas injection method	$V_{inj}^{Theo} = V_{inj} \times C_{inj} \times \frac{22.414 \cdot P_{std}}{R \cdot T_{std}} \times 10^{-6}$ (3.5)	V_{inj}^{Theo} = mass flow injected ($l\ s^{-1}$) V_{inj} = injected volumetric flow rate ($m^3\ s^{-1}$) R = universal constant of ideal gas (8.314 m^3 Pa K^{-1} mol^{-1}) T_{std} = 273.15 K; P_{std} = 101325 Pa

The calculation approaches of measured gas exchanges were from the classic method described for heat production calculation (McLean, 1972). The gas exchange rates in recovery methods were shown in Equation 3.6 to 3.8.

$$\dot{V}_{O2}^{Mea} = Q_{In.STPD}^{Air}(C_{O2}^{In} - (E/I) \times C_{O2}^{Ch}) \times 10^{-6} \tag{3.6}$$

$$\dot{V}_{CO2}^{Mea} = Q_{In.STPD}^{Air}((E/I) \times C_{CO2}^{Ch} - C_{CO2}^{In}) \times 10^{-6} \tag{3.7}$$

$$\dot{V}_{SF6}^{Mea} = Q_{In.STPD}^{Air}((E/I) \times C_{SF6}^{Ch} - C_{SF6}^{In}) \times 10^{-6} \tag{3.8}$$

Where

$\dot{V}_{O2}^{Mea}$, $\dot{V}_{CO2}^{Mea}$, $\dot{V}_{SF6}^{Mea}$ = oxygen consumption, carbon dioxide generation, and sulfur hexafluoride generation volumetric rate at dry basis (273.15 K and 101325 Pa) (m^3 s^{-1}).

$Q_{In.STPD}^{Air}$, $Q_{Ex.STPD}^{Air}$ = incoming/exhaust air flowrate at dry basis (273.15 K and 101325 Pa) (ml^3 s^{-1}).

E/I = ratio of exhaust air flowrate to incoming air flowrate.

C_{CO2}^{In}, C_{O2}^{In}, C_{SF6}^{In} = carbon dioxide, oxygen, sulfur hexafluoride gas concentration at incoming air, respectively (ppmv).

C_{CO2}^{Ch}, C_{O2}^{Ch}, C_{SF6}^{Ch} = carbon dioxide, oxygen, sulfur hexafluoride gas concentration of chamber air, respectively (ppmv).

The flow rates used in Equation 3.6 to 3.8 are under dry basis at standard condition. The measured ventilation rate needs to convert to this state according to psychrometric property equations (Eq. 3.9). The water vapor pressure was calculated using the dry-bulb temperature, relative humidity, and barometric pressure (Albright, 1990).

$$Q_{In.STPD}^{Air} = V_{In} \times (P_a - P_w)\left[\frac{273.16}{101325 \times (T_{db} + 273.16)}\right] \tag{3.9}$$

Where

V_{In} = incoming gas volumetric flow rate (ml^3 s^{-1}).

P_a = barometric pressure (Pa).

P_w = vapor pressure of moisture in the air (Pa).

T_{db} = dry-bulb temperature (^oC).

Exhaust air flowrate is calculated from incoming air flowrate (Eq. 3.10). This method assumes that nitrogen is neither generated nor consumed during the process.

$$E/I = \frac{Q_{Ex.STPD}^{Air}}{Q_{In.STPD}^{Air}} = \frac{1-(C_{CO2}^{In} + C_{O2}^{In}) \times 10^{-6}}{1-(C_{CO2}^{Ch} + C_{O2}^{Ch}) \times 10^{-6}} \tag{3.10}$$

Where

E/I = ratio of exhaust air flowrate to incoming air flowrate.

$Q_{In.STPD}^{Air}$ = incoming air flowrate at dry basis (273.15 K and 101325 Pa) (ml^3 s^{-1}).

$Q_{Ex.STPD}^{Air}$ = exhaust air flowrate at dry basis (273.15 K and 101325 Pa) (ml^3 s^{-1}).

3.3.4.2 Uncertainty analysis of estimate theoretical gas exchanges and measured gas exchanges

Uncertainty analysis is a useful strategy to evaluate integrity assessment method by quantifying systematic errors. It can identify which method is expected to have the lower systematic error. A well-documented uncertainty analysis of each component in REMS has been published, which described the potential sources and calculation methods of CGIM for REMS, and the analysis for this study followed a similar process for GRAV (Maia et al., 2015).

Based on the Equations 3.2 to 3.5, the uncertainty sources for mass injected or consumed calculation were weight and concentration measurement (Table 3.3).

Table 3.3. Summary of uncertainty calculations for theoretical mass injection or consumption.

Alcohol combustion method	Gravimetric gas injection method
$\Delta V_{gas}^{Theo} = \sqrt{(\frac{\partial V_{gas}^{Theo}}{\partial \Delta m_{ethanol}} \Delta m_{ethanol})^2 + (\frac{\partial V_{gas}^{Theo}}{\partial \Delta C_{ethanol}} \Delta C_{ethanol})^2}$ (3.11)	$\Delta V_{gra}^{Theo} = \sqrt{(\frac{\partial V_{gra}^{Theo}}{\partial \Delta m_{gas}} \Delta m_{gas})^2 + (\frac{\partial V_{gra}^{Theo}}{\partial \Delta C_{CO2}^{cyl}} \Delta C_{CO2}^{cyl})^2}$ (3.12)
ΔV_{gas}^{Theo}: estimate gas (O_2, CO_2) generation rate standard uncertainty (l/s) $\Delta m_{ethanol}$: standard uncertainty of weighting ethanol lamp (g) $\Delta C_{ethanol}$, : standard uncertainty of ethanol concentration ΔV_{gra}^{Theo} : standard uncertainty of CO_2 injection rate (g/s) Δm_{gas}: standard uncertainty of weighting gas cylinder (g) ΔC_{CO2}^{cyl} : standard uncertainty of CO_2 concentration	$\Delta m_{ethanol}$ or $\Delta m_{gas} = \sqrt{RES^2 + TOL^2}$ $\Delta C_{ethanol}$ or $\Delta C_{CO2}^{cyl} = \frac{PCT \times CV}{\sqrt{3}}$ RES: resolution (g) (one-half the resolution values of the digital indicator) TOL: tolerance of weight used (g) PCT: primary certified tolerance (%) CV: certified value.(100% for both CO_2 cylinder and ethanol concentration)

The calculations of measured gas exchanges using Equation 3.6 to 3.8 contain four error sources, which are incoming air flow rate, ratio of exhaust air flowrate to incoming air flowrate, incoming gas concentration and exhaust gas concentration (Eq. 3.13).

$$\Delta V_i = \sqrt{(\frac{\partial V_i}{\partial Q_{In.STPD}^{Air}} \Delta Q_{In.STPD}^{Air})^2 + (\frac{\partial V_i}{\partial VCF} \Delta VCF)^2 + (\frac{\partial V_i}{\partial C_i^{In}} \Delta C_i^{In})^2 + (\frac{\partial V_i}{\partial C_i^{Ch}} \Delta C_i^{Ch})^2} \quad (3.13)$$

Based on the Equation 3.9, the standard uncertainty of incoming air flow rate under standard condition was shown in Equation 3.14.

$$\Delta Q_{In.STPD}^{Air} = \sqrt{(\frac{\partial Q_{In.STPD}^{Air}}{\partial T_{db}} \Delta T_{db})^2 + (\frac{\partial Q_{In.STPD}^{Air}}{\partial Pa} \Delta P_a)^2 + (\frac{\partial Q_{In.STPD}^{Air}}{\partial V_{In}} \Delta V_{In})^2} \quad (3.14)$$

The ratio of exhaust air flowrate to incoming air flowrate was calculated based on oxygen concentration, carbon dioxide concentration, and incoming air flowrate. Therefore, the standard uncertainty could be estimated as shown in Equation 3.15:

$$\Delta(E/I) = \sqrt{(\frac{\partial(E/I)}{\partial C_{O2}^{In}} \Delta C_{O2}^{In})^2 + (\frac{\partial(E/I)}{\partial C_{CO2}^{In}} \Delta C_{CO2}^{In})^2 + (\frac{\partial(E/I)}{\partial C_{O2}^{Ch}} \Delta C_{O2}^{Ch})^2 + (\frac{\partial(E/I)}{\partial C_{CO2}^{Ch}} \Delta C_{CO2}^{Ch})^2} \quad (3.15)$$

The standard uncertainty of oxygen concentration and carbon dioxide concentration can be determined due to manufacturer’s specification (Maia et al., 2015). Since the gas analyzers used for oxygen and carbon dioxide are different, the standard uncertainty was different (Eq. 3.16).

$$\Delta C_{gas} = \sqrt{\left(\frac{SDPC}{1}\right)^2 + \left(\frac{REPI}{\sqrt{3}}\right)^2 + \left(\frac{PCT \cdot AC}{\sqrt{3}}\right)^2 + \left(\frac{RD}{\sqrt{3}}\right)^2 + \left(\frac{RES}{\sqrt{3}}\right)^2} \quad (3.16)$$

where

ΔC_{gas} = gas concentration combined standard uncertainty (% for O_2, ppm$_v$ for CO_2).

SDPC = standard deviation (*n* = 13) of post-calibration repeatability (±1%, % for O_2, ppm$_v$ for CO_2).

REPI = instrument repeatability from manufacturer (±1%, full scale, % for O_2, ppm$_v$ for CO_2).

PCT = primary certified tolerance (±2% of AC, % for O_2, ±1% ppm$_v$ for CO_2).

AC = actual concentration from manufacturer of primary certified tank (20.85% for O_2, 499.9 ppm$_v$ for CO_2).

RD = range drifts for measured gas concentration (±1% for 24 hours O_2, ±2.5% ppm$_v$ for three month CO_2).

RES = instrument resolution (0.1% for full scale O_2, 2 ppm$_v$ for CO_2).

3.3.4.3 Correction factor of ventilation and E/I

Constant gas injection method was designed to check the systematic error from ventilation rate measurement. By assuming the mass measured equals to the theoretical mass injected, the correction factor for ventilation was derived (Eq. 3.17). The data from the first five trials were applied to deduce the correction factors. The data from last three trials of each methods and each chamber were be used for checking the results after the correction.

$$K_{vent} = \frac{60 \times 22.414 \times V_{inj} \cdot C_{inj} \cdot P_{std}}{R \cdot T_{std} \cdot Q_{In.STPD}^{Air} (C_{SF6}^{Ch} - C_{SF6}^{In})} \quad (3.17)$$

where

K_{vent} = ventilation correction factors applied when recovery percentages of CGIM and GRAV is not within 100% ± 5%.

In addition, gravimetric gas injection method was applied to check the influence of CO_2 measurement to the ratio of exhaust air flowrate to incoming air flowrate. Alcohol combustion method was designed to check the influence of O_2 measurement to the ratio of exhaust air flowrate to incoming air flowrate. Based on the results, the correction factor for the ratio of exhaust air flowrate to incoming air flowrate was specified using oxygen balance in ACM (Eq. 3.18). The data from first five trials were applied to deduce the correction factors. The data from last three trials will be used for checking the results after the correction.

$$K_{E/I} = \frac{C^{In}_{O2}}{(E/I)\times C^{Ch}_{O2}} - \frac{\Delta m_{ethanol} \times C_{ethanol} \times 3 \times 22.414}{60 \times 46.0694 \times Q^{Air}_{In.STPD} \times (E/I) \times C^{Ch}_{O2}} \tag{3.18}$$

where

$K_{E/I}$ = correction factors in the ratio of exhaust air flowrate to incoming air flowrate applied when recovery percentages of respiratory quotient of ACM is not within 100% ± 5%.

3.4 Results and Discussion

Each of three recovery test methods may be evaluated for their advantages and disadvantages (Table 3.4). Alcohol combustion method can mimic ruminant respiratory quotient and simulate both gas production and gas consumption processes. However, this approach cannot reflect the influence of ethanol purity and evaporation when calculating ethanol mass consumption during the burning process. Gravimetric gas injection method uses a scale to measure the weight of a gas cylinder and a simple controller to keep a stable gas flow, but it needs more time or higher concentrated gas to accumulate enough weight change for measurement. Constant gas injection method does not require a long experimental time, but mass flow controller is more expensive because of the requirement of high accuracy. The tracer gas method needs to select a gas, with better results for inert gas (SF_6). This gas is desirable for the test due to no background influence and detectable at low concentration. Otherwise, the gas of interests, such as CH_4 and CO_2, can be applied since measuring them does not require additional gas analyzer.

Table 3.4. Comparisons of advantages and disadvantages for ACM, GRAV and CGIM.

Method	Gas	Advantages	Disadvantages	Reference
Alcohol combustion method	O_2, CO_2	Mimics ruminant respiratory quotient; Similar chemical exchange.	>100% recovery result for airflow resistance; Sensitive to impurity; Burning changes psychometric properties in chambers.	(Brown-Brandl et al., 2014; Cooper et al., 1991)
Gravimetric gas injection method	O_2, CO_2, N_2	Give stable gas output; Do not need regular calibration of instrument.	Accuracy of balance, Long duration measurement.	(Cooper et al., 1991; A. L. Hellwing et al., 2012)
Constant gas injection method	CH_4, SF_6	Low concentration gas; Short-term measurement; Less experiment operation.	Require accurate flow controller.	(Muñoz, Yan, Wills, Murray, & Gordon, 2012; Murray et al., 1999)

Uncertainty and reproducibility analysis evaluated equipment and operating procedures in the recovery tests (Table 3.5). The different equipment used for estimating theoretical gas exchanges introduced different uncertainty sources. Based on the instrument specifications and calibration reports, gravimetric gas injection method had the greatest uncertainty due to the precision of the scale for weighing gas cylinder, which contributes about 99% of the uncertainty. The compressed gas cylinder (4kg) is made of metal, which is relatively heavy compared to the gas. Although the cylinder is specifically selected to a reasonably affordable scale with better resolution (readability=0.5g), the relative portions of weight change is small compared to the total weight of cylinder. High accuracy (1% full-scale) mass flow controller of CGIM has the lower uncertainty, but it still contributes 93.2% to uncertainty. Although alcohol combustion method has least uncertainty in the gas production or consumption estimation, the weight measurement of ethanol still contributes about 93.7% of uncertainty.

The reproducibility is another indicator to evaluate the methods. Good reproducibility means that the temporal and spatial impacts are less, and the single measurement has better representativeness to the real value. As shown in Table 3.5, the recovery percentages of RQ in ACM have the greatest standard deviation. Constant gas injection method always has the least standard deviation. The expected measurement variability based on equipment is lowest for ACM but the actual variability had the greatest span. On the one hand, the experiment procedures of ACM and GRAV involve more activities than CGIM like recording weight and time. On the other hand, the activities, including opening doors, human movement, and respiration, will cause the fluctuation of incoming air ingredients. Using gases, like oxygen and nitrogen who have a high concentration in ambient air, were affected more seriously than the gas who have a low concentration in ambient air, like carbon dioxide or methane. The ambient air O_2

concentrations varied from 20.41% to 20.9% and CO_2 concentrations varied from 497 ppm_v to 606.9 ppm_v during 144 trials for all six chambers. Therefore, minimizing the activities in the space might improve the reproducibility and stabilize the ambient air during the experiment.

Table 3.5. Summary of uncertainty and reproducibility analysis for ACM, GRAV and CGIM.

Method	Uncertainty %[a]	Uncertainty in theoretical side		The range of SD from six chambers
		Uncertainty Sources	Relative Contribution%	
Alcohol combustion method	0.11	Weight measurement	93.7	RPs of RQ: (2, 4.32)
		Ethanol concentration	6.3	
Gravimetric gas injection method	17.6	Weight measurement	99.99	RPs of CO_2: (0.92, 2.92)
		Injection gas concentration	0.01	
Constant gas injection method	0.65	Injection flowrate	93.2	RPs of SF_6: (0.17, 1.98)
		Injection gas concentration	6.8	

[a] Mean uncertainty percentage of the theoretical gas exchange (production or consumption)
SD: Standard deviation of eight replicated trails for each chamber.

Alcohol combustion method can best simulate the respiration process by consuming O_2 and generating CO_2. As shown in Figure 3.3, O_2 recovered was consistently overestimated, and to a great extent than CO_2 recovered. Recovery percentages of RQ varied from 72% to 77% for six chambers. The uncertainty analysis was applied to the four variables in Equation 3.5 and 3.6. In the calculation of O_2 consumption, the major uncertainty was from gas concentration measurement (94%). The calculation of the ratio of exhaust air flowrate to incoming air flowrate contributed 6% to O_2 consumption. In the calculation of CO_2 production, gas concentration measurement contributed about 71%, ventilation measurement contributed about 21% and the ratio of exhaust air flowrate to incoming air flowrate contributed about 8%. In this REMS study, CO_2 concentration measurement has a standard uncertainty of about 71 ppm_v, and the standard uncertainty of O_2 concentration measurement is 3000ppm_v. The precision of gas analyzer could be one possible reason that made the RQs underestimated. In addition, the RPs of both gases were higher than 100%. The mathematical equations used in gas recovered terms involves one assumption in ventilation calculation. In one study, a 1% error in the ventilation estimation resulted in over 21% error in O_2 consumption calculation (Arch et al., 2006). Therefore, another possible systematic error might come from the ventilation measurement or the ratio of exhaust air flowrate to incoming air flowrate.

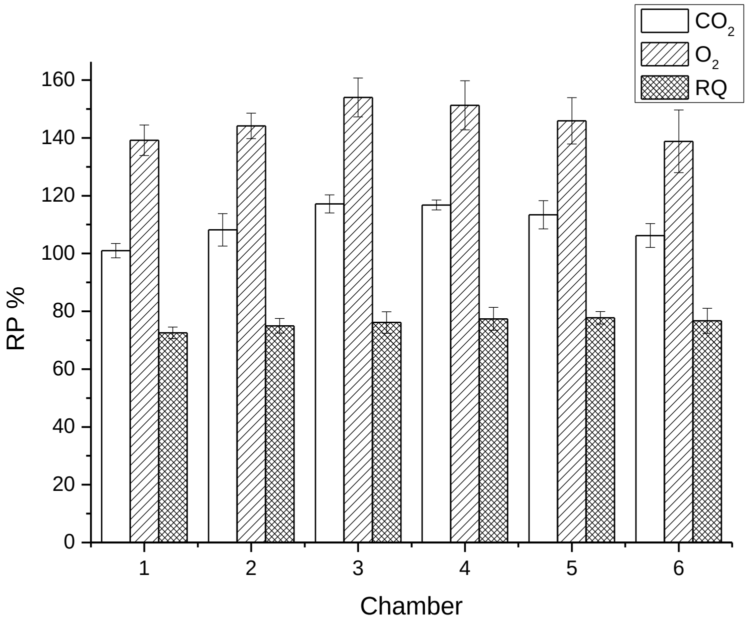


Figure 3.3. Mean with standard deviation of Recovery percentages (RP) for oxygen, carbon dioxide, and respiratory quotients (RQ) in ACM.

Constant injection method and gravimetric method were applied to identify error source. As shown in Table 3.3, constant gas injection method injected a small amount of SF_6 into the chamber that had negligible effects on the chamber gas composition. Therefore, the assumption, exhaust air flowrate equals to incoming air flowrate, was made to identify the effect of incoming ventilation. Gravimetric gas injection method consisted of CO_2 injection into the chamber. In this method, O_2 concentrations were assumed equal between incoming air and chamber air for studying the influence of CO_2 measurement.

Table 3.6. Mathematical assumptions in CGIM and GRAV for indentifying the error sources.

Method	Gas injected	Gas concentraion in chamber	Gas concentraion in barn	Assumption	Purpose
Constant gas injection method	SF_6, 4000 ppm_v	38.5ppm_v	0.128ppm_v	Air composition doesn't change during the experiment (VCF=1)	Identify the effect of incoming ventilation
Gravimetric gas injection method	CO_2, 99.99%	5800ppm_v	517ppm_v	CO_2 concentration change doesn't affect O_2 concentration change (VCF: $C_{O2}^{Ch} = C_{O2}^{In}$)	Identify the effect of CO_2 measurement

As shown in Figure 3.4, RPs of two gases in two methods had similar responses for the same chamber, which indicated the uncertainty of CO_2 measurement has less impact on the calculation of the ratio of exhaust air flowrate to incoming air flowrate. Eight replicated trials for chamber 2, 4, 5 and 6 showed the gas recovered was within 100% ± 5%. However, as for chamber 1 and chamber 3, gas recovered was lower than 95% for all replicated trials in both methods. Since the CGIM excluded the variation of the ratio of exhaust air flowrate to incoming air flowrate, the ventilation measurement of these two chambers needed to be corrected with an adjustment.

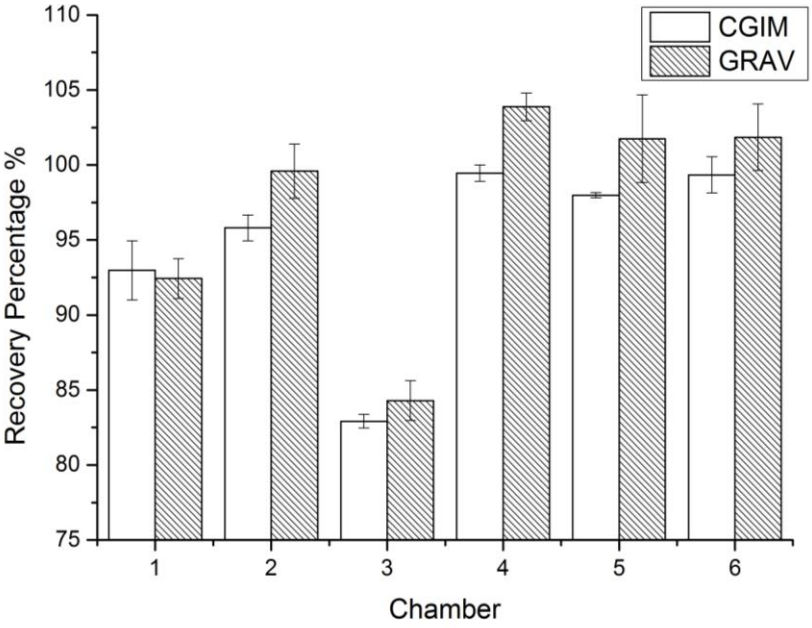


Figure 3.4. Mean with standard deviation of recovery percentage for the tracer gases in CGIM and RRAV under different assumptions.

The ratio of exhaust air flowrate to incoming air flowrate were corrected through reverse calculation of O_2 consumption equation (Eq. 3.18). As shown in Table 3.7, the correction factors for E/I approximately equaled to 1.01. Student's T-test showed these values for all six chambers are significant different than 1 ($P<0.001$), which claimed about 1% error in E/I calculation. Without any corrections, the exhaust air flowrate was lower than incoming air flowrate. But the exhaust air flowrate was adjusted higher than incoming air flowrate after applying these to the calculation. This systemic error was possibly caused by some unexpected and unmeasured ventilation.

Table 3.7. Summary of E/I correction factors for each chamber and each trial.

Chamber	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	Average
1	1.012	1.012	1.013	1.009	1.013	1.012
2	1.013	1.012	1.014	1.015	1.013	1.013
3	1.016	1.015	1.015	1.012	1.015	1.015
4	1.020	1.012	1.012	1.011	1.016	1.014
5	1.013	1.011	1.017	1.020	1.012	1.015
6	1.013	1.012	1.011	1.016	1.019	1.014

The measurement system results were corrected after the problems were identified. The calculated E/I correction factors were applied for all six chambers. The ventilation correction factors were applied to chamber 1 and chamber 3. As shown in Table 3.8, average RPs of all three methods were corrected within the range of 100% ± 5%. Considering the improvement of the RPs of RQ, this result also claimed the estimation of E/I is a sensitive factor in the gas exchange measurement (1% errors in E/I leaded to over 20% errors in gas measurement).

Table 3.8. Summary of average RQs (n=3) before and after applying correction factors to each chamber.

Chamber		Without correction （%）			With correction (%)			Ventilation correction factor	E/I correction factor
		RQ	CO_2	SF_6	RQ	CO_2	SF_6		
1	RP	73.24	91.6	93.3	97.99	99.98	101.57	1.08	1.01
2	RP	75.42	99.75	95.7	100.12	100.87	96.42	1	
3	RP	79.19	85.23	82.96	98.85	104.19	101.20	1.21	
4	RP	79.19	103.85	99.56	99.40	104.12	100.33	1	
5	RP	78.07	100.37	97.84	99.3	101.43	98.57	1	
6	RP	78.42	101.40	99.59	98.84	102.55	100.34	1	

3.5 Conclusion

Recovery tests can evaluate the system integrity without testing the performance of each component individually. Alcohol combustion method can mimic both gas consumption and production process of animals but has low reproducibility. Constant gas injection method has higher reproducibility and lower operation difficulty but cost more comparing with ACM and GRAV. Since the simulation of animal gas exchanges does not only focus on the measurement system integrity but also checking the temporal and spatial variations during the test, applying ACM first and followed by a tracer gas tests are recommended for a rigorous evaluation. Alcohol combustion method can generally check and expose the errors but cannot help operators to identify the error sources. The selection of tracer gas and concentration can help identify the error sources. The change of air ingredients is negligible when injecting lower concentration and inert gas, which focus the error from ventilation measurement. Otherwise, selecting the gas of interest with high concentration can help with determining the error from gas measurement devices.

This paper also discussed the approach to decrease the systematic errors. Different recovery method can identify various problems in measurement. The results from different recovery test method can be used for correcting the systematic errors. In the REMS study, constant gas injection method identified the ventilation error of the first and third chamber. Alcohol combustion method and gravimetric gas injection method were compared to exclude the error of CO_2 measurement and isolate the error from O_2 measurement in the calculation of the ratio between exhaust air flowrate and incoming air flowrate. Through a series of calculation, the RQs of ACM were improved to the desirable level ($\pm$ 5%).

Similar recovery tests could apply to any gas exchange measurement systems. Through selecting appropriate methods, operators can periodically check their system's integrity and identify the errors without checking each component individually. The systematic errors can be corrected following the methods described in this paper.

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CHAPTER 4: HEAT AND MOISTURE PRODUCTION OF BEEF CATTLE BASED ON ACCLIMATION PERIOD AND MODERN GENETICS

4.1 Abstract

Heat and moisture production can inform decisions for farm environmental controls and feed utilizations. Indirect calorimetry was applied to cattle using open-circuit chamber technology to explore. Two main factors that might cause a bias to heat and moisture production measurement results: acclimation and genotype. Previous studies reported different acclimation periods varying from 2 to 17 days, demonstrating the need to document an approach for determining acclimation period for a system before implementing it in a study. Based upon behavior and metabolism indicators, a two-day acclimation period was adequate for mature steers in the research mature steers in the chamber on the Beef Cattle and Sheep Field Laboratory at University of Illinois, Urbana-Champaign. Current research showed the beef cattle had a bias in feed intake higher than CIGR models. The heat and moisture production models were published at 1980s using feed intakes in the equation. The measured heat and moisture production were also compared with CIGR models for heat and moisture production predictions to assess representativeness. The average total heat production of mature steers (1.38 W/kg) and sensible heat production (1.20 W/kg) for mature steers were higher than the prediction results from CIGR models. The steers used in the experiments were not at the same stages as animals in CIGR models and were likely genetically quite different (fattening and breeding bulls), and the results support the idea that the existing models are an insufficient representation of heat and moisture production for modern beef cattle.

4.2 Introduction

Heat production (HP) is an important measure in animal metabolism research. It is a byproduct of metabolic activity as energy not capitalized in animal production (tissue gain, milk production, or egg production) (Nienaber et al., 2009). Therefore, measuring HP helps to understand how much energy is utilized from the feed, and it can indicate animal physiological status with regard to their environment. As a result, heat production can inform a decision about environmental controls: such as selecting fans and heaters, calculating heat gain and heat loss, establishing energy balance between inside and outside, etc (Albright, 1990).

Indirect calorimetry is a classic method to determine HP through measuring the by-product exchange rates. The feed metabolism (protein, carbohydrates, lipid, etc.) is a series of chemical reactions (McLean & Tobin, 1987). The oxidation and fermentation reactions of these materials consume oxygen and; generate carbon dioxide, urea nitrogen, and methane (Armsby, 1913). As shown in Equation 4.1, this process was well developed and modeled with a predictive equation (Brouwer, 1965). The total heat production (THP) can be indirectly determined by measuring the gas exchanges and urea nitrogen extraction.

$$\mathrm{THP} = 16.18\mathrm{O_2} + 5.02\mathrm{CO_2} - 2.17\mathrm{CH_4} - 5.99\mathrm{N} \tag{4.1}$$

Where

THP = total heat production rate (W).
O_2 = oxygen consumption rate (ml/s, STPD).
CO_2 = carbon dioxide production rate (ml/s, STPD).
CH_4 = methane production rate (ml/s, STPD).
N = nitrogen excretion rate (g/s).

This equation can be simplified by assuming some terms in constant values (McLean, 1972). The relationship between carbon dioxide production and oxygen consumption may be expressed as a respiratory quotient (RQ). Since the oxidation process of different materials will lead to different RQ values, analyzing RQ can describe the actually consumed materials of an animal (Brown-Brandl et al., 2003). If feed consumed is close to the maintenance level, the RQ will be low (Søren Pedersen et al., 2008). Methane comes from the microbial fermentation in the rumen, which accounts for 8-12% of digestible energy loss of cattle (Jentsch et al., 2007). The combined carbon dioxide and methane term contribute about 1.21% to HP in the Brouwer Equation (McLean, 1986). Urinary nitrogen is the product of protein oxidation process. It is determined by the chemical composition of the diet and varies from 13.7 g/day to 201.3 g/day for cattle (Dong et al., 2014). In the Brouwer equation, the urinary nitrogen may be estimated to be 0.032 (SD:0.01) g/l oxygen consumed (McLean, 1972).

Confirming the representativeness of heat and moisture production data is necessary for the confidence in measurements during a study. Previous studies have evaluated animal heat production using indirect calorimetry, including the effect of temperature, diets, floor types, body mass, diurnal variation, etc. (S Pedersen & Sällvik, 2002). Any change to the husbandry results in a challenge to homeostasis and requires a period to return to the baseline condition (Elton, 2001). Acclimation period has varied in previous indirect calorimetry work with cattle from 2 days to 17 days for different animals and measurement systems (Brown-Brandl et al., 2003; Brown-Brandl et al., 2005; Brown-Brandl et al., 2014; Webster et al., 1976). In order to measure animal HP data under the most representative conditions, it is essential to investigate whether the HP data were influenced by the environmental change of introduction to the metabolism chambers and how much time should be given for acclimation (Søren Pedersen et al., 2015).

Heat production models may be applied as a general criterion for engineering design and enterprise evaluation (National Research, 2012). These models were formulated to estimate heat production related with body weight, diet intake or daily gain (Johnson et al., 2012). Many factors influence HP such as genotype, diet, and ambient temperature (S Pedersen & Sällvik, 2002). The HP data of swine and chickens were collected 20 to 50 years ago, and recently shown an insufficient representation for the current animals (Chepete & Xin, 2004). Heat production equations used for cattle are based on data from the 1980s (Søren Pedersen et al., 2008). For the model of fattening steers, the range of daily gain is from 0.7 to 1.2 kg/day. Current studies already indicated the daily gain of finishing steers were over 1.6 kg/day (McGee et al., 2014). Therefore, it is necessary to evaluate if HP data of cattle is representative for modern genotypes and new environments.

The goals of this research is to evaluate the HP data during the acclimation period and for modern cattle. It includes investigating the response of metabolism during the acclimation period and testing the length of acclimation period needed. Moreover, the heat production data measured by indirect calorimetry method compared with the existing CIGR model to test the representativeness of HP from CIGR model. The calculation involved in this chapter were processed into MATLAB and described in Appendix C.

Objectives:

A. To evaluate animal behavior and metabolic indicators and to determine the acclimation period in a respiration chamber.
B. To evaluate the HP of modern beef cattle and assess the representativeness of current HP models.

4.3 Materials and Methods

4.3.1 Laboratory setup

The Ruminant Emission Measurement System (REMS) was applied to measure gas exchanges in the animal metabolism laboratory located in the Beef Cattle and Sheep Field Laboratory at the University of Illinois, Urbana-Champaign (Maia et al., 2015). This system is a positive pressure open-circuit respiratory system that secures cattle at the shoulder and captures eructation and respiration gas exchanges of beef cattle. The system was validated before and after experiments, using alcohol combustion test and constant gas injection method following the procedures described in chapter three. The experiments of animals were approved by the Institutional Animal Care and Use Committee (IACUC) under Protocol No. 11214 at University of Illinois, Urbana-Champaign.

4.3.2 Equipment

The cattle metabolism laboratory consists of six open stalls and six open-circuit chambers as shown in Figure 4.1. The gas measurement devices are a paramagnetic oxygen analyzer (600P, California Analytical Instrument Inc) and a photoacoustic gas monitor (INNOVA 1412, LumaSense Technologies Inc), which measure the gas concentrations including oxygen (O_2), carbon dioxide (CO_2), ammonia (NH_3), nitrous oxide (N_2O), methane (CH_4), sulfur hexafluoride (SF_6) and water vapor.

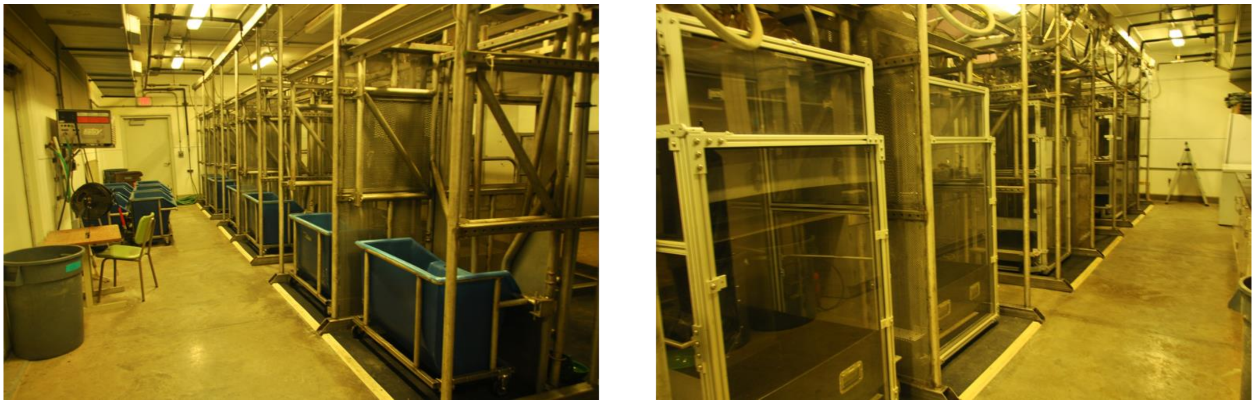

Figure 4.1. The animal metabolism laboratory, including six open stalls (left) and six chambers (right).

4.3.3 Animal husbandry

Three two-year-old crossbred steers (Angus x Simmental) with the body weight (BW) of 797.65 ± 74.65kg were moved to the stalls and given eight days of adaptation period to the laboratory environment before experiments. Feed contained corn silage, grass haylage, and ground corn supplement. Fresh water were provided ad libitum. The refused feed samples for each animal in each day were collected and weighed, then put into an oven at 105°C about 30 hours and weighed again to determine moisture content. The feedlot ration was fixed (DM: 10.89 kg, gross energy: 4.312kcal/g) during all 13 days of acclimation and testing. Animal water consumption was monitored every second using pulse flow meter (Turbotron series, Sika Corporation, Inc) and paired in data logger (Pulse101A, Omega Engineering, Inc). The pulses of water flow in the flowmeter were converted to the volumetric water flowrate.

4.3.4 Experiment procedures

Timeline: Three animals were randomly assigned to the REMS chambers for five days of measurement. On the first day of measurement, animals were moved into chamber at 8:45am. The measurement started at 9:00 am each day. After 23 hours (approx. 8:00 am) in each day, the system was shut down for an hour for maintenance including replacing filters, cleaning front door of each chamber, collecting refused feed, adding fresh feed, calibrating the oxygen analyzer and downloading the water consumption data logger.

Gas exchange measurement: The data from each instrument, including gas concentrations, temperatures, relative humidity and incoming air supplies, were measured and recorded through a LabView program, and subsequently processed for calculations by MATLAB R2014a. Data collection sampling was repeated and consisted of background, followed by chamber one to chamber four. At each sampling cycle, 10 samples were collected from the system for each background and chamber. The last five samples of each 10 samples were averaged and saved for that sampling cycle.

Animal behavior monitoring: Two cameras constantly recorded animal behaviors during the last day in stalls and all five days in chambers. Video was analyzed for the activity at the start of each minute. Animal behaviors were classified into four categories: eating, drinking, standing (excluding eating and drinking) and lying (excluding eating and drinking). The time spent on each behavior was manually summarized for each animal and each day.

4.3.5 Data analysis

Some heat production data and animal behavior data were lost when the steers pulled out of their chamber (11 h for No.682 at 7th June, 12h for No.682 and 8 h for No.737 at 11th June). The missing gas exchange data were omitted and daily metabolism indicators were normalized for 24 hours from the remaining data.

Paired t-tests were applied to check the difference between two consecutive days of metabolic indicators, including feed intake, water consumption, heat production, moisture production, methane emission and respiratory quotient. Differences considered significant at α =0.05 indicate the behavior change or metabolism alteration.

4.3.6 Calculation equations of metabolic indicators

There are four parts in Brouwer-Equation: oxygen consumption, carbon dioxide production, methane production, and nitrogen excretion. In this study, the nitrogen excretion is estimated by 0.032 (SD:0.01) g/l oxygen consumed (McLean, 1972). Therefore, the total heat production was calculated following Equation 4.2.

$$\mathrm{THP} = 16.18\mathrm{O_2} + 5.02\mathrm{CO_2} - 2.17\mathrm{CH_4} \tag{4.2}$$

The gas exchange rates are determined based on the nitrogen balance (Eq. 4.2 to 4.5). It is to establish the relationship between inlet airflow and exhaust airflow (Eq. 4.6).

$$O_2 = Q_{In.STPD}^{Air}(C_{O2}^{In} - (E/I) \times C_{O2}^{Ch}) \tag{4.3}$$

$$CO_2 = Q_{In.STPD}^{Air}((E/I) \times C_{CO2}^{Ch} - C_{CO2}^{In}) \tag{4.4}$$

$$CH_4 = Q_{In.STPD}^{Air}((E/I) \times C_{CH4}^{Ch} - C_{CH4}^{In}) \tag{4.5}$$

$$E/I = \frac{Q_{Ex.STPD}^{Air}}{Q_{In.STPD}^{Air}} = \frac{1 - (C_{CO2}^{In} + C_{O2}^{In} + C_{CH4}^{In}) \times 10^{-6}}{1 - (C_{CO2}^{Ch} + C_{O2}^{Ch} + C_{CH4}^{Ch}) \times 10^{-6}} \tag{4.6}$$

where

C_{CO2}^{In}, C_{O2}^{In}, C_{CH4}^{In} = carbon dioxide, oxygen, methane gas concentration at incoming air, respectively (ppm_v).
C_{CO2}^{Ch}, C_{O2}^{Ch}, C_{CH4}^{Ch} = carbon dioxide, oxygen, methane gas concentration of chamber air, respectively (ppm_v).
$Q_{In.STPD}^{Air}$, $Q_{Ex.STPD}^{Air}$ = incoming/exhaust air flowrate at dry basis (273.15 K and 101325 Pa) ($ml^3\ s^{-1}$).
E/I = ratio if exhaust air flow rate to incoming air flowrate.

Respiratory quotient (RQ) is another important indicator of animal energetics (Eq. 4.7).

$$RQ = \frac{CO_2}{O_2} \tag{4.7}$$

The measured ventilation rate was converted into flowrate at dry basis under standard temperature and pressure (Eq. 4.8). The moisture production can be calculated through psychrometric properties (Eq. 4.9), which was derived from the dry-bulb temperature, relative humidity, and barometric pressure (Albright, 1990). Sensible heat production is calculated by taking the difference between total heat production and latent heat production (Eq. 4.10).

$$Q_{In.STPD}^{Air} = V_{In} \times (P_a - P_w)[\frac{273.16}{101325 \times (T_{db} + 273.16)}] \tag{4.8}$$

$$MP = \frac{V_{In}\rho(KW_o - W_i)}{1000} \tag{4.9}$$

$$SHP = HP - MP \times h_{fg} \tag{4.10}$$

where

MP = moisture production rate (g/s).
SHP = sensible heat production rate (w/s).
P_w = vapor pressure of moisture in the air (Pa).
T_{db} = dry-bulb temperature (°C).
P_a = barometric pressure (Pa).
V_{In} = incoming gas volumetric flow rate ($ml^3\ s^{-1}$).
h_{fg} = the latent heat of water vaporization (2406 J/g at 40 °C).
ρ = incoming moisture air density ($g_{dry.air}/L_{moisture\ air}$).
W_o, W_i = humidity ratio of the outlet and inlet air (g H_2O/g dry air).

The accumulative heat production and moisture production of each animal were calculated by the integration of heat production and moisture production over the time, which was shown in Equation 4.11 and 4.12.

$$\mathrm{AHP} = \sum_{i=1}^{n-1}(t_{i+1} - t_i)(\mathrm{THP}_i + \mathrm{THP}_{i+1}) \tag{4.11}$$

$$\mathrm{AMP} = \sum_{i=1}^{n-1}(t_{i+1} - t_i)(\mathrm{MP}_i + \mathrm{MP}_{i+1}) \tag{4.12}$$

where

AHP = accumulative metabolic heat production over a period of time (J).
AMP = accumulative moisture production over a period of time (g).
n = number of measurements.
i = elapsed time (s).

Another method to calculate heat production of cattle is based on the body mass, daily gain, and energy intake. These equations were presented at CIGR handbook (S Pedersen & Sällvik, 2002). CIGR summarized animal heat and moisture production from different research centers and predicted these values through mathematical equations. Since there is no direct model specific to mature steers, the properties of experimental animals were applied to the heat production equations of fattening cattle and breeding bulls at 20 °C ambient environment, respectively (Eq. 4.13 and 4.14). The measured HP through indirect calorimetry will compare with the results from these equations.

$$\mathrm{HP} = 7.64m^{0.69} + G[\tfrac{23}{M} - 1][\tfrac{57.27 + 0.302m}{1 - 0.171G}] \tag{4.13}$$

$$\mathrm{HP} = 6m^{0.75} \tag{4.14}$$

Where

m = body mass of the animal (kg).
M = energy content of feed (MJ/kg.dry).
G = number of measurements.
i = daily gain (0.7-1.1 kg/day).

4.4 Results and Discussion

Behaviors and postures

Cattle behaviors changed measurably (Figure 4.2) on the first day after moving to chambers. Feed and water intake were not different (P= 0.169 and P=0.556, respectively), though the sample size was small and may not have been sufficient to detect a difference if it existed. Time spent on eating (P=0.009) and lying (P=0.028) of steers before and after being moved into the chamber were decreasing but was increasing for standing (P=0.046). Alteration of animal behaviors did not influence the feed and water consumption, but it did affect the time budget of activities.

The timing and quantities of meals might impact digestion processes and should be considered while planning studies. Steers adapted to the new environment, and the time spent on activities were stable after the first day. The time spent eating and lying showed a trend of increasing from the first to the second day in the chamber. Water consumption was higher the first day than the baseline (P=0.026), but no difference between the baseline day and the second day, indicating that the steers may have been playing with the water nozzle on the first day but less so after the novelty lessened.

Compared with other two steers, the time spent on each activity of No. 166 recovered to the baseline level after the second day. It has the lower weight than other two (BW: 723 kg versus 872.3kg and 855.9kg). It is possible that the activities of smaller steer was less restricted by chambers. At the last day of the experiment, the two big steers escaped from the chambers and were reluctant and difficult to re-secure. The size limitation of cattle might be a factor and should be considered in the measurement planning.

Metabolism indicators

Figure 4.2 also summarized the indicators of metabolism during the experiment. The differences of heat production, moisture production, methane emission and ammonia emission were not statistically significant during the gas exchange measurement period. Because the baseline was not quantified, it cannot be determined if these measures were not altered or if they needed longer than the period tested to return to baseline conditions. Since daily feed intake also did not vary greatly during the experiment, the behavior alteration did not have an influence on daily heat production. Respiratory quotient was different between the second day and the third day (means: 0.778 versus 0.871, P=0.032). Higher RQ means higher energy intake, which can be interpreted as steers digested more energy than the maintenance level (Søren Pedersen et al., 2008). Considering the behavior alteration at the first day were shown a difference, respiratory quotient might be more sensitive and could indicate metabolism recovery.

Animal heat production is not a constant value during a day and varies with the animal behavior and postures. Figure 4.3 to 4.5 summarize the heat production and time spent on the activities (eating, standing, lying) during the gas sampling interval (34 mins) for each individual steer. Heat production increased in the morning and decreased in the afternoon periodically. Steers spent most time on eating and drinking in the morning, but less time in the afternoon and night. This response is similar to the fluctuation of heat production. Therefore, a comprehensive heat production data needs a full-day measurement to take account of diurnal variation. In addition, the pattern (time and duration) of eating and drinking behavior are different for each animal between the first two days and last three days. During the last three days, the tendency of heat production was also shown a similar trend pattern in the last three days. Therefore, two-days acclimation period was reasonable for the mature steers in this study based on analysis of animal behaviors and hourly metabolic changes.

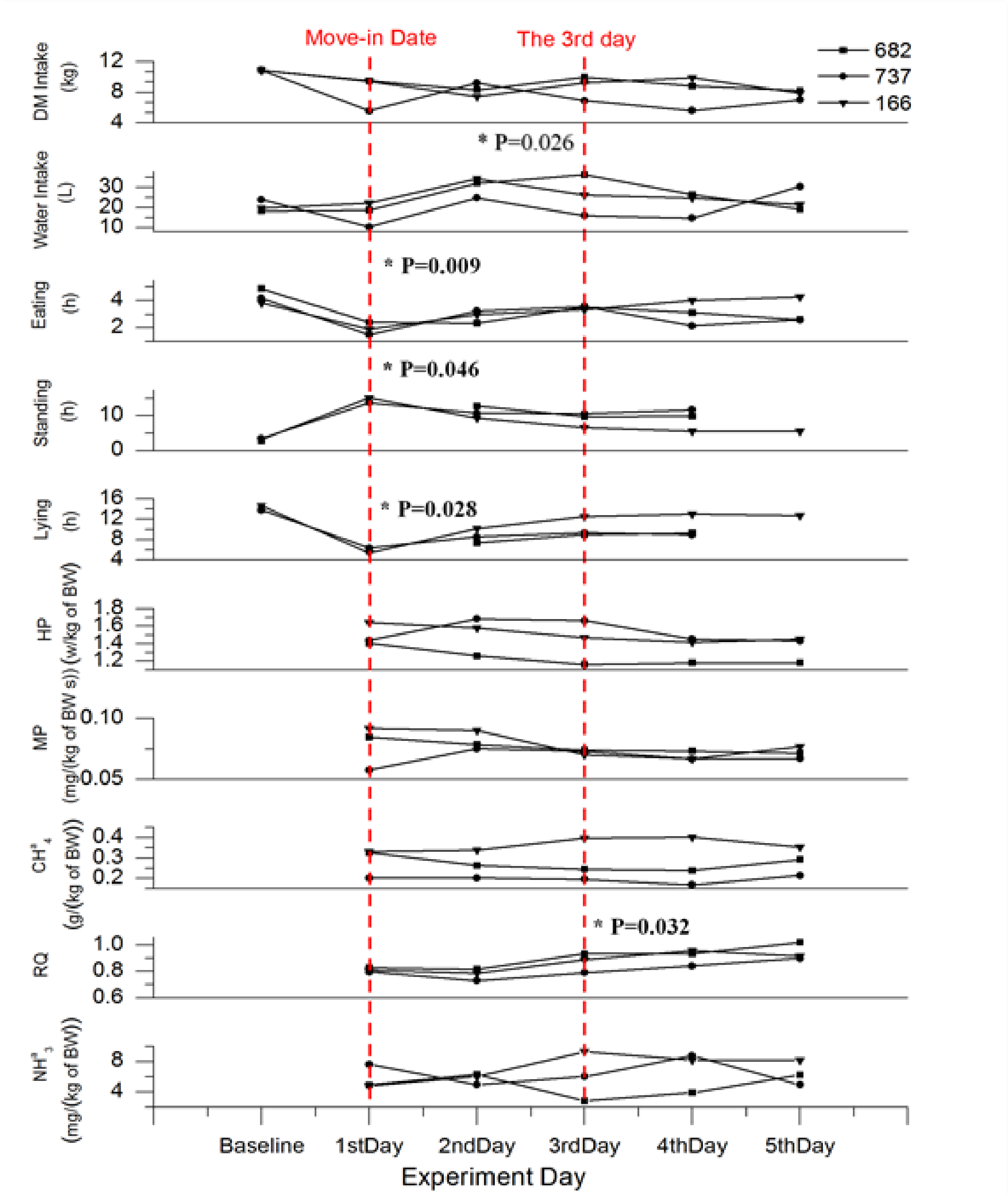

Figure 4.2. The summary of feed intake (DM: dry matter), water intake, the time spent in different activities and postures, and metabolism indicators for three steers (NO.682:872.3kg, NO.737:855.9kg, NO.166:723 kg) during the experiments. (1 day in stalls, 5 days in chambers). (*Denotes differences from previous day (α = 0.05); ^{a} Denote the daily gas emission)

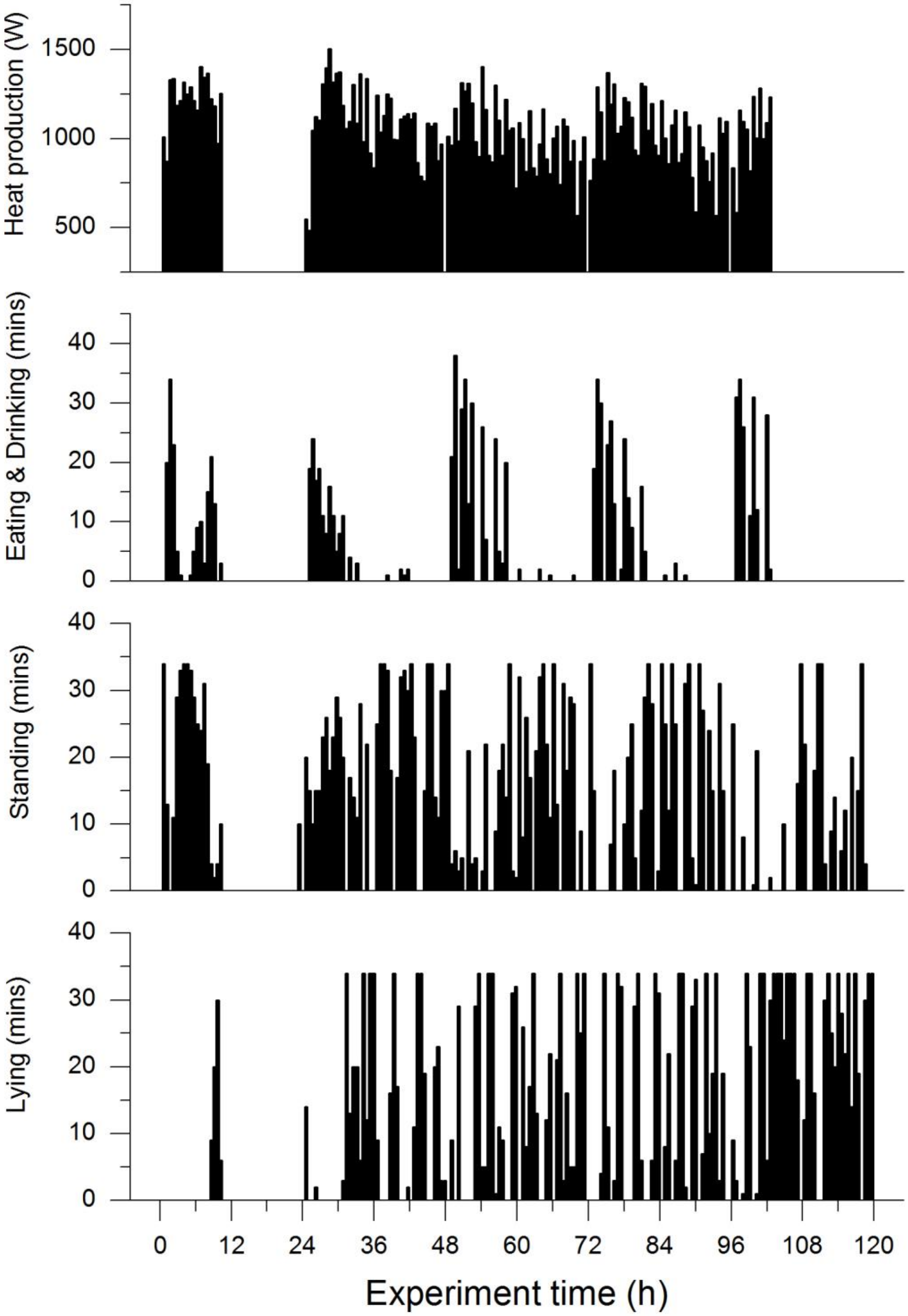

Figure 4.3. Summary of heat production and animal behaviors in 5 days for No.682 cattle (sampling intervals is 34 mins).

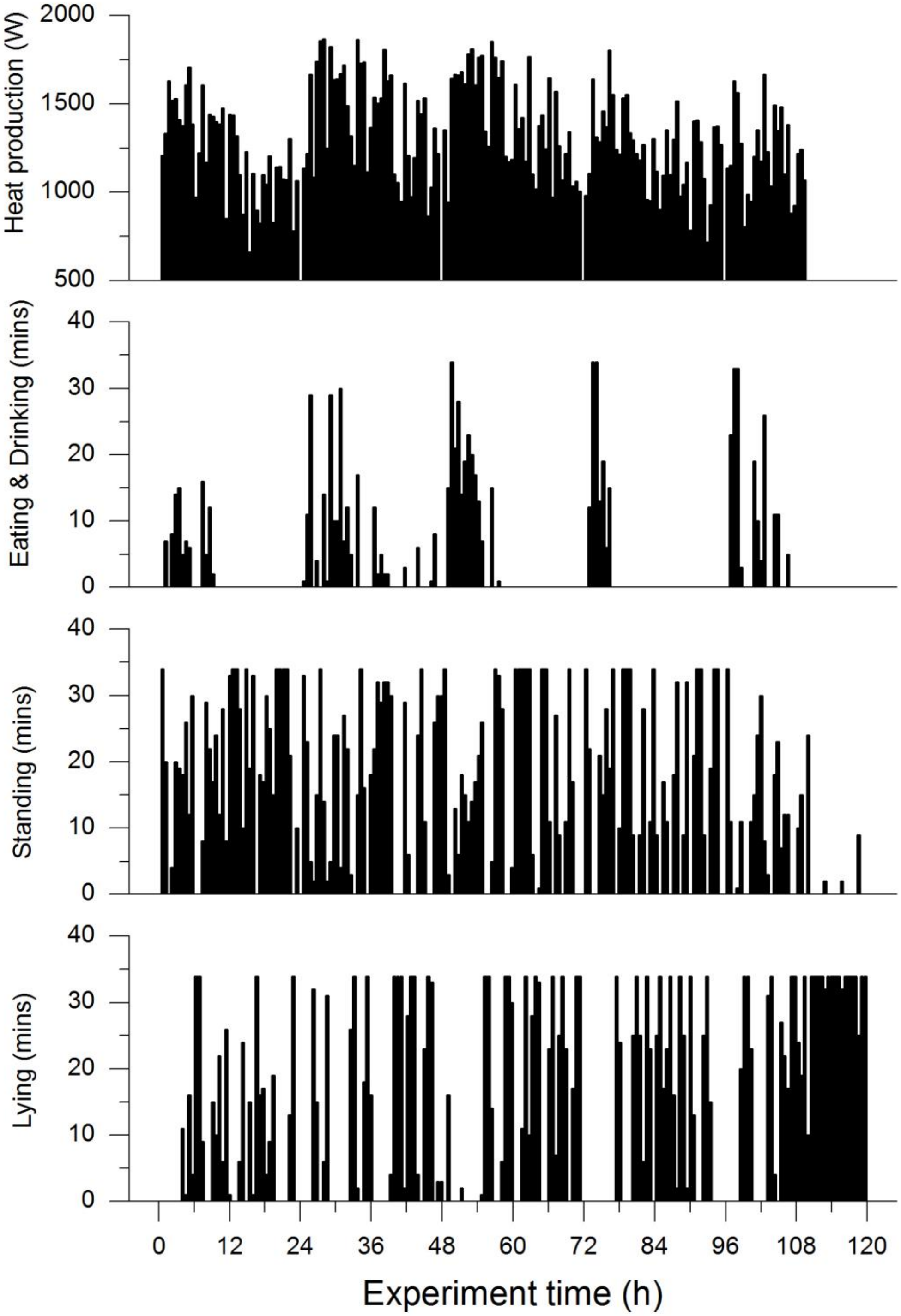

Figure 4.4. Summary of heat production and animal behaviors in 5 days for No.737 cattle (sampling intervals is 34 mins).

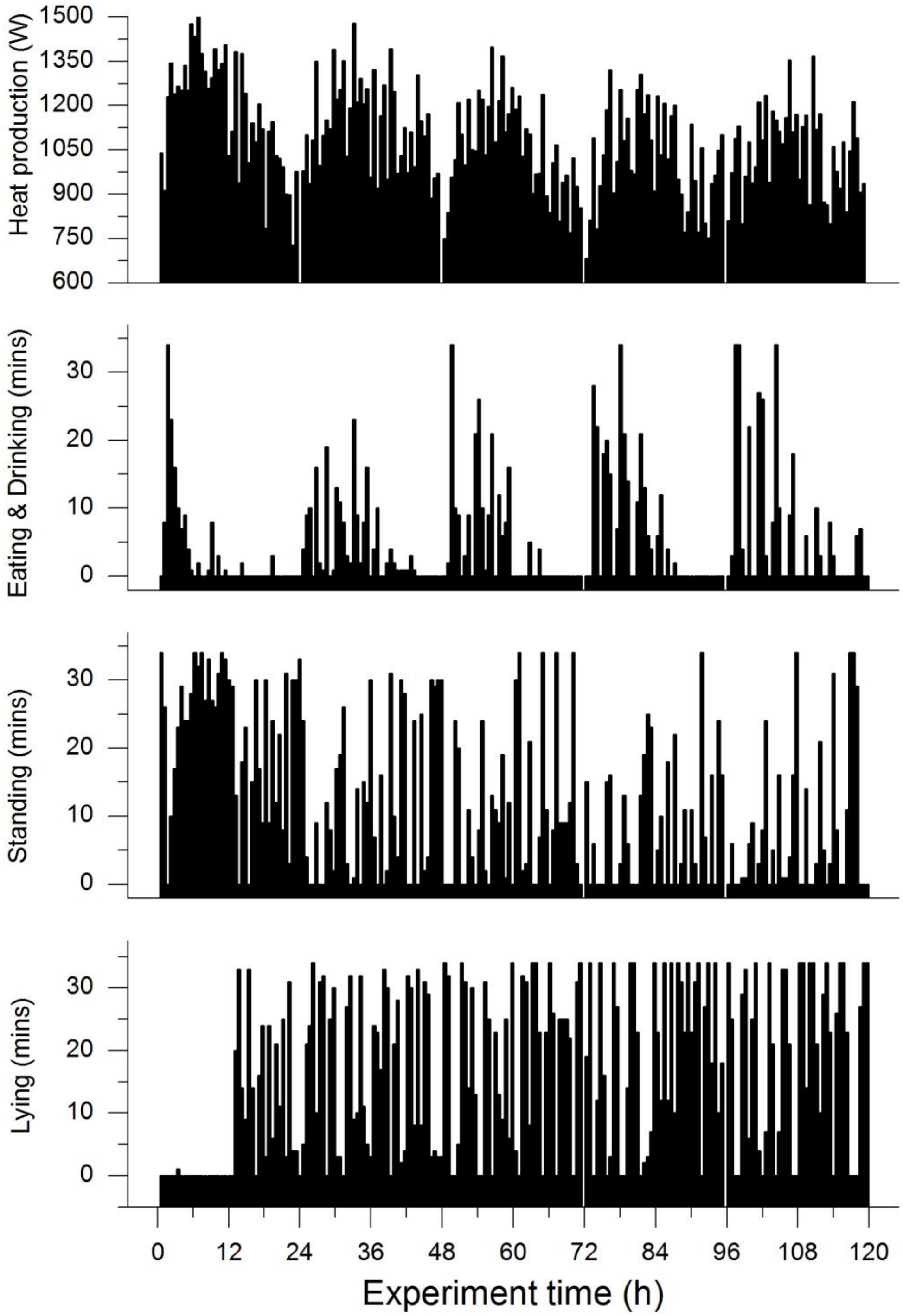

Figure 4.5. Summary of heat production and animal behaviors in 5 days for No.166 cattle (sampling intervals is 34 mins).

To evaluate if the current heat production value is representative, the data measured from REMS were compared with the results from the CIGR preditive models. As shown in Table 4.1, the previous research concluded the RQs of cattle varied from 0.9 to 1.2 (Søren Pedersen et al., 2008). The average RQ of three animals was within this range. There is no direct heat production data for mature steers. The first CIGR model (Eq. 4.13) is for fattening cattle and the second CIGR model (Eq. 4.14) is for breeding bulls, which are designed for animals up to 500kg. The THP and SHP (per kilogram body weight) from both CIGR models were lower than the measured value from REMS. Since the mature steers have less energy requirement than cattle in fattening and breeding, the heat production should be lower than those two animals. Therefore, CIGR models might underestimate the heat production for the current modern genetics.

Table 4.1. Summary of REMS results with CIGR models in 2012

	Feed Intake^1 (DW: kg/day)	Water Consumption^1 (L/day)	RQ^1	THP (w/kg)			SHP (w/kg)		
				REMS^1	CIGR^2	CIGR^3	REMS^1	CIGR^2	CIGR^3
Mean	7.93	22.79	0.91	1.38	0.96	1.12	1.20	0.62	0.73
SD	1.49	5.52	0.07	0.17	0.03	0.03	0.17	0.02	0.02

^{1}average data of last three days, ^{2}fattening cattle, ^{3}breeding bulls.

4.5 Conclusion

This research revealed animal behaviors (eating, standing and lying) were changed after introduction of cattle into the REMS chambers. The steers observed in the experiment were research animals and accustomed to the laboratory facility and interactions with humans. There were no differences observed in the daily heat production, moisture production, methane emission and ammonia emission. In addition, this study also shows the diurnal variation of heat production had similar performance with the time variation spending on eating. For the research interested at hourly metabolic responses and respiration quotient, two days of acclimation period is needed to get stable and representative data. This research documented a discussion about the variation of some indicators. Based on different research purposes and animals, operators can design the experiment plan to test acclimation before the formal experiment.

The second goal was to test the representativeness of HP predicted from the CIGR model. The heat production equations in CIGR was published in the 1980s. Heat and moisture production data for mature cattle showed a potential bias higher than CIGR models. Since the research animal is mature cattle instead of fattening cattle and breeding bulls and the common genetics 30 years ago are considerably different than today, more experiments are needed for further analyzing the heat production of cattle at different stages and with different genetics.

4.6 References

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CHAPTER 5: CONCLUSIONS AND FUTURE WORK

A ruminant emission measurement system was expanded to include the capabilities of cattle metabolism study, by monitoring heat production through indirect calorimetry. This thesis included a summary of the construction, simulation, and utilization.

The physical modification plan included two parts, upgrading the ventilation measurement and adding oxygen analyzer. The previous study concluded the ventilation measurement contribute a great amount of uncertainty. A digital pressure transducer replaced the inclined-vertical manometer for recording the pressure change during the experiment. An oxygen analyzer was added into the REMS gas analyzing subsystem, and associated electric circuiting and program controls were modified. Additionally, the calculation approaches used for the methane emission in the original system were modified for suitable ones for the heat production calculations.

After modification, the system needs a plan to evaluate the system reliability. Three recovery tests were selected from different viewpoints: alcohol combustion method, gravimetric gas injection method and constant gas injection method. Through varying the tracer gases and applying different assumption, these methods helped to identify the errors from ventilation measurement and exhaust gas calculation. These errors were corrected using the results of the recovery tests.

The upgraded REMS was applied to an animal test for evaluating animal metabolism and behavior during the acclimation period. The data measured was also used for evaluating current heat and moisture production predication model. The results showed two-days of acclimation period was enough to get stable results for research steers. In addition, the heat production data measured from REMS were higher than the predicted by the CIGR model. Since the cattle used were mature animals, further research are recommended to evaluate heat production data for cattle at different stages.

Oxygen measurement is always a challenge for indirect calorimetry studies. Currently, the fluctuation of oxygen concentration in ambient air is higher carbon dioxide and methane. Electric oxygen concentration signals are not stable as well as the carbon dioxide signals. The errors from oxygen measurement were corrected through a correction factor in this study. Another recommended experiment is about exploring an accurate and stable strategy to measure oxygen concentration. Minimizing the background air fluctuation and stabling oxygen signals can improve the measurement results.

APPENDIX A: ANALYSIS APPROACHES AND CODES OF ALCOHOL COMBUSTION METHOD

A.1 Introduction

MATLAB 2014a was used for processing the data from LabVIEW program “Recovery (1.3). VI”. A series of calculation codes were deduced to calculate respiratory quotient, mass recovered, mass injected, steady-state mass recovery percent, relative uncertainty, and absolute standard uncertainty of oxygen and carbon dioxide. The uncertainty analysis presented here was initially developed by Dr. Guilherme D.N. Maia and specific to the REMS.

“MR_std_uncertainty_ethanol.m” was the main function which can generate an excel file of the results. “MR_std_backward_ethanol.m” was used for calculating the correction factor of the ratio of exhaust air flowrate to incoming air flowrate under the assumption of mass recovered equals to mass injected. To execute main calculation codes, several support files were necessary. They were:

MR_importfile.m

std_uncertainty_om.m

std_uncertainty_Qairin.m

std_uncertainty_Correction.m

std_uncertainty_rho_inj

air_density_std_uncertainty.m

std_uncertainty_RP.m

A.2 Data Preparation

A.2.1 Data Process in LabVIEW Program

Program “Recovery (1.3). VI” would automatically generate a data file (.CVS) for each single chamber and each test. The data collection began with 10 background (building) concentration measurements, then followed by 40 chamber concentration measurements, and ended with 10 background chamber concentration measurements. The first 5 samples for each background were removed to avoid effects from transition between chamber and background. An estimated value was substituted with an average value of remaining 5 samples for both two background concentration measurements. Last 12 samples from chamber measurements will be used to represent gas concentration in steady state. The ethanol weight at the beginning and end of the experiment will be entered for further processing. There is a K factor for estimating whole process carbon dioxide generation, and oxygen consumption.

$$K = \frac{t_{12}}{\Delta t} \qquad (A.1)$$

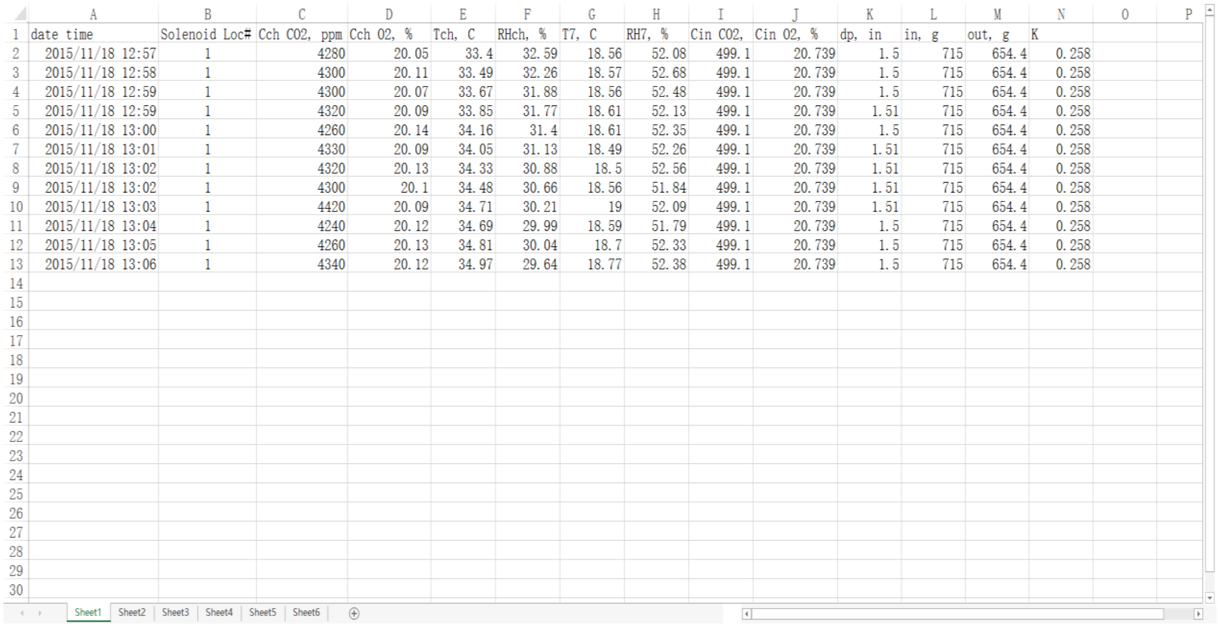
where

t_{12} = total time for 12 samples (s).

Δt = total time for whole ethanol burning process (s).

A.2.2 Reformat

LabVIEW generated a raw file under “C:\REMS\Data”, which was a comma-separated value text file (.CVS). Before importing the data into MATLAB, data were re-opened in Excel and each variable was automatically reformatted by separating into an independent column by Excel. Then, the date column and time column were combined together by using “date(year,month,date)+time” equation. This file contained all data from different chambers which were in time order. Next, the data were separated by a new sheet for each chamber. The file was saved as Excel file (.xls).



date time	Solenoid Loc#	Cch CO2, ppm	Cch O2, %	Tch, C	RHch, %	T7, C	RH7, %	Cin CO2,	Cin O2, %	dp, in	in, g	out, g	K
2015/11/18 12:57	1	4280	20.05	33.4	32.59	18.56	52.08	499.1	20.739	1.5	715	654.4	0.258
2015/11/18 12:58	1	4300	20.11	33.49	32.26	18.57	52.68	499.1	20.739	1.5	715	654.4	0.258
2015/11/18 12:59	1	4300	20.07	33.67	31.88	18.56	52.48	499.1	20.739	1.5	715	654.4	0.258
2015/11/18 12:59	1	4320	20.09	33.85	31.77	18.61	52.13	499.1	20.739	1.51	715	654.4	0.258
2015/11/18 13:00	1	4260	20.14	34.16	31.4	18.61	52.35	499.1	20.739	1.5	715	654.4	0.258
2015/11/18 13:01	1	4330	20.09	34.05	31.13	18.49	52.26	499.1	20.739	1.51	715	654.4	0.258
2015/11/18 13:02	1	4320	20.13	34.33	30.88	18.5	52.56	499.1	20.739	1.51	715	654.4	0.258
2015/11/18 13:02	1	4300	20.1	34.48	30.66	18.56	51.84	499.1	20.739	1.51	715	654.4	0.258
2015/11/18 13:03	1	4420	20.09	34.71	30.21	19	52.09	499.1	20.739	1.51	715	654.4	0.258
2015/11/18 13:04	1	4240	20.12	34.69	29.99	18.59	51.79	499.1	20.739	1.5	715	654.4	0.258
2015/11/18 13:05	1	4260	20.13	34.81	30.04	18.7	52.33	499.1	20.739	1.5	715	654.4	0.258
2015/11/18 13:06	1	4340	20.12	34.97	29.64	18.77	52.38	499.1	20.739	1.5	715	654.4	0.258

Figure A.1. The template of ACM data before processing into the MATLAB.

A.3 MR_std_uncertainty_ethanol.m

```
function MR_std_uncertainty_ethanol(name)
%      Jiangong Li (3-27-2016)
%       Department of Agricultural and Biological Engineering
%       University of Illinois at Urbana-Champaign
%
% file naming and deleting of old files and matlab data
ext = '.xlsx';
output = strcat(name,'_out',ext);
delete(output);
warning('off','all');

% loop for chambers one thru six corresponding to Sheet1 thru
Sheet6 in
% excel file; thus, j=1:6
for j=1:6
%% Data input
    % Reads columns of data from excel file and stores them in a
matrix
    % which is then arranged into its appropriate variables
    % read data from mass recovery test
    data_mat = MR_importfile(name,j);
    % convert datenum to h
    dt = [0 cumsum(diff(data_mat(:,1).*24))'];
    % chamber CO2 concentration at steady-state
    Cco2_ch = data_mat(:,3)/1000000;
    % chamber O2 concentration at steady-state
    Co2_ch = data_mat(:,4)/100;
    % chamber temperature (deg C)
    T_ch = data_mat(:,5);
    % chamber relative humidity (%)
    RH_ch = data_mat(:,6);
    % incoming temperature (deg C)
    T_in = data_mat(:,7);
    % incoming relative humidity (%)
    RH_in = data_mat(:,8);
    % mean incoming CO2 concentration before and after steady-
state
    Cco2_in = data_mat(:,9)/1000000;
    % mean incoming O2 concentration before and after steady-
state
    Co2_in = data_mat(:,10)/100;
    % orifice meter differential pressure (in wc)
    dP = data_mat(:,11);
    % Mass before experiment (g)
    mass_i = data_mat(:,12);
```

```
    % Mass after experiment (g)
    mass_f = data_mat(:,13);
    % Conversion factor
    k=data_mat(:,14);
    chamber = strcat('Chamber',' ',num2str(j),' successfully
imported');
    disp(chamber);
    %%
    % loop for every row in the Sheet[j] i.e. for each steady-
state data
    % point
    for i=1:length(dt)
        %% SYMBOLIC REPRESENTATION
        % Prefix guide
        % D = absolute std uncertainty
        % C_ = contribution
        % chamber CO2 concentration
        syms Cco2ch DCco2ch Cco2rec_Cch
        % chamber O2 concentration
        syms Co2ch DCo2ch Co2rec_Cch
        % incoming CO2(background) concentration
        syms Cco2in DCco2in Cco2rec_Cin
        % incoming O2(background) concentration
        syms Co2in DCo2in Co2rec_Cin
        % incoming temperature
        syms Tin DTin
        % Incoming ventilation rate at standard temperature and
pressure
        syms Qairin DQairin Crec_co2_Qairin Crec_o2_Qairin
        % Exhaust ventilation rate at standard temperature and
pressure
        syms Qairout DQairout Crec_co2_Qairout Crec_o2_Qairout
        % ventilation rate
        syms Qin DQin
        % Constant: barometric pressure
        syms pb Dpb
        % Saturated vapor pressure
        syms es
        % initial ethanol amount
        syms massi Dmassi Cinj_o2_massi Cinj_co2_massi
        % finial ethanol amount
        syms massf Dmassf Cinj_o2_massf Cinj_co2_massf
        % O2 consumption, CO2 production, respiratory quotient
        syms Vo2 Vo2_W Crq_o2_int Dm_o2_int
        % CO2 production
        syms Vco2 Vco2_W Crq_co2_int Dm_co2_int
        % estimated O2 consumption, CO2 production
```

```
        syms PVco2 PVo2w PVco2w DPVo2 DPVco2
        % Recovery of O2, CO2
        syms Dco2rec Do2rec
        % Concentration of ethanol
        syms ye Dye Cinj_o2_ye Cinj_co2_ye
        % Respiratory Quotient and recovery percent
        syms RQ DRQ O2RP CO2RP DO2RP DCO2RP K correct Dcorr
        %Saturated vapor pressure of incoming air
        %es=610.78*exp(Tin/(Tin+237.3)*17.2694);

        %O2 consumption (lph)
        Vo2=(Qairin*Co2in-correct*Qairin*Co2ch)*60;
        %CO2 production (lph)
        Vco2=(correct*Qairin*Cco2ch-Qairin*Cco2in)*60;
        %estimated O2 consumption (l)
        PVo2= ((massi-massf)*ye/46.0694)*3*K*22.414;
        %estimated CO2 consumption (l)
        PVco2= ((massi-massf)*ye/46.0694)*2*K*22.414;
        %Respiratory Quotient
        RQ=Vco2_W/Vo2_W;

        % Inject mass for O2 uncertainty parameter contributions
        Cinj_o2_massi =(Dmassi*diff(PVo2,massi))^2;
        Cinj_o2_massf=(Dmassf*diff(PVo2,massf))^2;
        Cinj_o2_ye=(Dye*diff(PVo2,ye))^2;
        % Inject mass for CO2 uncertainty parameter
contributions
        Cinj_co2_massi=(Dmassi*diff(PVco2,massi))^2;
        Cinj_co2_massf=(Dmassf*diff(PVco2,massf))^2;
        Cinj_co2_ye=(Dye*diff(PVco2,ye))^2;
        %recovered CO2 flow std uncertainty parameter
contributions
        Cco2rec_Cch=(DCco2ch*diff(Vco2,Cco2ch))^2;
        Cco2rec_Cin=(DCco2in*diff(Vco2,Cco2in))^2;
        Crec_co2_Qairin=(DQairin*diff(Vco2,Qairin))^2;
        Crec_co2_Qairout=(Dcorr*diff(Vco2,correct))^2;
        %recovered O2 flow std uncertainty parameter
contributions
        Co2rec_Cch=(DCo2ch*diff(Vo2,Co2ch))^2;
        Co2rec_Cin=(DCo2in*diff(Vo2,Co2in))^2;
        Crec_o2_Qairin=(DQairin*diff(Vo2,Qairin))^2;
        Crec_o2_Qairout=(Dcorr*diff(Vo2,correct))^2;
        %Individual contributions to mass recovery percent std
        %Uncertainty after integration over steady-state
        Crq_co2_int=(Dm_co2_int*diff(RQ,Vco2_W))^2;
        Crq_o2_int=(Dm_o2_int*diff(RQ,Vo2_W))^2;
        DRQ=sqrt(Crq_co2_int+Crq_o2_int);
```

```
        %Std uncertainty of recovered CO2 flow

Dco2rec=sqrt(Cco2rec_Cch+Cco2rec_Cin+Crec_co2_Qairin+Crec_co2_Qa
irout);
        %Std uncertainty of recovered O2 flow

Do2rec=sqrt(Co2rec_Cch+Co2rec_Cin+Crec_o2_Qairin+Crec_o2_Qairout
);
        %Std uncertainty of estimated O2 flow
        DPVo2=sqrt(Cinj_o2_massi+Cinj_o2_massf+Cinj_o2_ye);
        %Std uncertainty of estimated CO2 flow
        DPVco2=sqrt(Cinj_co2_massi+Cinj_co2_massf+Cinj_co2_ye);

        %Store data corresponding to row[i] into symbolic
variable for
        %Evolution later
        Tin=T_in(i);
        Co2in=Co2_in(i);
        Cco2in=Cco2_in(i);
        Co2ch=Co2_ch(i);
        Cco2ch=Cco2_ch(i);
        massi= mass_i(i);
        massf=mass_f(i);
        K=k(i);
        pb= 100815;
        ye=1;

        %Ventilation rate std uncertainty function
        %Vin= ventilation rate (m^3 s^-1)
        %DVin= ventilation rate std uncertainty (m^3 s^-1)
        [Qin,
DQin]=std_uncertainty_om(dP(i),j,T_in(i),RH_in(i));
        %Qairin= ventilation rate (lpm)
        %DQairin= ventilation rate std uncertainty (lpm)
        [Qairin, DQairin] = std_uncertainty_Qairin
(Qin,DQin,pb,Tin,RH_in(i));
        %Qairin= ventilation rate (lpm)
        %DQairin= ventilation rate std uncertainty (lpm)
        [Corr, DCorr] = std_uncertainty_Correction
(Co2in,Cco2in,Co2ch,Cco2ch,j);
        correct=Corr;
        Dcorr=DCorr;
        %Temperature uncertainty
        DTin=.5;
        Dpb=1;
        Dmassf=0.0497;
        Dmassi=0.0497;
```

```
        Dye=0.000289;

        %Individual components of co2 gas analyzer std
uncertainty
        %Post calibration repeatability
        rep_ga_pc=(0.005*.016)^2;
        %Instrument repeatability
        rep_ga_insch=((0.01*Cco2_ch(i)*1000000)/(3^0.5))^2;
        rep_ga_insin=((0.01*Cco2_in(i)*1000000)/(3^0.5))^2;
        % primary certified tolerance
        pct_ga=((0.01*40)/(3^0.5))^2;
        % range drift
        rd_ga_ch=((0.025*Cco2_ch(i)*1000000)/(3^0.5))^2;
        rd_ga_in=((0.025*Cco2_in(i)*1000000)/(3^0.5))^2;
        % resolution
        res_ga=((5*0.006)/(3^0.5))^2;
        % propagation of gas analyzer std uncertainty (1)

         DCco2ch=sqrt(rep_ga_pc+rep_ga_insch+pct_ga+rd_ga_ch+res
         _ga)/1000000;

         DCco2in=sqrt(rep_ga_pc+rep_ga_insin+pct_ga+rd_ga_in+res
         _ga)/1000000;

        % individual components of O2 analyzer std uncertainty
        % post calibration repeatability
        rep_oa_pc=(0.051553)^2;
        % instrument repeatability
        rep_oa_insch=((0.22)/(3^0.5))^2;
        rep_oa_insin=((0.22)/(3^0.5))^2;
        % primary certified tolerance
        pct_oa=((20.7*0.02)/(3^0.5))^2;
        % range drift
        rd_oa_ch=((0.01*Co2_ch(i)*100)/(3^0.5))^2;
        rd_oa_in=((0.01*Co2_in(i)*100)/(3^0.5))^2;
        % resolution
        res_oa=((0.001*22)/(3^0.5))^2;
        % propagation of gas analyzer std uncertainty (1)

DCo2ch=sqrt(rep_oa_pc+rep_oa_insch+pct_oa+rd_oa_ch+res_oa)/100;

DCo2in=sqrt(rep_oa_pc+rep_oa_insin+pct_oa+rd_oa_in+res_oa)/100;

        % reproducibility determined as std dev of SSMRP
        MR_rep=[1.7114 1.6135 2.1768 3.2204 1.2861 1.4367];
        %MR_rep=[1.7114 1.6135 2.1768 3.2204];
        rep=MR_rep(j);
```

```
        %% EVALUATE EQUATIONS
        format long;
        % calculate incoming air flow rate at standard
temperature
        Qairins(i) =Qairin;
        % calculate exhaust air flow rate at standard
temperature
        Qairouts(i) = correct;
        %calculate O2 consumption
        Vo2s(i)= eval(Vo2);
        %calculate CO2 production
        Vco2s(i)=eval(Vco2);
        % Calculate estimated O2 consumption
        PVo2s(i)= eval(PVo2);
        % Calculate estimated CO2 consumption
        PVco2s(i)= eval(PVco2);

        % calculate std uncertainty of incoming air flow rate
(l)
        DQairins(i)=DQairin;
        % calculate std uncertainty of exhaust air flow rate (l)
        DQairouts(i)=Dcorr;
        % calculate std uncertainty of recovered CO2 flow (l)
        Dco2recs(i)=eval(Dco2rec);
        % calculate std uncertainty of recovered O2 flow (l)
        Do2recs(i)=eval(Do2rec);
        % calculate std uncertainty of Estimated O2 flow (l)
        DPVo2s(i)=eval(DPVo2);
        % calculate std uncertainty of Estimated CO2 flow (l)
        DPVco2s(i)=eval(DPVco2);
        %% PREPARE OUTPUT MATRICES
        % collect parameter value used in calculations
        value(1,i)=Tin;
        value(2,i)=Qin*60000;
        value(3,i)=Co2in*100;
        value(4,i)=Co2ch*100;
        value(5,i)=Cco2in*100;
        value(6,i)=Cco2ch*100;
        value(7,i)=massi;
        value(8,i)=massf;
        value(9,i)=Qairins(i);
        value(10,i)=Qairouts(i);
        value(11,i)=Vo2s(i);
        value(12,i)=Vco2s(i);
        value(13,i)=PVo2s(i);
        value(14,i)=PVco2s(i);
```

```
        % collect parameter absolute std uncertainty
        u_abs(1,i)=DTin;
        u_abs(2,i)=DQin*60000;
        u_abs(3,i)=DCo2in*100;
        u_abs(4,i)=DCo2ch*100;
        u_abs(5,i)=DCco2in*100;
        u_abs(6,i)=DCco2ch*100;
        u_abs(7,i)= DQairins(i);
        u_abs(8,i)= DQairouts(i);
        u_abs(9,i)=Dco2recs(i);
        u_abs(10,i)= Do2recs(i);
        u_abs(11,i)=DPVo2s(i);
        u_abs(12,i)=DPVco2s(i);
        u_abs(13,i)=Dye*100;

        % contributions to recovered co2 std uncertainty

m_rec_co2total=eval(Cco2rec_Cch+Cco2rec_Cin+Crec_co2_Qairin+Crec
_co2_Qairout);
        % evaluate individual contributions
        contribution(1,i)=100*eval(Cco2rec_Cch)/m_rec_co2total;
        contribution(2,i)=100*eval(Cco2rec_Cin)/m_rec_co2total;

contribution(3,i)=100*eval(Crec_co2_Qairin)/m_rec_co2total;

contribution(4,i)=100*eval(Crec_co2_Qairout)/m_rec_co2total;

        % contributions to recovered o2 std uncertainty

m_rec_o2total=eval(Co2rec_Cch+Co2rec_Cin+Crec_o2_Qairin+Crec_o2_
Qairout);
        % evaluate individual contributions
        contribution(5,i)=100*eval(Co2rec_Cch)/m_rec_o2total;
        contribution(6,i)=100*eval(Co2rec_Cin)/m_rec_o2total;

contribution(7,i)=100*eval(Crec_o2_Qairin)/m_rec_o2total;

contribution(8,i)=100*eval(Crec_o2_Qairout)/m_rec_o2total;
        % co2 flow recovered std uncertainty
        contribution(9,i)=100*Dco2recs(i)/Vco2s(i);
        % o2 flow recovered std uncertainty
        contribution(10,i)=100*Do2recs(i)/Vo2s(i);

        % contributions to injected mass of o2 flow std
uncertainty

m_inj_o2_total=eval(Cinj_o2_massi+Cinj_o2_massf+Cinj_o2_ye);
```

```
        % evaluate individual contributions

contribution(11,i)=100*eval(Cinj_o2_massi)/m_inj_o2_total;

contribution(12,i)=100*eval(Cinj_o2_massf)/m_inj_o2_total;
contribution(13,i)=100*eval(Cinj_o2_ye)/m_inj_o2_total;
        % mass flow injected of o2 std uncertainty
contribution(14,i)=100*DPVo2s(i)/Vo2s(i);

        % contributions to injected mass of co2 flow std
uncertainty

m_inj_co2_total=eval(Cinj_co2_massi+Cinj_co2_massf+Cinj_co2_ye);
        % evaluate individual contributions

contribution(15,i)=100*eval(Cinj_co2_massi)/m_inj_co2_total;

contribution(16,i)=100*eval(Cinj_co2_massf)/m_inj_co2_total;

contribution(17,i)=100*eval(Cinj_co2_ye)/m_inj_co2_total;
        % mass flow injected of co2 std uncertainty
contribution(18,i)=100*DPVco2s(i)/Vco2s(i);
    end
    %% RQ ANALYSIS AND STD UNCERTAINTY ANALYSIS
    % calculate total mass recovered by integrating of SS period
(l)
    Vco2_W=trapz(dt,Vco2s);
    Vo2_W=trapz(dt,Vo2s);
    % calculate total O2 and CO2 recovered std uncertainty
assume root-sum
    % square
    for h=1:length(dt)-1
        Dmo2_temp1=(0.5*Do2recs(h)*dt(h));
        Dmo2_temp2=(0.5*Do2recs(h)*dt(h+1));
        Dmo2_temp3=(0.5*Do2recs(h+1)*dt(h));
        Dmo2_temp4=(0.5*Do2recs(h+1)*dt(h+1));
        Dm_o2_temp(h)=(-Dmo2_temp1+Dmo2_temp2-
Dmo2_temp3+Dmo2_temp4)^2;

        Dmco2_temp1=(0.5*Dco2recs(h)*dt(h));
        Dmco2_temp2=(0.5*Dco2recs(h)*dt(h+1));
        Dmco2_temp3=(0.5*Dco2recs(h+1)*dt(h));
        Dmco2_temp4=(0.5*Dco2recs(h+1)*dt(h+1));
        Dm_co2_temp(h)=(-Dmco2_temp1+Dmco2_temp2-
Dmco2_temp3+Dmco2_temp4)^2;
    end
    % mass recovered std uncertainty
```

```
    Dm_o2_int=sqrt(sum(Dm_o2_temp));
    % mass injected std uncertainty
    Dm_co2_int=sqrt(sum(Dm_co2_temp));
    DPVco2_W=mean(u_abs(12));
    DPVo2_W=mean(u_abs(11));
    PVo2w=mean(value(13));
    PVco2w=mean(value(14));

[O2RP,CO2RP,DO2RP,DCO2RP]=std_uncertainty_RP(Vco2_W,Vo2_W,PVo2w,
PVco2w,Dm_o2_int,Dm_co2_int,DPVco2_W,DPVo2_W);
    % evaluate
    RQ=eval(RQ);
    DRQ=eval(DRQ);
    O2RP=O2RP;
    DO2RP=DO2RP;
    CO2RP=CO2RP;
    DCO2RP=DCO2RP;

    % preparing for output to excel
    Result_contribution(1,1)=Vo2_W;
    Result_contribution(2,1)=Dm_o2_int;
    Result_contribution(3,1)=Vco2_W;
    Result_contribution(4,1)=Dm_co2_int;
    Result_contribution(5,1)=RQ;
    Result_contribution(6,1)=DRQ;
    Result_contribution(7,1)=O2RP*100;
    Result_contribution(8,1)=DO2RP*100;
    Result_contribution(9,1)=CO2RP*100;
    Result_contribution(10,1)=DCO2RP*100;

    %% FORMATING FOR OUTPUT
    A={'Values'; 'Temperature incoming (C)';'Flow rate
(lpm)';...
    'O2 Concentration incoming (%)';'O2 Concentration chamber
(%)’;...
    'CO2 Concentration incoming (%)';'CO2 Concentration chamber
(%)’;...
    'Initial ethanol mass (g)';'Final ethanol mass (g)’...
    'Standard incoming air flow rate (lpm)';'Standard exhaust
air flow rate (lpm)';...
    'O2 consumption (lpm)';'CO2 production (lpm)';...
    'Estimate O2 consumption (l) '; 'EstimateCO2 production
(l)'};
    xlswrite(output,A,j,'A1');
    xlswrite(output,value,j,'B2');
```

```
    B={'Absolute standard uncertainty'; 'u(Temperature incoming)
(C))';...
    'u(Flow rate (lpm))';'u(O2 Concentration incoming£¨%£©)';...
    'u(O2 Concentration chamber(%))';'u(CO2 Concentration
incoming£¨%£©)';...
    'u(CO2 Concentration chamber (1)';'u(Standard incoming air
flow rate(lpm))';...
    'u(Standard exhaust air flow rate(lpm))';'u(CO2 consumption
(lpm))';...
    'u(O2 production (lpm))';...
    'u(Estimate O2 consumption (l))';'u(Estimate CO2 production
(l))';'u(Ethanol concentration (%))'};
    q=strcat('A',num2str(length(A)+2));
    w=strcat('B',num2str(length(A)+3));
    xlswrite(output,B,j,q);
    xlswrite(output,u_abs,j,w);

    C={'Relative contributions'; 'u(CO2 Concentration chamber)
(%))';...
    'u(CO2 Concentration incoming (%))';'u(Standard incoming air
flow rate (%))';...
    'u(Standard exhaust air flow rate (%))';'u(O2 Concentration
chamber (%))';...
    'u(O2 Concentration incoming (%))';'u(Standard incoming air
flow rate (%)';...
    'u(Standard exhaust air flow rate (%))';'Rel std for CO2
u(Mass flow recovered (%))';...
    'Rel std for O2 u (Mass flow recovered (%))';...
    'u(Initial ethanol mass for O2 (%))';'u(Final ethanol mass
for O2 (%))';...
    'u(Ethanol concentration for O2 (%))'; 'Rel std for O2
u(Mass flow injected (%))';...
    'u(Initial ethanol mass for CO2 (%))';'u(Final ethanol mass
for CO2 (%))';...
    'u(Ethanol concentration for CO2 (%))'; 'Rel std for CO2
u(Mass flow injected (%))'};
    e=strcat('A',num2str(length(A)+length(B)+3));
    s=strcat('B',num2str(length(A)+length(B)+4));
    xlswrite(output,C,j,e);
    xlswrite(output,contribution,j,s);

    D={'Recovery';'O2 recovered (l)';'u(O2 recovered (l))';...
    'CO2 recovered (l)';'u(CO2 recovered (l))';...
    'Respiratory Quotient'; 'u(Respiratory Quotient)';...
    'O2 recovery percent (%)'; 'u(O2 recovery percent) (%)';...
    'CO2 recovery percent (%)'; 'u(CO2 recovery percent) (%)'};
    v=strcat('A',num2str(length(A)+length(B)+length(C)+4));
```

```
    h=strcat('B',num2str(length(A)+length(B)+length(C)+5));
    xlswrite(output,D,j,v);
    xlswrite(output,Result_contribution,j,h);
    % variables
    save(strcat(output,'.mat'));
end
end
```

A.4 std_uncertainty_Qairin.m

```
%% INCOMING AIR FLOWRATE STD UNCERTAINTY at STPD
%       Jiangong Li (4-07-2016)
%       Department of Agricultural and Biological Engineering
%       University of Illinois at Urbana-Champaign
%
%       calculates the dry basis air flowrate under standard
temperature and
%       pressure and its associated std uncertainty
function [Qairin, DQairin] = std_uncertainty_Qairin
(Qin,DQin,pb,Tin,RHi)
% Prefix guide
% D = absolute std uncertainty
% C_ = contribution
%INPUTS
   %Tin= incoming temperature (C)
   %pb= Barometric pressure (pa)
   %Qin= incoming ventilation rate (m^3 s^-1)
   %DQin= incoming ventilation rate std uncertainty (m^3 s^-1)
%OUTPUTS
   %Qairin= ventilation rate (lpm)
   %DQairin= ventilation rate std uncertainty (lpm)

% ventilation rate
syms Qini DQini Cin_Qin
% Constant: barometric pressure
syms Dpb Cin_pb pbi
% incoming temperature
syms DTin Crec_Tin Cin_Tin Tini
% Incoming ventilation rate at standard temperature and pressure
syms Qairin DQairin
syms Cin_Tin Cin_pb Cin_Qin
syms  Cin_RH RH delta_RH

% constants to calculate saturated water vapor partial pressure
constants
% apply for temperature range: 0<T<200 deg C
A1 = -5.8002206E+03; A2 = +1.3914993; A3 = -48.640239E-03;
```

```
A4 = +41.764768E-06; A5 = -14.452093E-09; A6 = 0;
A7 = +6.5459673;

% Incoming air flow rate at standard temperature (lpm)
Qairin =60000*Qini*((pbi
(RH/100)*(exp((A1./(Tini))+A2+(A3.*(Tini))+(A4.*((Tini).^2))+(A5
.*...
((Tini).^3))+(A6.*((Tini).^4))+(A7.*(log((Tini)))))))/101325)*(2
73.16/Tini);

DTin=.5;
Dpb=1;
delta_RH=3; % relative humidity accuracy (%)
% incoming air flow rate std uncertainty parameter contributions
Cin_Tin=(DTin*diff(Qairin,Tini))^2;
Cin_pb=(Dpb*diff(Qairin,pbi))^2;
Cin_Qin=(DQini*diff(Qairin,Qini))^2;
Cin_RH=(delta_RH*diff(Qairin,RH))^2;
% std uncertainty of incoming air flow rate
DQairin=sqrt(Cin_Tin+Cin_pb+Cin_Qin+Cin_RH);
Qini=Qin;
DQini=DQin;
pbi=pb;
Tini=Tin+273.15;
RH=RHi;
% calculate Incoming air flow rate
Qairin=eval(Qairin);
% calculate Incoming air flow rate std uncertainty
DQairin=eval(DQairin);
end
```

A.5 std_uncertainty_Correction.m

```
%% CORRECTION FACTOR FOR EXHAUST AIR FLOWRATE STD UNCERTAINTY
%     Jiangong Li (5-01-2016)
%     Department of Agricultural and Biological Engineering
%     University of Illinois at Urbana-Champaign
%
%     calculates the gas cylinder mixture density and its
associated std
%     uncertainty
function [Corr, DCorr] = std_uncertainty_Correction
Co2in, Cco2in, Co2ch, Cco2ch, j)
% Prefix guide
% D = abosolute std uncertainty
% C_ = contribution
%INPUTS
```

```
   %Qairin= incoming ventilation rate (lpm)
   %DQairin= incoming ventilation std uncertainty (lpm)
   %Co2in= incoming oxygen concentration
   %Cco2in= incoming carbon dioxide
   %Co2ch= chamber oxygen concentration
   %Cco2ch= chamber carbon dioxide concentration
%OUTPUTS
   %Corr= correction factor for exhaust air flowrate
   %DCorr= correction factor for exhaust air flowrate std
   uncertainty %
%Exhaust ventilation rate at standard temperature and pressure
syms Corr DCorr
% chamber CO2 concentration
syms Cco2cho DCco2ch Cout_Cco2ch
% chamber O2 concentration
syms Co2cho DCo2ch Cout_Co2ch
% incoming CO2(background) concentration
syms Cco2ino DCco2in Cout_Cco2in
% incoming O2(background) concentration
syms Co2ino DCo2in Cout_Co2in a m_rec_total

kfactor = [1.01 1.01 1.01 1.01 1.01 1.01];

% individual components of co2 gas analyzer std uncertainty
% post calibration repeatability
rep_ga_pc=(0.005*.016)^2;
% instrument repeatability
rep_ga_insch=((0.01*Cco2cho*1000000)/(3^0.5))^2;
rep_ga_insin=((0.01*Cco2ino*1000000)/(3^0.5))^2;
% primary certified tolerance
pct_ga=((0.01*40)/(3^0.5))^2;
% range drift
rd_ga_ch=((0.025*Cco2cho*1000000)/(3^0.5))^2;
rd_ga_in=((0.025*Cco2ino*1000000)/(3^0.5))^2;
% resolution
res_ga=((5*0.006)/(3^0.5))^2;
% propagation of gas analyzer std uncertainty (1)
DCco2ch=sqrt(rep_ga_pc+rep_ga_insch+pct_ga+rd_ga_ch+res_ga)/1000
000;
DCco2in=sqrt(rep_ga_pc+rep_ga_insin+pct_ga+rd_ga_in+res_ga)/1000
000;

% individual components of O2 analyzer std uncertainty
% post calibration repeatability
rep_oa_pc=(0.051553)^2;
% instrument repeatability
rep_oa_insch=((0.22)/(3^0.5))^2;
```

```
rep_oa_insin=((0.22)/(3^0.5))^2;
% primary certified tolerance
pct_oa=((20.7*0.02)/(3^0.5))^2;
% range drift
rd_oa_ch=((0.01*Co2cho*100)/(3^0.5))^2;
rd_oa_in=((0.01*Co2ino*100)/(3^0.5))^2;
% resolution
res_oa=((0.001*22)/(3^0.5))^2;
% propagation of gas analyzer std uncertainty (l)
DCo2ch=sqrt(rep_oa_pc+rep_oa_insch+pct_oa+rd_oa_ch+res_oa)/100;
DCo2in=sqrt(rep_oa_pc+rep_oa_insin+pct_oa+rd_oa_in+res_oa)/100;
% Exhaust air flow rate at standard temperature
Corr =a*(1-Co2ino-Cco2ino)/(1-Co2cho-Cco2cho);

% exhaust air flow rate std uncertainty parameter contributions
Cout_Cco2in=( DCco2in*diff(Corr,Cco2ino))^2;
Cout_Co2in=( DCo2in*diff(Corr,Co2ino))^2;
Cout_Cco2ch=( DCco2ch*diff(Corr,Cco2cho))^2;
Cout_Co2ch=( DCo2ch*diff(Corr,Co2cho))^2;

% std uncertainty of exhaust air flow rate
DCorr=sqrt(Cout_Cco2in+Cout_Co2in+Cout_Cco2ch+Cout_Co2ch);

Co2ino=Co2in;
Cco2ino=Cco2in;
Co2cho=Co2ch;
Cco2cho=Cco2ch;
a=kfactor(j);

% calculate Incoming air flow rate
Corr=eval(Corr);
% calculate Incoming air flow rate std uncertainty
DCorr=eval(DCorr);
end
```

APPENDIX B: ANALYSIS APPROACHES AND CODES OF GRAVIMETRIC GAS INJECTION METHOD

B.1 Introduction

MATLAB 2014a was used for processing the data from LabVIEW program “Recovery (1.3). VI”. A series of calculation codes were deduced to calculate the mass recovered, mass injected, steady-state mass recovery percent, relative uncertainty and absolute standard uncertainty. The uncertainty analysis presented here was initially developed by Dr. Guilherme D.N. Maia and specific to the REMS.

“MR_std_uncertainty_gravimetrics.m” was the main code which can generate an excel file of the results. This program was designed to evaluate the carbon dioxide measurement. Therefore, the oxygen concentrations inside the chamber were assumed the same as the oxygen concentrations at ambient air. To execute main calculation codes, several support files were necessary. They were:

MR_importfile.m

std_uncertainty_om.m

std_uncertainty_Qairin.m

std_uncertainty_Qairout.m

air_density_std_uncertainty.m

B.2 Data Preparation

B.2.1 Data Process in LabVIEW Program

Program "Recovery (1.3). VI" would automatically generate a data file (.CVS) for each single chamber and each test. The data collection began with 10 background (building) concentration measurements, then followed by 40 chamber concentration measurements, and ended with 10 background chamber concentration measurements. The first 5 samples for each background were removed to avoid effects from transition between chamber and background. An estimated value was substituted with an average value of remaining 5 samples for both two background concentration measurements. Last 12 samples from chamber measurements will be used to represent gas concentration in steady state. The difference in weight of gas cylinder at the beginning and end of the experiment will be recorded for further processing. The concentration of gas cylinder and estimate flow rate need to be entered in the excel file. The mass represents the weight change of cylinder in 12 sample period which could be calculated as followed:

$$\text{mass} = \frac{t_{12}}{\Delta t}(m_i - m_f) \tag{B.1}$$

where

t_{12} = total time for 12 samples (s).

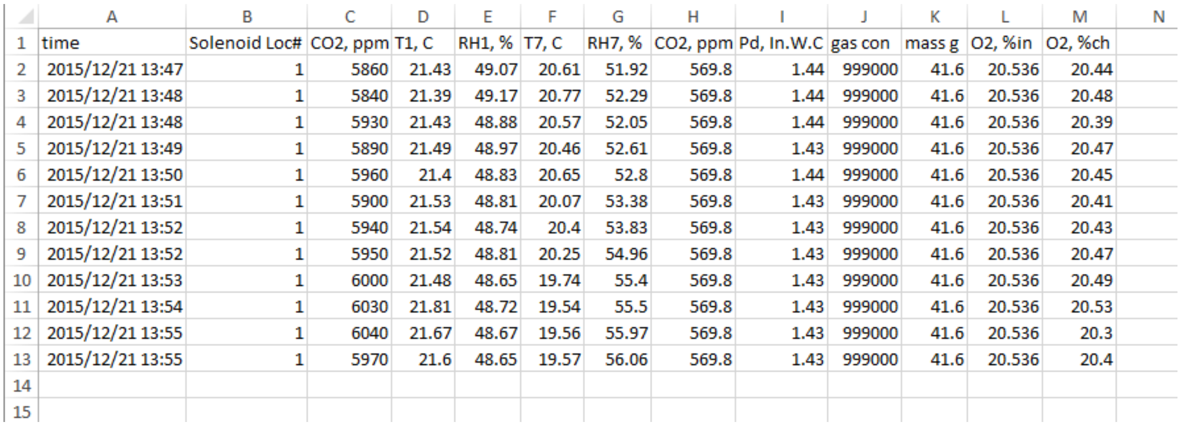
Δt = total time for whole ethanol burning process (s).

m_i = initial cylinder weight (g).

m_f = final cylinder weight (g).

B.2.2 Reformat

LabVIEW generated a raw file under "C:\REMS\Data", which was a comma-separated value text file (.CVS). Before importing the data into MATLAB, data were re-opened in Excel and each variable was automatically reformatted by separating into an independent column by Excel. Then, the date column and time column were combined together by using "date(year,month,date)+time" equation. This file contained all data from different chambers which were in time order. Next, the data were separated by to a new sheet for each chamber. The file was saved as Excel file (.xls).



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	time	Solenoid Loc#	CO2, ppm	T1, C	RH1, %	T7, C	RH7, %	CO2, ppm	Pd, In.W.C	gas con	mass g	O2, %in	O2, %ch	
2	2015/12/21 13:47	1	5860	21.43	49.07	20.61	51.92	569.8	1.44	999000	41.6	20.536	20.44	
3	2015/12/21 13:48	1	5840	21.39	49.17	20.77	52.29	569.8	1.44	999000	41.6	20.536	20.48	
4	2015/12/21 13:48	1	5930	21.43	48.88	20.57	52.05	569.8	1.44	999000	41.6	20.536	20.39	
5	2015/12/21 13:49	1	5890	21.49	48.97	20.46	52.61	569.8	1.43	999000	41.6	20.536	20.47	
6	2015/12/21 13:50	1	5960	21.4	48.83	20.65	52.8	569.8	1.44	999000	41.6	20.536	20.45	
7	2015/12/21 13:51	1	5900	21.53	48.81	20.07	53.38	569.8	1.43	999000	41.6	20.536	20.41	
8	2015/12/21 13:52	1	5940	21.54	48.74	20.4	53.83	569.8	1.43	999000	41.6	20.536	20.43	
9	2015/12/21 13:52	1	5950	21.52	48.81	20.25	54.96	569.8	1.43	999000	41.6	20.536	20.47	
10	2015/12/21 13:53	1	6000	21.48	48.65	19.74	55.4	569.8	1.43	999000	41.6	20.536	20.49	
11	2015/12/21 13:54	1	6030	21.81	48.72	19.54	55.5	569.8	1.43	999000	41.6	20.536	20.53	
12	2015/12/21 13:55	1	6040	21.67	48.67	19.56	55.97	569.8	1.43	999000	41.6	20.536	20.3	
13	2015/12/21 13:55	1	5970	21.6	48.65	19.57	56.06	569.8	1.43	999000	41.6	20.536	20.4	
14														
15														

Figure B.1. The template of GRAV data before processing into the MATLAB.

B.3 MR_std_uncertainty_gravimetrics.m

```
%% STEADY-STATE MASS RECOVERY TEST ANALYSIS AND STD UNCERTIANTY
%   Jiangong Li (11-22-2015)
%   Department of Agricultural and Biological Engineering
%   University of Illinois at Urbana-Champaign
% calculates SSMRP and its associated std uncertainty
%INPUTS
    %name= data file w/o extension (assumes '.xlsx' file type)
function MR_std_uncertainty_gravimetrics(name)
% file naming and deleting of old files and matlab data
ext = '.xlsx';
output = strcat(name,'_out',ext);
delete(output);
warning('off','all');
% loop for chambers one thru six corresponding to Sheet1 thru
Sheet6 in
% excel file; thus, j=1:6
for j=1:6
    %% Data input
    % Reads columns of data from excel file and stores them in a
matrix
    % which is then arranged into its appropriate variables
    % read data from mass recovery test
    data_mat = MR_importfile(name,j);
    % convert datenum to h
    dt = [0 cumsum(diff(data_mat(:,1).*24))'];
    % chamber concentration at steady-state (ppmv)
```

```
    C_ch = data_mat(:,3);
    % chamber temperature (deg C)
    T_ch = data_mat(:,4);
    % chamber relative humidity (%)
    RH_ch = data_mat(:,5);
    % incoming temperature (deg C)
    T_in = data_mat(:,6);
     % incoming relative humidity (%)
    RH_in = data_mat(:,7);
    % mean incoming concentration before and after steady-state
(ppmv)
    C_in = data_mat(:,8);
    % orifice meter differential pressure (in wc)
    dP = data_mat(:,9);
    % gas cylinder concentration (ppmv)
    C_cyl = data_mat(:,10);
    mass = data_mat(:,11);
    % incoming O2 concentration (%)
    C_ino = data_mat(:,12);
    % chamber O2 concentration (%)
    C_cho = data_mat(:,13);
    chamber = strcat('Chamber',' ',num2str(j),' successfully
imported');
    disp(chamber);
    %%
    % loop for every row in the Sheet[j] i.e. for each steady-
state data
    for i=1:length(dt)
        %% SYMBOLIC REPRESENTATION
        % Prefix guide
        % D = absolute std uncertainty
        % C_ = contribution
        % chamber concentration
        syms Cch DCch Crec_Cch
        % incoming (background) concentration
        syms Cin DCin Crec_Cin
        % chamber temperature
        syms Tch DTch Crec_Tch
        % incoming temperature
        syms Tin DTin Crec_Tin
        % ventilation rate
        syms Qin DQin
        % ventilation rate STPD
        syms Qairout DQairout Qairin DQairin Crec_Qout Crec_Qin
        % chamber moist air density
        syms rhoch Drhoch Crec_rhoch
        % incoming moist air density
```

```
        syms rhoin Drhoin Crec_rhoin
        % Constant: barometric pressure
        syms pb
        % Constant: molecular weight
        syms M MMtg MMn2
        % Constant: universal gas constant and time
        syms R t
        % std temperature and pressure
        syms Tstd pstd
        % injected gas cylinder concentration
        syms Ccyl DCcyl Cinj_Ccyl
        % mass flow injected
        syms m_inj_grav Dm_inj_grav m_inj_gravf Dm_inj_gravf
        % mass injected
        syms m_inj_int Dm_inj_int Cmrp_minj_int
        % mass flow recovered
        syms m_rec Dm_rec
        % mass recovered
        syms m_rec_int Dm_rec_int Cmrp_mrec_int
        % steady-state mass recovery percent
        syms MRP_grav DMRP_grav
        %CO2 concentration
        syms ytg Dytg Cinj_grav_ytg
        %N2 concentration
        syms yn2 Dyn2 Cinj_grav_yn2
        % mass of cylinder
        syms Mass DMass Cinj_grav_Mass Crec_Minj
        syms massf Dmassf Cinj_grav_massf
        % uncertainty of MRP
        syms Cmrp_grav_mrec_int Cmrp_grav_minj_grav rep
        %% EQUATION FOR MR ANALYSIS
        % gravimetric injected gas mass equation (g)
        m_inj_grav = Mass*((ytg*MMtg)/(ytg*MMtg+yn2*MMn2));
        % recovered gas mass flow equation (g h^-1)
        m_rec=((Qairout*Cch-Qairin*Cin)*60*1E-6)*44.01/22.414;
        % steady-state mass recovery percent (%)
        MRP_grav = 100*(m_rec_int/m_inj_gravf);
      %% PARAMETER INDIVIDUAL CONTRIBUTION

        % injected mass std uncertainty parameter contributions
        % (gravimetric)
        Cinj_grav_Mass=(DMass*diff(m_inj_grav,Mass))^2;
        Cinj_grav_ytg=(Dytg*diff(m_inj_grav,ytg))^2;
        Cinj_grav_yn2=(Dyn2*diff(m_inj_grav,yn2))^2;

        % recovered mass flow std uncertainty parameter
contributions
```

```
        Crec_Cch=(DCch*diff(m_rec,Cch))^2;
        Crec_Cin=(DCin*diff(m_rec,Cin))^2;
        Crec_Qin=(DQairin*diff(m_rec,Qairin))^2;
        Crec_Qout=(DQairout*diff(m_rec,Qairout))^2;
        % individual contributions to mass recovery percent std
        % uncertainty after integration over steady-state

Cmrp_grav_mrec_int=(Dm_rec_int*diff(MRP_grav,m_rec_int))^2;

Cmrp_grav_minj_grav=(Dm_inj_gravf*diff(MRP_grav,m_inj_gravf))^2;
        %% STD UNCERTAINTY EQNS
        % std uncertainty of injected gas mass (gravimetric)

Dm_inj_grav=sqrt(Cinj_grav_Mass+Cinj_grav_ytg+Cinj_grav_yn2);
        % std uncertainty of recovered gas mass flow
        Dm_rec=sqrt(Crec_Cch+Crec_Cin+Crec_Qin+Crec_Qout);
        % std uncertainty of ss mass recovery percent
gravimetric
        DMRP_grav=sqrt(Cmrp_grav_mrec_int+Cmrp_grav_minj_grav);
        %% DETERMINATION OF STD UNCERTAINTY FOR EACH PARAMETER
        % store data corresponding to row[i] into symbolic
variable for
        % evaluation later
        Tin=T_in(i);
        Cch=C_ch(i);
        Cin=C_in(i);
        Ccyl=C_cyl(i);
        Mass=mass(i);
        DMass = 0.6455;
        ytg=Ccyl(1)/1E6;
        yn2=1-ytg;
        Dytg =(0.01*ytg)/sqrt(3);
        Dyn2 =(0.01*yn2)/sqrt(3);
        DTin=.5;
        MMtg=44.01;
        MMn2=28.01;
        Cino=C_ino(i);
        Ccho=C_cho(i);
        % define constants with assumed negligible contribution
to std uncertainty
        % barometric pressure (Pa) (ASHRAE 2013, Chp 1, Eqn 3)
        pb=98639.3086;
        % std temperature (K)
        Tstd=273.15;
        % std pressure (Pa)
        pstd=101325;
        % ideal gas constant (m^3 Pa K^-1 mol^-1)
```

```
        R=8.314;
        % molecular mass of CO2 (g mol^-1)
        M=44.01;
        t=dt(12);
        % individual components of gas analyzer std uncertainty
        % post calibration repeatability
        rep_pc=(0.005*.016)^2;
        % instrument repeatability
        rep_insch=((0.01*C_ch(i))/(3^0.5))^2;
        rep_insin=((0.01*C_in(i))/(3^0.5))^2;
        % primary certified tolerance
        pct=((0.01*40)/(3^0.5))^2;
        % range drift
        rd_ch=((0.025*C_ch(i))/(3^0.5))^2;
        rd_in=((0.025*C_in(i))/(3^0.5))^2;
        % resolution
        res=((5*0.006)/(3^0.5))^2;
        % propagation of gas analyzer std uncertainty
        DCch=sqrt(rep_pc+rep_insch+pct+rd_ch+res);
        DCin=sqrt(rep_pc+rep_insin+pct+rd_in+res);
        % ventilation rate std uncertainty function
        [Qin,
DQin]=std_uncertainty_om(dP(i),j,T_in(i),RH_in(i));
        [Qairin, DQairin] = std_uncertainty_Qairin
(Qin,DQin,pb,T_in(i),RH_in(i));
        [Qairout, DQairout] = std_uncertainty_Qairout
(Qairin,DQairin,Ccho,Cino,Cch,Cin, DCch,DCin);
        %% EVALUATE EQUATIONS
        format long;
        % calculate gas mass flow recovered
        m_recs(i)=eval(m_rec);
        % calculate gas mass flow recovered std uncertainty
        Dm_recs(i)=eval(Dm_rec);
        % calculate gas mass flow injected
        m_inj_gravs(i)=eval(m_inj_grav);
        %% PREPARE OUTPUT MATRICES
        % collect parameter value used in calculations
        value(1,i)=Mass;
        value(2,i)=Tin;
        value(3,i)=Cch;
        value(4,i)=Cin;
        value(5,i)=Qin*60000;
        value(6,i)=Qairin;
        value(7,i)=Qairout;
        value(8,i)=m_recs(i);
        value(9,i)=Ccyl;
        value(10,i)= m_inj_gravs(i);
```

```
        value(11,i)=ytg;
        value(12,i)=yn2;
        % collect parameter absolute std uncertainty
        u_abs(1,i)=DTin;
        u_abs(2,i)=DCch;
        u_abs(3,i)=DCin;
        u_abs(4,i)=DQin*60000;
        u_abs(5,i)=DQairin;
        u_abs(6,i)=DQairout;
        u_abs(7,i)=Dm_recs(i);
        u_abs(8,i)=DMass;
        u_abs(9,i)=Dytg;
        u_abs(10,i)=Dyn2;
        % contributions to recovered mass flow std uncertainty
        m_rec_total=eval(Crec_Cch+Crec_Cin+Crec_Qin+Crec_Qout);
        % evaluate individual contributions
        contribution(1,i)=100*eval(Crec_Cch)/m_rec_total;
        contribution(2,i)=100*eval(Crec_Cin)/m_rec_total;
        contribution(3,i)=100*eval(Crec_Qin)/m_rec_total;
        contribution(4,i)=100*eval(Crec_Qout)/m_rec_total;
        % mass flow recovered std uncertainty
        contribution(5,i)=100*Dm_recs(i)/m_recs(i);
        m_inj_grac_total = eval(Cinj_grav_Mass+Cinj_grav_ytg+...
        Cinj_grav_yn2);
contribution(6,i)=100*eval(Cinj_grav_Mass)/m_inj_grac_total;
contribution(7,i)=100*eval(Cinj_grav_ytg)/m_inj_grac_total;
contribution(8,i)=100*eval(Cinj_grav_yn2)/m_inj_grac_total;
    end
    %% SSMRP ANALYSIS AND STD UNCERTAINTY ANALYSIS
    % calculate total mass recovered by integrating of SS period
    save('test.mat');
    m_rec_int=trapz(dt,m_recs);
    m_inj_gravf=mean(value(10,:));
    Dm_inj_gravf = eval(Dm_inj_grav);
    % calculate total mass recovered std uncertainty assume
root-sum
    % square
    for h=1:length(dt)-1
       Dmrec_temp1=(0.5*Dm_recs(h)*dt(h));
       Dmrec_temp2=(0.5*Dm_recs(h)*dt(h+1));
       Dmrec_temp3=(0.5*Dm_recs(h+1)*dt(h));
       Dmrec_temp4=(0.5*Dm_recs(h+1)*dt(h+1));
       Dm_rec_temp(h)=(-Dmrec_temp1+Dmrec_temp2-
   Dmrec_temp3+Dmrec_temp4)^2;
    end
    % mass recovered std uncertainty
    Dm_rec_int=sqrt(sum(Dm_rec_temp));
```

```
    % evaluate
    MRP_grav=eval(MRP_grav);
    DMRP_grav=eval(DMRP_grav) ;
    % preparing for output to excel
    mrp_total=eval(Cmrp_grav_minj_grav+Cmrp_grav_mrec_int);
    mrp_contribution(1,1)=m_rec_int;
    mrp_contribution(2,1)=Dm_rec_int;

mrp_contribution(3,1)=100*eval(Cmrp_grav_mrec_int)/mrp_total;
    mrp_contribution(4,1)=m_inj_gravf;
    mrp_contribution(5,1)=Dm_inj_gravf;

mrp_contribution(6,1)=100*eval(Cmrp_grav_minj_grav)/mrp_total;
    mrp_contribution(7,1)=MRP_grav;
    mrp_contribution(8,1)=DMRP_grav;
    mrp_contribution(9,1)=100*(DMRP_grav/MRP_grav);
     A={'Values'; 'Mass injected (g)';'Temperature incoming
(C)';...
         'Concentration chamber (ppmv)';'Concentration incoming
(ppmv)'; ...
         'Flow incoming (lpm)’; ...
         'Flow incoming STPD (lpm)';'Flow exhaust STPD (lpm)’;
...
         'Mass flow recovered (g h^-1)';'Concentration injected
(ppmv)’; ...
          'Mass injected (g)’; ...
          'Concentration of co2'; 'Concentration of N2'};
     xlswrite(output,A,j,'A1');
     xlswrite(output,value,j,'B2');
    B={'Absolute standard uncertainty'; 'u(Temperature incoming
   (C))';'u(Concentration chamber (ppmv))';...
         'u(Concentration incoming (ppmv))';'u(Flow incoming
(lpm))';...
         'u(Flow incoming STPD (lpm))';...
         'u(Flow exhaust STPD (lpm))';...
         'u(Mass flow recovered (g h^-1))';...
         'u(mass (g))'; 'u(Concentration of co2)';...
         'u(Concentration of N2)'};
     q=strcat('A',num2str(length(A)+2));
     w=strcat('B',num2str(length(A)+3));
     xlswrite(output,B,j,q);
     xlswrite(output,u_abs,j,w);
     C={'Relative contributions'; 'u(Concentration chamber
(%))';...
        'u(Concentration incoming (%))';'u(Flow incoming STPD
(%))';...
```

```
         'u(Flow exhaust STPD (%))'; 'Rel std u(Mass flow
recovered (%))';'u(Mass (%))';...
        'u(CO2 concentration (%))';'u(N2 concentration (%))' };
     e=strcat('A',num2str(length(A)+length(B)+3));
     s=strcat('B',num2str(length(A)+length(B)+4));
     xlswrite(output,C,j,e);
     xlswrite(output,contribution,j,s);
     D={'Mass recovery'; 'Mass recovered (g)';'u(Mass recovered
(g))';...
         'Mass recovered contribution (%)';
        'Mass grav(g)';'u(mass grav(g))';...
         'Mass grav contribution (%)'; 'Mass grav percent
(%)';...
         'u(Mass grav percent) (%)'; 'Rel std uncertainty (%)'};
     v=strcat('A',num2str(length(A)+length(B)+length(C)+4));
     h=strcat('B',num2str(length(A)+length(B)+length(C)+5));
     xlswrite(output,D,j,v);
     xlswrite(output,mrp_contribution,j,h);
    save(strcat(output,'.mat'));
end
end
```

APPENDIX C: ANALYSIS APPROACHES AND CODES OF CONSTANT GAS INJECTION METHOD

C.1 Introduction

MATLAB 2014a was used to process the data from LabVIEW program “Recovery (1.3). VI”. A series of calculation codes were deduced to calculate the mass recovered, mass injected, steady-state mass recovery percent, relative uncertainty and absolute standard uncertainty. The previous version were designed by Brett Carlos Ramirez which used moisture mass balance to establish the relationship between incoming moisture air flowrate and exhaust moisture air flowrate. The uncertainty analysis presented here was initially developed by Dr. Guilherme D.N. Maia and specific to the REMS.

“MR_std_uncertainty_SF6.m” was the main function which can generation an excel file of recovery results. This program was designed to evaluate the ventilation measurement. Therefore, the ratio of exhaust air flowrate to incoming air flowrate was assumed to be a constant (k=1). “MR_std_backward_SF6.m” was used for backward calculating the correction factor of ventilation under the assumption of mass recovered equals to mass injected. To execute main calculation codes, several support files were necessary：

MR_importfile.m

air_density_std_uncertainty.m

std_uncertainty_Qairin.m

std_uncertainty_rho_inj.m

std_uncertainty_om.m

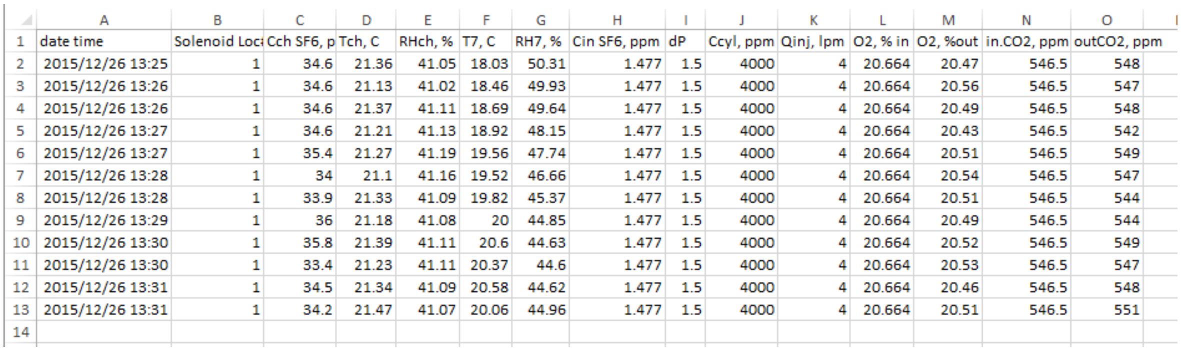
C.2 Data Preparation

C.2.1 Data Process in LabVIEW Program

Program “Recovery (1.3). VI” would automatically generate a data file (.CVS) for each single chamber and each test. The data collection began with 10 background (building) concentration measurements, then followed by 40 chamber concentration measurements and ended with 10 background chamber concentration measurements. The first 5 samples for each background were removed to avoid effects from transition between chamber and background. An estimated value was substituted with an average value of remaining 5 samples for both two background concentration measurements. Last 12 samples from chamber measurements will be used to represent gas concentration in steady state.

C.2.2 Reformat

LabVIEW generated a raw file under “C:\REMS\Data”, which was a comma-separated value text file (.CVS). Before importing the data into MATLAB, data were re-opened in Excel and each variable was automatically reformatted by separating into an independent column by Excel. Then, the date column and time column were combined together by using “date(year,month,date)+time” equation. This file contained all data from different chambers which were in time order. Next, the data were separated by to a new sheet for each chamber. The file was saved as Excel file (.xls).



	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O
1	date time	Solenoid Loc	Cch SF6, p	Tch, C	RHch, %	T7, C	RH7, %	Cin SF6, ppm	dP	Ccyl, ppm	Qinj, lpm	O2, % in	O2, %out	in.CO2, ppm	outCO2, ppm
2	2015/12/26 13:25	1	34.6	21.36	41.05	18.03	50.31	1.477	1.5	4000	4	20.664	20.47	546.5	548
3	2015/12/26 13:26	1	34.6	21.13	41.02	18.46	49.93	1.477	1.5	4000	4	20.664	20.56	546.5	547
4	2015/12/26 13:26	1	34.6	21.37	41.11	18.69	49.64	1.477	1.5	4000	4	20.664	20.49	546.5	548
5	2015/12/26 13:27	1	34.6	21.21	41.13	18.92	48.15	1.477	1.5	4000	4	20.664	20.43	546.5	542
6	2015/12/26 13:27	1	35.4	21.27	41.19	19.56	47.74	1.477	1.5	4000	4	20.664	20.51	546.5	549
7	2015/12/26 13:28	1	34	21.1	41.16	19.52	46.66	1.477	1.5	4000	4	20.664	20.54	546.5	547
8	2015/12/26 13:28	1	33.9	21.33	41.09	19.82	45.37	1.477	1.5	4000	4	20.664	20.51	546.5	544
9	2015/12/26 13:29	1	36	21.18	41.08	20	44.85	1.477	1.5	4000	4	20.664	20.49	546.5	544
10	2015/12/26 13:30	1	35.8	21.39	41.11	20.6	44.63	1.477	1.5	4000	4	20.664	20.52	546.5	549
11	2015/12/26 13:30	1	33.4	21.23	41.11	20.37	44.6	1.477	1.5	4000	4	20.664	20.53	546.5	547
12	2015/12/26 13:31	1	34.5	21.34	41.09	20.58	44.62	1.477	1.5	4000	4	20.664	20.46	546.5	548
13	2015/12/26 13:31	1	34.2	21.47	41.07	20.06	44.96	1.477	1.5	4000	4	20.664	20.51	546.5	551
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Figure C.1. The template of CGIM data before processing into the MATLAB.

C.3 MR_std_uncertainty_SF6.m

```
%% STEADY-STATE MASS RECOVERY TEST ANALYSIS AND STD UNCERTIANTY
%  Brett C. Ramirez (05-02-2014)/Revised by Jiangong Li (06-01-
2016)
%  Department of Agricultural and Biological Engineering
%  University of Illinois at Urbana-Champaign
%  calculates SSMRP and its associated std uncertainty
%  name= data file w/o extension (assumes '.xlsx' file type)
function MR_std_uncertainty_SF6(name)
% file naming and deleting of old files and matlab data
ext = '.xlsx';
output = strcat(name,'_out',ext);
delete(output);
warning('off','all');
% loop for chambers one thru six corresponding to Sheet1 thru
Sheet6 in
% excel file; thus, j=1:6
for j=1:6
    %% Data input
    % Reads columns of data from excel file and stores them in a
matrix
    % which is then arranged into its appropriate variables
    % read data from mass recovery test
    data_mat = MR_importfile(name,j);
    % convert datenum to h
    dt = [0 cumsum(diff(data_mat(:,1).*24))'];
    % chamber concentration at steady-state (ppmv)
    C_ch = data_mat(:,3);
    % chamber temperature (deg C)
    T_ch = data_mat(:,4);
    % chamber relative humidity (%)
    RH_ch = data_mat(:,5);
    % incoming temperature (deg C)
    T_in = data_mat(:,6);
```

```
     % incoming relative humidity (%)
    RH_in = data_mat(:,7);
    % mean incoming concentration before and after steady-state
(ppmv)
    C_in = data_mat(:,8);
    % orifice meter differential pressure (in wc)
    dP = data_mat(:,9);
    % gas cylinder concentration (ppmv)
    C_cyl = data_mat(:,10);
    % injected gas flow rate (lpm -> m^3 s^-1)
    Q_inj = data_mat(:,11)./60000;
    % incoming O2 concentration
    C_ino = data_mat(:,12)/100;
    % chamber O2 concentration
    C_cho = data_mat(:,13)/100;
    % incoming CO2 concentration at steady-state (ppmv)
    C_inCO2= data_mat(:,14)/1000000;
    % chamber concentration at steady-state (ppmv)
    C_chCO2 = data_mat(:,15)/1000000;
    chamber = strcat('Chamber',' ',num2str(j),' successfully
imported');
    disp(chamber);
    %%
    % loop for every row in the Sheet[j] i.e. for each steady-
state data
    % point
    for i=1:length(dt)
        %% SYMBOLIC REPRESENTATION
        % Prefix guide
        % D = absolute std uncertainty
        % C_ = contribution
        % chamber concentration
        syms Cch DCch Crec_Cch
        % incoming (background) concentration
        syms Cin DCin Crec_Cin
        % chamber temperature
        syms Tch DTch
        % incoming temperature
        syms Tin DTin Cinj_Tin
        % ventilation rate
        syms Qin DQin Crec_Qin Qairin DQairin Qairout  DQairout
        % injected volumetric flowrate
        syms Qinj DQinj Crec_Qinj Cinj_Qinj
        % Constant: barometric pressure
        syms pb
        % Constant: molecular weight
        syms M
```

```
        % Constant: universal gas constant
        syms R
        % std temperature and pressure
        syms Tstd pstd
        % injected gas cylinder concentration
        syms Ccyl DCcyl Cinj_Ccyl
        % mass flow injected
        syms m_inj Dm_inj
        % mass injected
        syms m_inj_int Dm_inj_int Cmrp_minj_int
        % mass flow recovered
        syms m_rec Dm_rec
        % mass recovered
        syms m_rec_int Dm_rec_int Cmrp_mrec_int
        % steady-state mass recovery percent
        syms MRP DMRP
        %% EQUATION FOR MR ANALYSIS
        % injected gas mass flow equation (L h^-1)
        m_inj=Qinj*((Tin+273.15)/Tstd)*(pstd/pb)*3600*Ccyl*1E-
6*((22.414*pb)...
            /(R*(Tin+273.15)));
        % recovered gas mass flow equation (L h^-1)
        m_rec=(1.01*(Qairout)*Cch-Qairin*Cin)*60*1E-6;
        % steady-state mass recovery percent (%)
        MRP=100*(m_rec_int/m_inj_int);

        %% PARAMETER INDIVIDUAL CONTRIBUTION
        % injected mass flow std uncertainty parameter
contributions
        Cinj_Qinj=(DQinj*diff(m_inj,Qinj))^2;
        Cinj_Ccyl=(DCcyl*diff(m_inj,Ccyl))^2;
        Cinj_Tin=(DTin*diff(m_inj,Tin))^2;
        % recovered mass flow std uncertainty parameter
contributions
        Crec_Cch=(DCch*diff(m_rec,Cch))^2;
        Crec_Cin=(DCin*diff(m_rec,Cin))^2;
        Crec_Qinj=(DQinj*diff(m_rec,Qinj))^2;
        Crec_Qin=(DQairin*diff(m_rec,Qairin))^2;

        % individual contributions to mass recovery percent std
        % uncertainty after integration over steady-state
        Cmrp_mrec_int=(Dm_rec_int*diff(MRP,m_rec_int))^2;
        Cmrp_minj_int=(Dm_inj_int*diff(MRP,m_inj_int))^2;

        %% STD UNCERTAINTY EQNS

        % std uncertainty of injected gas mass flow
```

```
        Dm_inj=sqrt(Cinj_Ccyl+Cinj_Tin+Cinj_Qinj);
        % std uncertainty of recovered gas mass flow
        Dm_rec=sqrt(Crec_Cch+Crec_Cin+Crec_Qinj+Crec_Qin);
        % std uncertainty of ss mass recovery percent
        DMRP=sqrt(Cmrp_mrec_int+Cmrp_minj_int);
        %DMRP=sqrt(Cmrp_mrec_int+Cmrp_minj_int);

        %% DETERMINATION OF STD UNCERTAINTY FOR EACH PARAMETER
        % store data corresponding to row[i] into symbolic
variable for
        % evaluation later
        Tin=T_in(i);
        Cch=C_ch(i);
        Cin=C_in(i);
        Qinj=Q_inj(i);
        Ccyl=C_cyl(i);
        Co2in=C_ino(i);
        Cco2in=C_inCO2(i);
        Co2ch=C_cho(i);
        Cco2ch=C_chCO2(i);
        % define constants with assumed negligible contribution
to
        % std uncertainty
        % barometric pressure (Pa) (ASHRAE 2013, Chp 1, Eqn 3)
        pb=101117;
        % std temperature (K)
        Tstd=273.15;
        % std pressure (Pa)
        pstd=101325;
        % ideal gas constant (m^3 Pa K^-1 mol^-1)
        R=8.314;
        % ventilation rate std uncertainty function
        [Qin,
DQin]=std_uncertainty_om(dP(i),j,T_in(i),RH_in(i));
        [Qairin, DQairin] = std_uncertainty_Qairin
(Qin,DQin,pb,T_in(i),RH_in(i));
        [Qairout, DQairout] = std_uncertainty_Qairout (Qairin,
DQairin, Co2in, Cco2in, Co2ch, Cco2ch);
        % temperature uncertainty
        DTin=.5;
        % primary certified gas cylinder concentration std
uncertainty

DCcyl=sqrt((.01*C_cyl(i))/sqrt(3)+((.01*C_cyl(i))/sqrt(3)));
        % inject gas flow std uncertainty
        DQinj=4.19716E-07;
```

```
% individual components of gas analyzer std uncertainty
% post calibration repeatability
rep_pc=(0.005*.016)^2;
% instrument repeatability
rep_insch=((0.01*C_ch(i))/(3^0.5))^2;
rep_insin=((0.01*C_in(i))/(3^0.5))^2;
% primary certified tolerance
pct=((0.01*40)/(3^0.5))^2;
% range drift
rd_ch=((0.025*C_ch(i))/(3^0.5))^2;
rd_in=((0.025*C_in(i))/(3^0.5))^2;
% resolution
res=((5*0.006)/(3^0.5))^2;

% propagation of gas analyzer std uncertainty
DCch=sqrt(rep_pc+rep_insch+pct+rd_ch+res);
DCin=sqrt(rep_pc+rep_insin+pct+rd_in+res);
%% EVALUATE EQUATIONS
format long;
% calculate gas mass flow recovered
m_recs(i)=eval(m_rec);
% calculate gas mass flow recovered std uncertainty
Dm_recs(i)=eval(Dm_rec);
% calculate gas mass flow injected
m_injs(i)=eval(m_inj);
% calculate gas mass flow injected std uncertainty
Dm_injs(i)=eval(Dm_inj);
%% PREPARE OUTPUT MATRICES
% collect parameter value used in calculations
value(1,i)=Tin;
value(2,i)=Cch;
value(3,i)=Cin;
value(4,i)=Qin*60000;
value(5,i)=Qinj*60000;
value(6,i)=Qairin;
value(7,i)=m_recs(i);
value(8,i)=Tin;
value(9,i)=Ccyl;
value(10,i)=Qinj*60000;
value(11,i)=m_injs(i);
% collect parameter absolute std uncertainty
u_abs(1,i)=DCin;
u_abs(2,i)=DQin;
u_abs(3,i)=DQairin*60000;
u_abs(4,i)=DQinj*60000;
u_abs(5,i)=Dm_recs(i);
u_abs(6,i)=DTin;
```

```
        u_abs(7,i)=DCcyl;
        u_abs(8,i)=DQinj*60000;
        u_abs(9,i)=Dm_injs(i);
        % contributions to recovered mass flow std uncertainty
        m_rec_total=eval(Crec_Cch+Crec_Cin+Crec_Qinj+...
            Crec_Qin);
        % evaluate individual contributions
        contribution(1,i)=100*eval(Crec_Cch)/m_rec_total;
        contribution(2,i)=100*eval(Crec_Cin)/m_rec_total;
        contribution(3,i)=100*eval(Crec_Qin)/m_rec_total;
        contribution(4,i)=100*eval(Crec_Qinj)/m_rec_total;
        % mass flow recovered std uncertainty
        contribution(5,i)=100*Dm_recs(i)/m_recs(i);
         % contributions to injected mass flow std uncertainty
        m_inj_total=eval(Cinj_Qinj+Cinj_Ccyl+Cinj_Tin);
        % evaluate individual contributions
        contribution(6,i)=100*eval(Cinj_Tin)/m_inj_total;
        contribution(7,i)=100*eval(Cinj_Ccyl)/m_inj_total;
        contribution(8,i)=100*eval(Cinj_Qinj)/m_inj_total;
        % mass flow injected std uncertainty
        contribution(9,i)=100*Dm_injs(i)/m_injs(i);
    end
    %% SSMRP ANALYSIS AND STD UNCERTAINTY ANALYSIS
    % calculate total mass recovered by integrating of SS period
    m_rec_int=trapz(dt,m_recs);
    m_inj_int=trapz(dt,m_injs);
    % calculate total mass recovered std uncertainty assume
root-sum
    % square
    for h=1:length(dt)-1
    Dmrec_temp1=(0.5*Dm_recs(h)*dt(h));
    Dmrec_temp2=(0.5*Dm_recs(h)*dt(h+1));
    Dmrec_temp3=(0.5*Dm_recs(h+1)*dt(h));
    Dmrec_temp4=(0.5*Dm_recs(h+1)*dt(h+1));
    Dm_rec_temp(h)=(-Dmrec_temp1+Dmrec_temp2-
Dmrec_temp3+Dmrec_temp4)^2;

    Dminj_temp1=(0.5*Dm_injs(h)*dt(h));
    Dminj_temp2=(0.5*Dm_injs(h)*dt(h+1));
    Dminj_temp3=(0.5*Dm_injs(h+1)*dt(h));
    Dminj_temp4=(0.5*Dm_injs(h+1)*dt(h+1));
    Dm_inj_temp(h)=(-Dminj_temp1+Dminj_temp2-
Dminj_temp3+Dminj_temp4)^2;
    end
    % mass recovered std uncertainty
    Dm_rec_int=sqrt(sum(Dm_rec_temp));
    % mass injected std uncertainty
```

```
    Dm_inj_int=sqrt(sum(Dm_inj_temp));
    % evaluate
    MRP=eval(MRP);
    DMRP=eval(DMRP);
    % preparing for output to excel
    % before computing reproducibility uncomment the following
line
    mrp_total=eval(Cmrp_mrec_int+Cmrp_minj_int);
    mrp_contribution(1,1)=m_rec_int;
    mrp_contribution(2,1)=Dm_rec_int;
    mrp_contribution(3,1)=100*eval(Cmrp_mrec_int)/mrp_total;
    mrp_contribution(4,1)=m_inj_int;
    mrp_contribution(5,1)=Dm_inj_int;
    mrp_contribution(6,1)=100*eval(Cmrp_minj_int)/mrp_total;
    mrp_contribution(7,1)=MRP;
    mrp_contribution(8,1)=DMRP;
    mrp_contribution(9,1)=(DMRP/MRP)*100;
    func_out(1,j)=MRP;
    func_out(2,j)=DMRP;
    %% FORMATING FOR OUTPUT
    A={'Values’; ‘Temperature incoming (C)';...
        'Concentration chamber (ppmv)';'Concentration incoming
(ppmv)'; ...
        'Flow incoming (lpm)';'Flow injected STPD (lpm)’; ...
        'Flow incoming STPD (lpm)';'Mass flow recovered (g h^-
1)’; ...
        'Temperature incoming (C)';'Concentration injected
(ppmv)’; ...
        'Flow injected (lpm)'; 'Mass flow injected (l h^-1)'};
    xlswrite(output,A,j,'A1');
    xlswrite(output,value,j,'B2');
     B={'Absolute standard uncertainty'; 'u(Concentration
incoming    (ppmv))';'u(Flow incoming (lpm))';'u(Flow incoming
STPD (lpm))';...
        'u(Flow injected STPD(lpm))';'u(Mass flow recovered (g
h^-1))';...
        'u(Temperature incoming (C))';'u(Concentration injected
(ppmv))';...
        'u(Flow injected STPD (lpm))'; 'u(Mass flow injected (l
h^-1))'};
    q=strcat('A',num2str(length(A)+2));
    w=strcat('B',num2str(length(A)+3));
    xlswrite(output,B,j,q);
    xlswrite(output,u_abs,j,w);
    C={'Relative contributions'; 'u(Concentration chamber
(%))';...
```

```
        'u(Concentration incoming (%))';'u(Flow incoming STPD
(%))';...
        'u(Flow injected STPD (%))';'Rel std u(Mass flow
recovered (%))';...
        'u(Temperature incoming (%))';'u(Concentration injected
(%))';...
        'u(Flow injected STPD (%))'; 'Rel std u(Mass flow
injected (%))'};
    e=strcat('A',num2str(length(A)+length(B)+3));
    s=strcat('B',num2str(length(A)+length(B)+4));
    xlswrite(output,C,j,e);
    xlswrite(output,contribution,j,s);
    D={'Mass recovery'; 'Mass recovered (l)';'u(Mass recovered
(l))';...
        'Mass recovered contribution (%)';'Mass injected (l)’;
...
        'u(Mass injected(l))';'Mass injected contribution
(%)';...
        'Mass recovery percent (%)'; 'u(Mass recovery percent)
(%)';...
        'Rel std uncertainty (%)'};
    v=strcat('A',num2str(length(A)+length(B)+length(C)+4));
    h=strcat('B',num2str(length(A)+length(B)+length(C)+5));
    xlswrite(output,D,j,v);
    xlswrite(output,mrp_contribution,j,h);
    % variables
    save(strcat(output,'.mat'));
end
end
```

APPENDIX D: CALCULATION APPROACHES AND CODES OF HEAT PRODUCTION AND GAS EXCHANGES

D.1 Introduction

MATLAB 2014a was used in processing the data from LabVIEW program “Study (1.4). VI”. The heat production calculation followed Brouwer-Equation, which were described in chapter 4. The codes were deduced to calculate the heat production, moisture production, respiratory quotient, methane emission, oxygen consumption, carbon dioxide emission and ammonia emission. For each parameter mentioned above, three integrity methods were used, which were Trapezoidal integration, Left Riemann Sum integration, and Approximation integration. In addition, the hourly heat production, moisture production, and respiratory quotient data were presented in the results.

“REMS_HP_calc.m” was used for calculating heat and moisture production. “REMS_ER_RQ_calc.m” was used for calculating methane, ammonia, oxygen, carbon dioxide, and respiratory quotient. To execute these calculation, several support files were necessary:

MR_importfile.m
std_uncertainty_QairSTPD.m
air_humidity_ratio.m
daily_E_std_uncertainty.m
daily_RQ_std.m
subsamp_HPR_integration.m
subsamp_MPR_integration.m
leftsum.m

D.2 Data Preparation

D.2.1 Data Process in LabVIEW Program

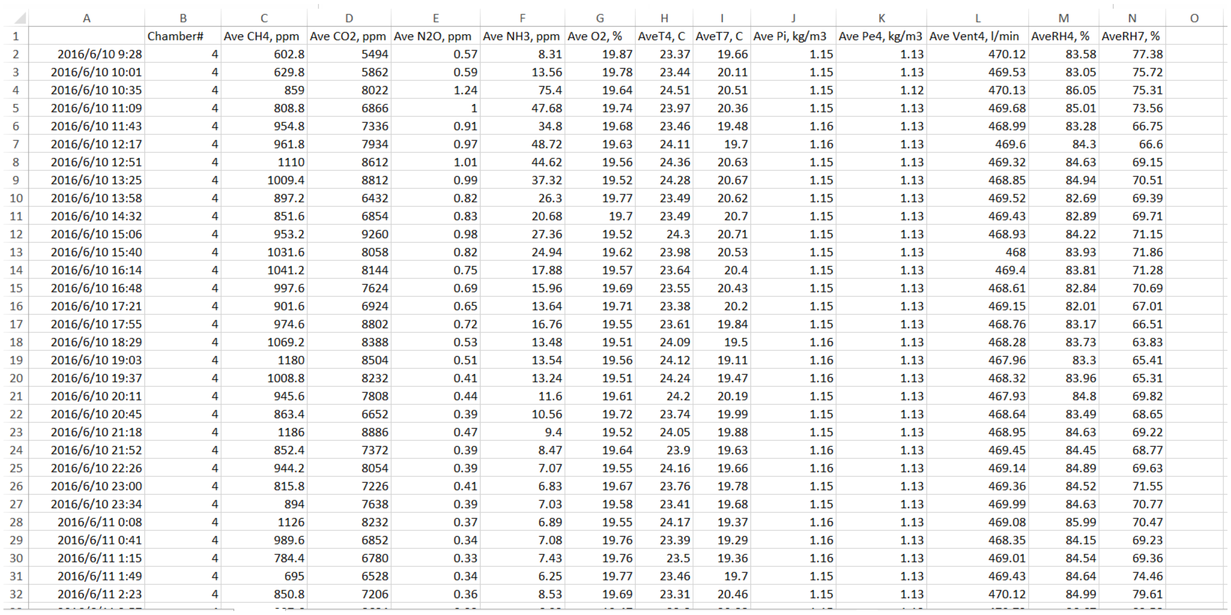
After data were collected and saved by program “Study (1.4). VI”, data still needed further preparation before processing into MATLAB. The data collection began with 10 background (building) concentration measurements. Feed was only introduced once at the beginning of the experiment, and enough food was provided for 24 hours. Each animal was monitored for 24 hours. Feed was placed into the chamber when system started sampling next chamber. This was to ensure the motion of adding feed does not influence sampling arriving at steady state.

Program “Study (1.4). VI” automatically save two types of data for further data processing. Raw data was automatically saved under “C:\REMS\Data”. The file included each individual samples for each animal and each chamber in 43.33s. Another file was automatically saved under “C:\REMS\EData”, which was the average value for each animal in one cycle measurement. Since the system cycled through all chambers before returning to the first chamber, each value represented the average value for each cycle time which depended on sample sequences.

The first 5 samples for each background and chamber sample were removed to avoid effects from transition between chamber and background. An estimated value was substituted for those samples in calculations. The remaining 5 samples from the background were averaged to obtain a single background concentration. Linear interpolation was done between each background and provided background concentrations for the full 24-hour period, used to calculate emissions rates. The remaining five gas concentrations from each chamber were averaged to represent the gas concentration for one cycle measurement.

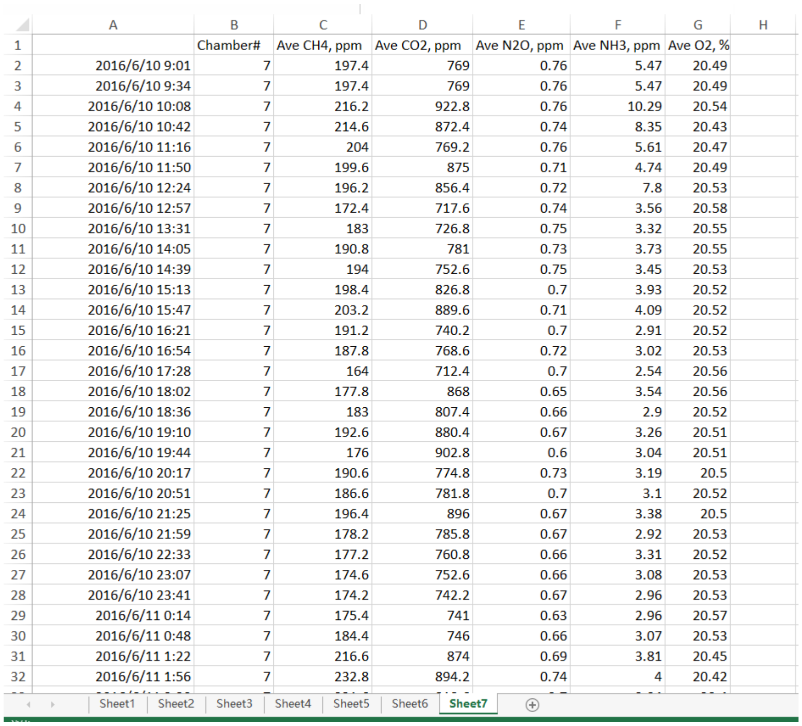
D.2.2 Reformat

LabVIEW generated a raw file including all 24 hours' measurement under "C:\REMS\EData", which was a comma-separated value text file (.CVS). Before importing the data into MATLAB, data were re-opened in Excel and each variable was automatically reformatted by separating into an independent column by Excel. Then, the date column and time column were combined together by using "date(year,month,date)+time" equation. This file contained all data from different chambers which were in time order. Next, the data were separated by chamber using data sort by the chamber column and copied to a new sheet for each chamber. Background samples were also put into an individual sheet. The file was saved as Excel file (.xls).



	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1		Chamber#	Ave CH4, ppm	Ave CO2, ppm	Ave N2O, ppm	Ave NH3, ppm	Ave O2, %	AveT4, C	AveT7, C	Ave Pi, kg/m3	Ave Pe4, kg/m3	Ave Vent4, l/min	AveRH4, %	AveRH7, %
2	2016/6/10 9:28	4	602.8	5494	0.57	8.31	19.87	23.37	19.66	1.15	1.13	470.12	83.58	77.38
3	2016/6/10 10:01	4	629.8	5862	0.59	13.56	19.78	23.44	20.11	1.15	1.13	469.53	83.05	75.72
4	2016/6/10 10:35	4	859	8022	1.24	75.4	19.64	24.51	20.51	1.15	1.12	470.13	86.05	75.31
5	2016/6/10 11:09	4	808.8	6866	1	47.68	19.74	23.97	20.36	1.15	1.13	469.68	85.01	73.56
6	2016/6/10 11:43	4	954.8	7336	0.91	34.8	19.68	23.46	19.48	1.16	1.13	468.99	83.28	66.75
7	2016/6/10 12:17	4	961.8	7934	0.97	48.72	19.63	24.11	19.7	1.16	1.13	469.6	84.3	66.6
8	2016/6/10 12:51	4	1110	8612	1.01	44.62	19.56	24.36	20.63	1.15	1.13	469.32	84.63	69.15
9	2016/6/10 13:25	4	1009.4	8812	0.99	37.32	19.52	24.28	20.67	1.15	1.13	468.85	84.94	70.51
10	2016/6/10 13:58	4	897.2	6432	0.82	26.3	19.77	23.49	20.62	1.15	1.13	469.52	82.69	69.39
11	2016/6/10 14:32	4	851.6	6854	0.83	20.68	19.7	23.49	20.7	1.15	1.13	469.43	82.89	69.71
12	2016/6/10 15:06	4	953.2	9260	0.98	27.36	19.52	24.3	20.71	1.15	1.13	468.93	84.22	71.15
13	2016/6/10 15:40	4	1031.6	8058	0.82	24.94	19.62	23.98	20.53	1.15	1.13	468	83.93	71.86
14	2016/6/10 16:14	4	1041.2	8144	0.75	17.88	19.57	23.64	20.4	1.15	1.13	469.4	83.81	71.28
15	2016/6/10 16:48	4	997.6	7624	0.69	15.96	19.69	23.55	20.43	1.15	1.13	468.61	82.84	70.69
16	2016/6/10 17:21	4	901.6	6924	0.65	13.64	19.71	23.38	20.2	1.15	1.13	469.15	82.01	67.01
17	2016/6/10 17:55	4	974.6	8802	0.72	16.76	19.55	23.61	19.84	1.15	1.13	468.76	83.17	66.51
18	2016/6/10 18:29	4	1069.2	8388	0.53	13.48	19.51	24.09	19.5	1.16	1.13	468.28	83.73	63.83
19	2016/6/10 19:03	4	1180	8504	0.51	13.54	19.56	24.12	19.11	1.16	1.13	467.96	83.3	65.41
20	2016/6/10 19:37	4	1008.8	8232	0.41	13.24	19.51	24.24	19.47	1.16	1.13	468.32	83.96	65.31
21	2016/6/10 20:11	4	945.6	7808	0.44	11.6	19.61	24.2	20.19	1.15	1.13	467.93	84.8	69.82
22	2016/6/10 20:45	4	863.4	6652	0.39	10.56	19.72	23.74	19.99	1.15	1.13	468.64	83.49	68.65
23	2016/6/10 21:18	4	1186	8886	0.47	9.4	19.52	24.05	19.88	1.15	1.13	468.95	84.63	69.22
24	2016/6/10 21:52	4	852.4	7372	0.39	8.47	19.64	23.9	19.63	1.16	1.13	469.45	84.45	68.77
25	2016/6/10 22:26	4	944.2	8054	0.39	7.07	19.55	24.16	19.66	1.16	1.13	469.14	84.89	69.63
26	2016/6/10 23:00	4	815.8	7226	0.41	6.83	19.67	23.76	19.78	1.15	1.13	469.36	84.52	71.55
27	2016/6/10 23:34	4	894	7638	0.39	7.03	19.58	23.41	19.68	1.15	1.13	469.99	84.63	70.77
28	2016/6/11 0:08	4	1126	8232	0.37	6.89	19.55	24.17	19.37	1.16	1.13	469.08	85.99	70.47
29	2016/6/11 0:41	4	989.6	6852	0.34	7.08	19.76	23.39	19.29	1.16	1.13	468.35	84.15	69.23
30	2016/6/11 1:15	4	784.4	6780	0.33	7.43	19.76	23.5	19.36	1.16	1.13	469.01	84.54	69.36
31	2016/6/11 1:49	4	695	6528	0.34	6.25	19.77	23.46	19.7	1.15	1.13	469.43	84.64	74.46
32	2016/6/11 2:23	4	850.8	7206	0.36	8.53	19.69	23.31	20.46	1.15	1.13	470.12	84.99	79.61

Figure D.1. The data template of chamber for heat production calculation before processing into the MATLAB.



	A	B	C	D	E	F	G	H
1		Chamber#	Ave CH4, ppm	Ave CO2, ppm	Ave N2O, ppm	Ave NH3, ppm	Ave O2, %	
2	2016/6/10 9:01	7	197.4	769	0.76	5.47	20.49	
3	2016/6/10 9:34	7	197.4	769	0.76	5.47	20.49	
4	2016/6/10 10:08	7	216.2	922.8	0.76	10.29	20.54	
5	2016/6/10 10:42	7	214.6	872.4	0.74	8.35	20.43	
6	2016/6/10 11:16	7	204	769.2	0.76	5.61	20.47	
7	2016/6/10 11:50	7	199.6	875	0.71	4.74	20.49	
8	2016/6/10 12:24	7	196.2	856.4	0.72	7.8	20.53	
9	2016/6/10 12:57	7	172.4	717.6	0.74	3.56	20.58	
10	2016/6/10 13:31	7	183	726.8	0.75	3.32	20.55	
11	2016/6/10 14:05	7	190.8	781	0.73	3.73	20.55	
12	2016/6/10 14:39	7	194	752.6	0.75	3.45	20.53	
13	2016/6/10 15:13	7	198.4	826.8	0.7	3.93	20.52	
14	2016/6/10 15:47	7	203.2	889.6	0.71	4.09	20.52	
15	2016/6/10 16:21	7	191.2	740.2	0.7	2.91	20.52	
16	2016/6/10 16:54	7	187.8	768.6	0.72	3.02	20.53	
17	2016/6/10 17:28	7	164	712.4	0.7	2.54	20.56	
18	2016/6/10 18:02	7	177.8	868	0.65	3.54	20.56	
19	2016/6/10 18:36	7	183	807.4	0.66	2.9	20.52	
20	2016/6/10 19:10	7	192.6	880.4	0.67	3.26	20.51	
21	2016/6/10 19:44	7	176	902.8	0.6	3.04	20.51	
22	2016/6/10 20:17	7	190.6	774.8	0.73	3.19	20.5	
23	2016/6/10 20:51	7	186.6	781.8	0.7	3.1	20.52	
24	2016/6/10 21:25	7	196.4	896	0.67	3.38	20.5	
25	2016/6/10 21:59	7	178.2	785.8	0.67	2.92	20.53	
26	2016/6/10 22:33	7	177.2	760.8	0.66	3.31	20.52	
27	2016/6/10 23:07	7	174.6	752.6	0.66	3.08	20.53	
28	2016/6/10 23:41	7	174.2	742.2	0.67	2.96	20.53	
29	2016/6/11 0:14	7	175.4	741	0.63	2.96	20.57	
30	2016/6/11 0:48	7	184.4	746	0.66	3.07	20.53	
31	2016/6/11 1:22	7	216.6	874	0.69	3.81	20.45	
32	2016/6/11 1:56	7	232.8	894.2	0.74	4	20.42	

Figure D.2. The data template of barn for heat production calculation before processing into the MATLAB.

D.3 REMS_HP_calc.m

```
%% HEAT PRODUCTION CALCULATION
%    Jiangong Li (04-28-2016)
%    Department of Agricultural and Biological Engineering
%    University of Illinois at Urbana-Champaign
%
%    Calculates heat production for moisture balance for 24 h
%    sampling representation
%  OUTPUTS
%data_out= has daily E and u(E) in matrix in matlab
%INPUTS
%  name {''}= data filename w/o extension in a cell (.xlsx)
%%
function [data_out]=REMS_HP_calc(name)
%% Initializing
% close open figures
close all;
% disable warning messages
warning('off','all')
% define data file extension
ext = '.xlsx';
% convert file name to matrix string
name = cell2mat(name);
```

```
% create output file name (appends '_out')
output = strcat(name,'_out',ext);
% delete existing output file
delete(output)
% delete existing variables
delete(name)
%% ER Uncertainty [u(ER)]
% symbolic representation for each parameter for ER uncertainty
syms Tch Tin O2 CO2 CH4 Vdot_in din dch...
     R pb pstd Tstd o2 co2 ch4
% constants
% symbolic representation for abs std uncertainty
syms Vin_STDP RHin RHch Wi Wch rhoin
% symbolic representation for the contributions
syms C_Tch C_Tin C_Cch C_Cin C_Vdot_in C_din C_dch ER1
% symbolic representation for oxygen and carbon dioxide
concentration
syms C_ch_o2 C_ch_co2 C_in_o2 C_in_co2  C_ch_ch4 C_in_ch4
syms HPR AlphaS alphaS MP

O2=Vin_STDP*(C_in_o2-alphaS*C_ch_o2)*1E-6;
CO2=Vin_STDP*(C_ch_co2-alphaS*C_in_co2)*1E-6;
CH4=Vin_STDP*(C_ch_ch4-alphaS*C_in_ch4)*1E-6;
AlphaS=1.01*(1-(C_in_o2+C_in_co2+C_in_ch4)*1E-6)/(1-
(C_ch_o2+C_ch_co2+C_ch_ch4)*1E-6);
% heat production rate (w)
HPR=16.18*o2+5.02*co2-2.17*ch4;
%moisture production rate (mg/s)
MP=1000*Vdot_in*rhoin*(alphaS*Wch-Wi)/60;

%% Constants
% barometric pressure (Pa) (ASHRAE 2013, Chp1, Eqn 3)
pb = 100914;
% ideal gas constant (m^3 Pa mol^-1 K)
R = 8.3144621;
% constant (K) for ER eqn
K =(pb*1E-6)/R;
% molar mass of GHG (kg mol^-1)
%     CH4     CO2     NH3      N2O      O2
MM =[0.01604 0.04401 0.044013 0.017031 0.032];
% time interval (s) to compare to the daily emissions
rep = [1:1:22]*3600;
kfactor = [1.08 1 1.21 1 1 1];
% row header for data organization
row_head = {'Chamber 1', 'Chamber 2', 'Chamber 3', 'Chamber 4’,
...
    'Chamber 5', 'Chamber 6'};
```

```
row_head1 = {'time (h)','Chamber 1 (w)', 'Chamber 2 (w) ',
'Chamber 3 (w)', 'Chamber 4 (w)’, ...
    'Chamber 5 (w)', 'Chamber 6 (w)'};
row_head2 = {'Chamber (mg/s)','Chamber 1 (mg/s)', 'Chamber 2
(mg/s) ', 'Chamber 3 (mg/s)', 'Chamber 4 (mg/s)’, ...
    'Chamber 5 (mg/s)', 'Chamber 6 (mg/s)'};
%% Import Data
% loop thru 7 sheets {CH1... CH6, bckgnd} and store data in
cells
for chamber = 1:7
    % import data for one chamber
    data_mat = importfile2(name,chamber);
    % convert dd/mm/yyyy hh:mm:ss to do cumulative sum £¨s)
    time_mat{1,chamber} = [0
cumsum(diff(data_mat(:,1).*24*3600))'];
    % store vectors of GHG concentration data in cell rows
(ppmv)
    for ii = 1:4
        C_mat{ii,chamber} = data_mat(:,ii+2);
    end
    % O2 concentration (ppmv)
        C_mat{5,chamber} = data_mat(:,7)*10000;
    if chamber ~= 7
        % mean incoming temperature (deg C)
        T_in_mat{1,chamber} = data_mat(:,9);
        % mean chamebr temperature (deg C)
        T_ch_mat{1,chamber} = data_mat(:,8);
        % mean incoming moist air density (kg m^-3)
        rho_in_mat{1,chamber} = data_mat(:,10);
        % mean chamber moist air density (kg m^-3)
        rho_ch_mat{1,chamber} = data_mat(:,11);
        % mean incoming ventilation rate (lpm)
        vent_mat{1,chamber} = data_mat(:,12);
        % mean chamber relative humidity (%)
        RH_ch_mat{1,chamber} = data_mat(:,13);
        % mean incoming relative humidity (%)
        RH_in_mat{1,chamber} = data_mat(:,14);
    end
end
%% ER & Associated Uncertainty Calculation
% loop thru {CH1... CH6}
for chamber = 1:4
    % loop for every row (h) of data
    for h = 1:length(time_mat{1,chamber})
        Vdot_in = vent_mat{1,chamber}(h);
        RHin = RH_in_mat{1,chamber}(h);
        Tin = T_in_mat{1,chamber}(h);
```

```
         Rhoin =rho_in_mat{1,chamber}(h);
         RHch = RH_ch_mat{1,chamber}(h);
         Tch = T_ch_mat{1,chamber}(h);
         % ventilation rate at STPD
        [Qairin] = std_uncertainty_QairSTPD
(Vdot_in,pb,Tin,RHin);
        Vin_STDP=kfactor(chamber)*Qairin;
        [wi] = air_humidity_ratio (Tin, RHin,pb);
        [wch] = air_humidity_ratio (Tch, RHch,pb);
        % loop thru 5 GHGs (ii)
        for ii = 1:5
        % linear interpolation for background gas concentration
        C_i{ii,chamber}(h)=
interp1(time_mat{1,7},C_mat{ii,7},...
                time_mat{1,chamber}(h));
        end
        C_in_o2=C_i{5,chamber}(h);
        C_ch_o2=C_mat{5,chamber}(h);
        C_ch_co2=C_mat{2,chamber}(h);
        C_in_co2=C_i{2,chamber}(h);
        C_ch_ch4=C_mat{1,chamber}(h);
        C_in_ch4=C_i{1,chamber}(h);
        Wi=wi;
        Wch=wch;
        rhoin=Rhoin;
        % HP (w)
        alphaS=eval(AlphaS);
        int_MP(h,chamber)=eval(MP);
        o2=eval(O2);
        co2=eval(CO2);
        ch4=eval(CH4);
        int_HP(h,chamber)=eval(HPR);
    end
end
% initialize empty vectors
int_meth = [];
data_out = [];
for chamber = 1:4
    %% Integration Methods Analysis
    % redefine cumsum of time as x
    x = time_mat{1,chamber};
     % redefine HPR as z
    y=int_HP(:,chamber);
    z=int_MP(:,chamber);
    %calculate HPR for each chamber of different integration
methods
    [int_hpr,int_str_hpr]=subsamp_HPR_integration(x,y);
```

```
    [int_mpr,int_str_mpr] = subsamp_MPR_integration(x,z);

    for j=1:3
    hpr_mat(j,chamber)= int_hpr(j,1);
    mpr_mat(j,chamber)= int_mpr(j,1);
    end

    % clear vector data for next iteration
    y = [];
    x = [];
end
%% Preparing Output
for i = 1:3
     for chamber = 1:4
        int_hpr_out{i,chamber+1} =hpr_mat(i,chamber);
        int_hpr_out{i,1} = int_str_hpr{i,1};
        int_mpr_out{i,chamber+1} =mpr_mat(i,chamber);
        int_mpr_out{i,1} = int_str_mpr{i,1};
    end
end

for chamber = 1:4
    for i = 1:length(time_mat{1,chamber});
        in_HP_out{i,chamber+1} =int_HP(i,chamber);
        in_HP_out{i,1} = i*0.84;
        in_MP_out{i,1} = i*0.84;
        in_MP_out{i,chamber+1} =int_MP(i,chamber);
    end
end

%% Write to Excel File
xlswrite(output,row_head,1,'B1')
xlswrite(output,int_hpr_out,1,'A2')
xlswrite(output,row_head1,1,'A6')
xlswrite(output,in_HP_out,1,'A7')

xlswrite(output,row_head,2,'B1')
xlswrite(output,int_mpr_out,2,'A2')
xlswrite(output,row_head2,2,'A6')
xlswrite(output,in_MP_out,2,'A7')
range_count = 1;
% for j = 1:no_int_meth-1
%     range_str = strcat('A',num2str(range_count));
%     xlswrite(output,p_diff{1,j},3,range_str)
%     range_count = range_count+no_int_meth+1;
% end
% save variables
```

```
save(name)
end
```

D.4 REMS_ER_RQ_calc.m

```
%% [data_out]=REMS_ER_RQ_calc(name,graph)
%       Jiangong Li (08-24-2015)
%       Department of Agricultural and Biological Engineering
%       University of Illinois at Urbana-Champaign
%
%       Calculate respiratory quotient quit and perform
subsampling for 24 h
%       sampling representation
%
%       OUTPUTS
%       data_out    = has daily E and u(E) in matrix in matlab
%
%       INPUTS*
%       name        = data filename w/o extension in a cell
(.xlsx)
%       graph       = controls plotting: 1-on; 0-off
% * data is from LabVIEW Project (4) and must be preformatted
%%

function [data_out]=REMS_ER_RQ_calc(name,graph)
%% Initializing
% close open figures
close all;
% disable warning messages
warning('off','all')
% define data file extension
ext = '.xlsx';
% convert file name to matrix string
name = cell2mat(name);
% create output file name (appends '_out')
output = strcat(name,'_rqNout',ext);
% delete existing output file
delete(output)
% delete existing variables
delete(name)
%% ER Uncertainty [u(ER)]
% symbolic representation for each parameter for ER uncertainty
syms Tch Tin O2 CO2 CH4 NH3 Vdot_in din dch...
    R pb pstd Tstd  % constants
% symbolic representation for abs std uncertainty
```

```
syms Vin_STDP
% symbolic representation for the contributions
syms C_Tch C_Tin C_Cch C_Cin C_Vdot_in C_din C_dch ER1
% symbolic representation for oxygen and carbon dioxide
concentration
syms C_ch_o2 C_ch_co2 C_in_o2 C_in_co2  C_ch_ch4 C_in_ch4
C_ch_NH3 C_in_NH3
syms HPR AlphaS alphaS

O2=Vin_STDP*(C_in_o2-alphaS*C_ch_o2)*1E-6;
CO2=Vin_STDP*(C_ch_co2-alphaS*C_in_co2)*1E-6;
CH4=Vin_STDP*(C_ch_ch4-alphaS*C_in_ch4)*1E-6;
NH3=Vin_STDP*(C_ch_NH3-alphaS*C_in_NH3)*1E-6;
AlphaS=1.01*(1-(C_in_o2+C_in_co2+C_in_ch4)*1E-6)/(1-
(C_ch_o2+C_ch_co2+C_ch_ch4)*1E-6);


%% Constants
% barometric pressure (Pa) (ASHRAE 2013, Chp1, Eqn 3)
pb = 98639.3086;
% ideal gas constant (m^3 Pa mol^-1 K)
R = 8.3144621;
% constant (K) for ER
K =(pb*1E-6)/R;
% molar mass of GHG (kg mol^-1)
%     CH4     CO2     N2O      NH3      O2
MM =[0.01604 0.04401 0.044 0.017 0.032];
% time interval (s) to compare to the daily emissions
rep = [1:1:22]*3600;
kfactor = [1.08 1 1.21 1 1 1];
% row header for data organization
row_head = {'Chamber 1', 'Chamber 2', 'Chamber 3', 'Chamber
4',...
    'Chamber 5', 'Chamber 6'};
%% Import Data
% loop thru 7 sheets {CH1... CH6, bckgnd} and store data in
cells
for chamber = 1:7
    % import data for one chamber
    data_mat = importfile2(name,chamber);
    % convert dd/mm/yyyy hh:mm:ss to do cumulative sum £¨s)
    time_mat{1,chamber} = [0
cumsum(diff(data_mat(:,1).*24*3600))'];
    % store vectors of GHG conc data in cell rows (ppmv)
    for ii = 1:4
        C_mat{ii,chamber} = data_mat(:,ii+2);
    end
```

```
    % O2 concentration
        C_mat{5,chamber} = data_mat(:,7)*10000;
    % none of the following data is for bckgnd (chambers only)
    if chamber ~= 7
        % mean incoming temperature (deg C)
        T_in_mat{1,chamber} = data_mat(:,9);
        % mean chamber temperature (deg C)
        T_ch_mat{1,chamber} = data_mat(:,8);
        % mean incoming moist air density (kg m^-3)
        rho_in_mat{1,chamber} = data_mat(:,10);
        % mean chamber moist air density (kg m^-3)
        rho_ch_mat{1,chamber} = data_mat(:,11);
        % mean incoming ventilation rate (lpm)
        vent_mat{1,chamber} = data_mat(:,12);
        % mean chamber relative humidity (%)
        RH_ch_mat{1,chamber} = data_mat(:,13);
        % mean incoming relative humidity (%)
        RH_in_mat{1,chamber} = data_mat(:,14);
    end
end
%% ER & Associated Uncertainty Calculation
% loop thru {CH1... CH6}
for chamber = 1:4
    % loop for every row (h) of data
    for h = 1:length(time_mat{1,chamber})
        Vdot_in = vent_mat{1,chamber}(h);
        RHin = RH_in_mat{1,chamber}(h);
        Tin = T_in_mat{1,chamber}(h);
        din = rho_in_mat{1,chamber}(h);
        dch = rho_ch_mat{1,chamber}(h);
        Tch = T_ch_mat{1,chamber}(h);
        RHch = RH_ch_mat{1,chamber}(h);
         % u(chamber temperature)
         DTch = 0.5;
         % u(incoming temperature)
         DTin = 0.5;
         % ventilation rate at STPD
        [Qairin] = std_uncertainty_QairSTPD
(Vdot_in,pb,Tin,RHin);
        Vin_STDP=kfactor(chamber)*Qairin;

        % loop thru 5 GHGs (ii)
        for ii = 1:5
        % linear interpolation for background gas concentration
            C_i{ii,chamber}(h) =
interp1(time_mat{1,7},C_mat{ii,7},...
                time_mat{1,chamber}(h));
```

```
        end
        C_in_o2=C_i{5,chamber}(h);
        C_ch_o2=C_mat{5,chamber}(h);
        C_ch_co2=C_mat{2,chamber}(h);
        C_in_co2=C_i{2,chamber}(h);
        C_ch_ch4=C_mat{1,chamber}(h);
        C_in_ch4=C_i{1,chamber}(h);
        C_ch_NH3=C_mat{4,chamber}(h);
        C_in_NH3=C_i{4,chamber}(h);

         % HP (w)
         alphaS=eval(AlphaS);
         o2(h,chamber)=eval(O2);
         co2(h,chamber)=eval(CO2);
         ch4(h,chamber)=eval(CH4);
         nh3(h,chamber)=eval(NH3);
       interval_rq(h,chamber)=co2(h,chamber)/o2(h,chamber);
    end
end
% initialize empty vectors
int_meth = [];
data_out = [];
for chamber = 1:4
    %% Integration Methods Analysis
    % redefine cum sum of time as x
    x = time_mat{1,chamber};
    % redefine ER as y (only CH4)
    y = ch4(:,chamber);
     % redefine HPR as z
    w=o2(:,chamber);
    t=co2(:,chamber);
    z=nh3(:,chamber);
    % estimation of sampling interval
    interval_out = 50.3*60;
    % calculate ER for each of the different integration methods
    [int_meth_rtrn,int_meth_str_rtrn] =
daily_E_std_uncertainty(x,y);

    [int_rq,int_str_rq]=daily_RQ_std(x,w,t,z);
   for j=1:3
    int_meth(j,chamber)= int_meth_rtrn(j,1);
    end
     for j=1:12
    rq_mat(j,chamber)= int_rq(j,1);
    end
    % plot data
    if graph == 1
```

```
ER_plot(time_mat{1,chamber}./3600,ER{1,chamber}.*3600,chamber,na
me);
    end
    % clear vector data for next iteration
    y = [];
    x = [];
end
%% Preparing Output

for j = 1:3
    for chamber = 1:4
        int_meth_out{1,chamber+1} = row_head{1,chamber};
        int_meth_out{j+1,chamber+1} = int_meth(j,chamber);
        int_meth_out{j+1,1} =int_meth_str_rtrn{j,1};
    end
end

for i = 1:12
     for chamber = 1:4
        int_rq_out{i,chamber+1} =rq_mat(i,chamber);
        int_rq_out{i,1} = int_str_rq{i,1};
    end
end
for chamber = 1:4
    for i = 1:length(time_mat{1,chamber});
        in_timerq_out{i,1} = i*0.84;
    end
end
row_head1 = {'time (h)','Chamber 1', 'Chamber 2  ', 'Chamber 3
', 'Chamber 4 ',...
    'Chamber 5 ', 'Chamber 6 '};
%% Write to Excel File
xlswrite(output,int_meth_out,1,'A1')
xlswrite(output,int_rq_out,1,'A5')
xlswrite(output,row_head1,1,'A18')
xlswrite(output, in_timerq_out,1,'A19')
xlswrite(output, interval_rq,1,'B19')

range_count = 1;
save(name)
end
```

APPENDIX E: ELECTRICAL CIRCUIT MAP AND CONNECTIONS IN CONTROL BOX

REMS has six open-circuit chambers and involves a lot of controls and measurements. The controller could govern the measurement of each chamber including gas concentration, temperature, humidity, pressure different across orifice meter in air supply pipes, and sampling sequence. As shown in Figure E.1. Each line represents one kind of connection between chamber and control equipment. The damage of each line will cause a serious problem in this system.

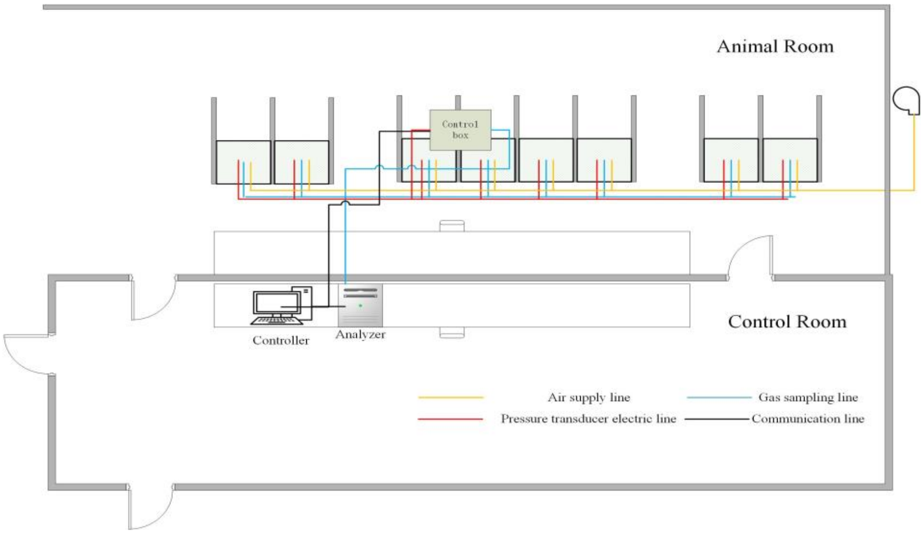


Figure E.1. Layout of the connections across different rooms.

Communication line is used for the data exchange between controller and analyzer, controller and control box, control box and analyzer. The disconnection of these lines lead to the data losing for all six chamber. Gas sampling line represents the gas sampling from chamber to analyzer. If any connected joint in this line was not tight, the gas concentration measured will be not accurate. Conducting recovery rest before each test will prevent this happened. Pressure transducer electric line is to transmit electric signal from pressure transducer to data acquisition board in control box. Any disconnection in this line will make the ventilation data invalid for a chamber. Air supply line represents the fresh air supply pipes from ambient to chamber. The problem in this line will also lead to the useless data in ventilation and could cause health problem for animals.

The control box is an integrity of controlling and data acquisition. As shown in Figure E.2, it includes power supply, data acquisition board, relay board, solenoids, USB extension board, and LED indicators. The maintenance of this system includes a periodic check of every pin in this box. Figure E.3, E.4, and E.5 are the layout of each device, which could help identify the connection between each component.

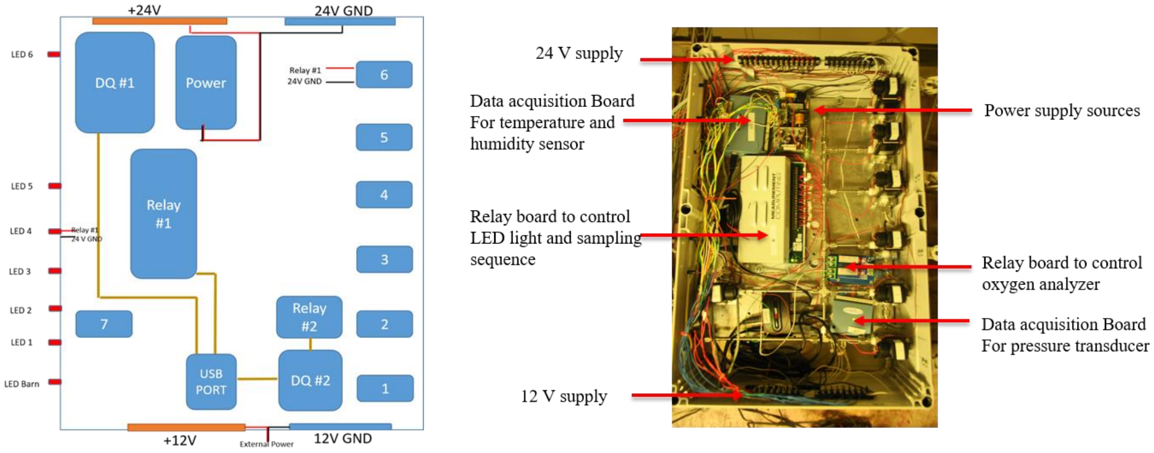


Figure E.2. Layout of devices in the control box.

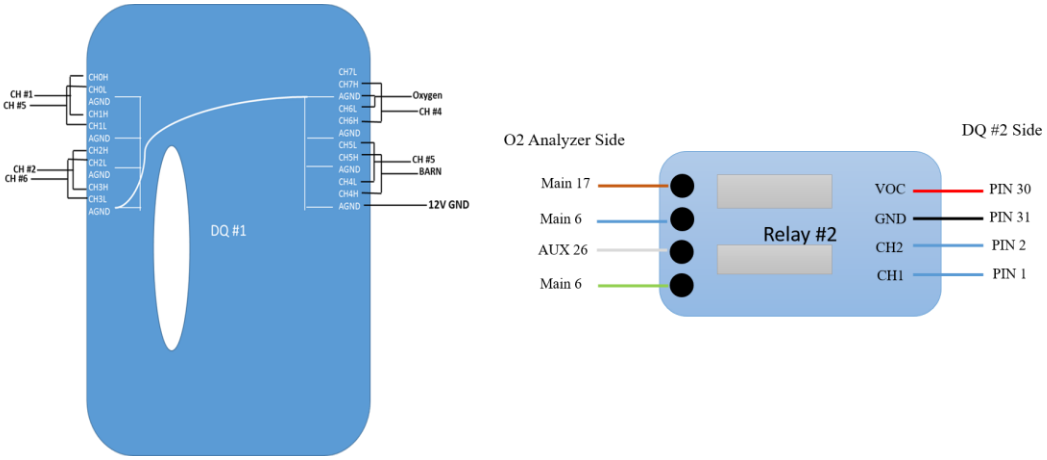


Figure E.3. Connections between temperature/humidity sensors, oxygen analyzer, and N.2 relay board to N.1 data acquisition board.

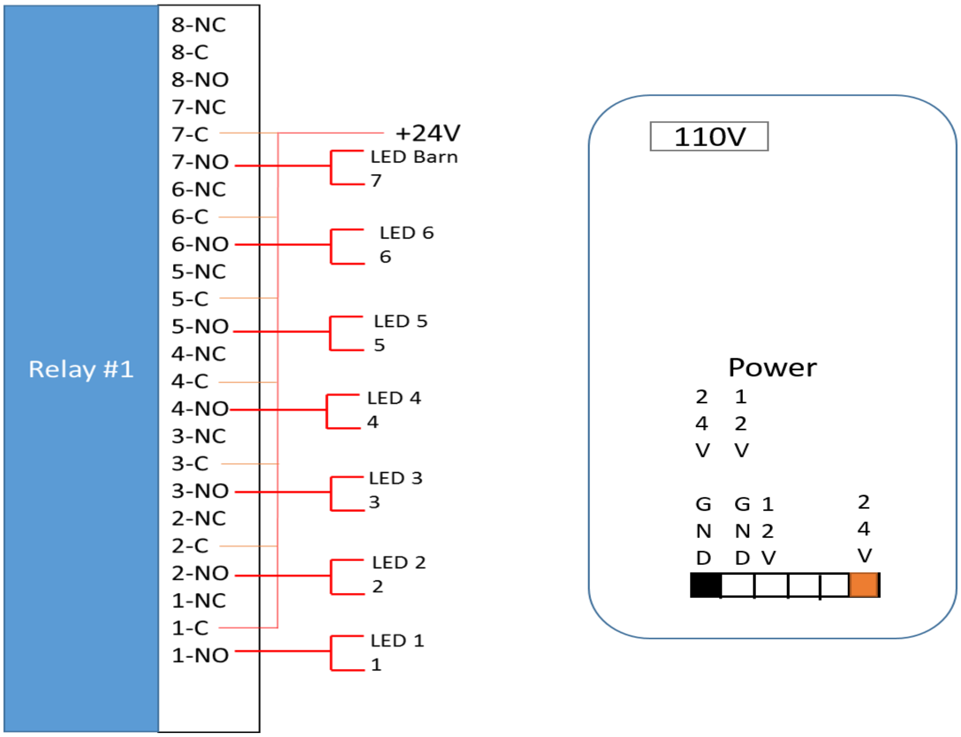


Figure E.4. Connections between power, solenoids, indicator and NO.1 relay board.

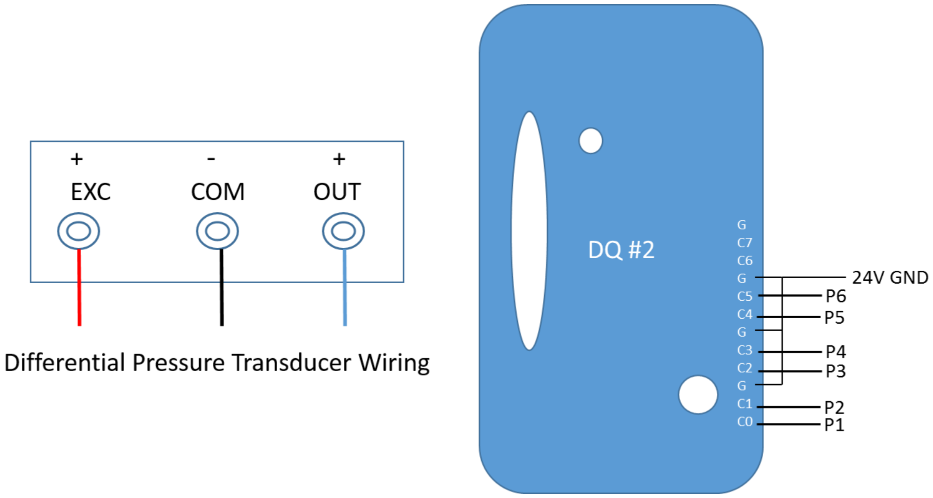


Figure E.5. Connections between pressure transducers and N.2 data acquisition board.