DESIGN AND CONSTRUCTION OF A MEDIUM-SCALE AUTOMATED DIRECT MEASUREMENT RESPIROMETRIC SYSTEM TO ASSESS AEROBIC BIODEGRADATION OF POLYMERS

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Packaging – Master of Science

2013

ABSTRACT

DESIGN AND CONSTRUCTION OF A MEDIUM-SCALE AUTOMATED DIRECT MEASUREMENT RESPIROMETRIC SYSTEM TO ASSESS AEROBIC BIODEGRADATION OF POLYMERS

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A medium-scale automated direct measurement respirometric (DMR) system was designed and built to assess the aerobic biodegradation of up to 30 materials in triplicate simultaneously. Likewise, a computer application was developed for rapid analysis of the data generated. The developed DMR system was able to simulate different testing conditions by varying temperature and relative humidity, which are the major exposure conditions affecting biodegradation. Two complete tests for determining the aerobic biodegradation of polymers under composting conditions were performed to show the efficacy and efficiency of both the DMR system and the DMR data analyzer. In both cases, cellulose reached 70% mineralization at 139 and 45 days. The difference in time for cellulose to reach 70% mineralization was attributed to the composition of the compost and water availability, which highly affect the biodegradation rate. Finally, among the tested materials, at least 60% of the organic carbon content of the biodegradable polymers was converted into carbon dioxide by the end of the test.

To my parents Roberto and Clementina,

brother and sister, Roberto Carlos and Denise

ACKNOWLEDGMENTS

I would like to acknowledge those who without their support, contribution, encouragement and inspiration during my study at Michigan State University, this thesis would not have been possible.

I would like to express my deep gratitude to Dr. Susan Selke and Dr. Rafael Auras, my research supervisors, for their patient guidance, enthusiastic encouragement and useful critiques during the planning and development of this work. Dr. Susan Selke comments and criticisms taught me about critical thinking and reasoning. I would like to express my very great appreciation to Dr. Auras who encouraged me to always give the best of me and taught me that every single problem has a simple solution if we try harder and think differently. I would also like to thank Dr. Liu for her valuable and constructive suggestions.

I would like to offer my special thanks to Dr. Kijchavengkul for his guidance in building the equipment, and for his help in developing the computer application for controlling and operating the equipment. His willingness to give his time so generously has been very much appreciated.

I wish to acknowledge the help provided by the members of Dr. Auras' research group (RAA group) who are not only partners but friends. Special thanks should be given to Tuan, Ning, Siyuan, Hayati, Jing, Marcelo, and Rodolfo. I would also like to extend my thanks to Rijosh, Oh, Torn, and the undergraduate students: Matt, Alyssa, Drew, Tommy, Tony, and Kelly for their invaluable help and time.

I am very thankful to my friends: Juliana, Marcelo, and Cristal for their support, encouragement and inspiration. Advice given by Cristal and Marcelo has been a great help in developing the computer application for analyzing the data.

I would like to acknowledge CIATEQ (Centro de Tecnología Avanzada), and CONACYT (Consejo Nacional de Ciencia y Tecnología), without their financial support my M.S. studies would not have been completed.

I would like to express my gratitude to the faculty from School of Packaging, their teaching guidance, and advices have not only helped me understand and improve my research, but also inspired me to continue working in the researching area. I would also like to extend my gratitude to the staff from School of Packaging for their support and constant willingness to help.

Finally, I wish to thank my family, especially my parents, Roberto and Clementina, and siblings, Roberto Carlos y Denise, for their love and continuous support and encouragement, and for believing in me. I have no words to express my gratitude to them. I would also like to extend my gratitude to those who unintentionally I did not mention.

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KEY TO SYMBOLS AND ABBREVIATIONS

Ω	=	ohms
A	=	area under the curve in equation 5-4
$A(CO_2)_B$	=	average cumulative mass of CO_2 evolved from the blank in equation 5-7
$A(CO_2)_T$	=	average cumulative mass of CO_2 evolved from the sample in equation 5-7
C	=	carbon
C	=	concentration of CO_2 evolved during the measurement interval in equation 5-3
[C]	=	actual concentration of CO_2 of each sample in equation 5-1
$[C]_n$	=	concentration of CO_2 at time t_n in equation 5-4
$[C]_{n-1}$	=	concentration of CO_2 at time t_{n-1} in equation 5-4
c	=	response concentration of CO_2 as measured by the NDIR analyzer in equation 5-1
$C(CO_2)$	=	cumulative mass of CO_2 in equation 5-5
$C(CO_2)_H$	=	cumulative mass of CO_2 at time t_H in equation 5-6
$C(CO_2)_L$	=	cumulative mass of CO_2 at time t_L in equation 5-6
$C(CO_2)_{n-1}$	=	cumulative mass of CO_2 until time t_{n-1} in equation 5-5

C/N	=	carbon-nitrogen ratio
$Ca(OH)_2$	=	hydrated lime
cc	=	cubic centimeters
CH_4	=	methane
cm	=	centimeters
CO_2	=	carbon dioxide
C_{TOT}	=	proportion of total organic carbon in the total mass of test material
d	=	days
DAS	=	data acquisition system
DC	=	direct current
DMR	=	direct measurement respirometric
$E(CO_2)$	=	mass of evolved carbon dioxide in equation 5-3
$E(CO_2)_n$	=	mass of CO_2 evolved from the sample at time t_n in equation 5-5
EMC	=	electronic manifold card
EPA	=	U.S. Environmental Protection Agency
F	=	air flow rate
g	=	grams
GMCP3M	=	gamma irradiated cellophane with 3 months of storage

GMCP6M	=	gamma irradiated cellophane with 6 months of storage
GND	=	ground
GPC	=	gel permeation chromatography
H_2O	=	water
$I(CO_2)$	=	interpolated cumulative mass of CO2 at time t_I in equation 5-6
IR	=	infrared light/radiation
ISO	=	International Organization for Standardization
k	=	calibration factor
L	=	liters
LDPE	=	low density polyethylene
LLDPE	=	linear low density polyethylene
LPM	=	liter per minute
mA	=	milliamperes
MFC	=	mass flow controller
mg	=	milligrams
% Mineralization	=	percent carbon molecules converted to CO_2 in equation 5-7
ml/min	=	milliliters per minute

mm	=	millimeters
MSU	=	Michigan State University
MSW	=	municipal solid waste
M_{TOT}	=	mass of test material
NaOH	=	sodium hydroxide
NDIR	=	non-dispersive infrared gas analyzer
NMR	=	nuclear magnetic resonance
NPN	=	negative-positive-negative transistor
O_2	=	oxygen
O_3	=	ozone
PE	=	polyethylene
PET	=	polyethylene terephthalate
ppm	=	parts per million
R^2	=	R-square
RH	=	relative humidity
sccm	=	standard cubic centimeters per minute
SEM	=	scanning electron microscopy
SoP	=	School of Packaging

STP	=	standard temperature and pressure
T	=	measurement interval
TGA	=	thermogravimetric analysis
t_H	=	immediate higher value of the time interval in equation 5-6
$Th(CO_2)$	=	theoretical amount of evolved carbon dioxide
t_I	=	time interval in equation 5-6
t_L	=	immediate lower value of the time interval in equation 5-6
t_n	=	time at which each measurement was done in equation 5-4
t_{n-1}	=	time in which the previous measurement was done in equation 5-4
TS_n	=	time stamp at time t_n in equation 5-2
TS_o	=	time stamp at time to corresponding to the time at which the experiment started in equation 5-2
UV	=	ultraviolet light/radiation
V	=	volts
W	=	watts

CHAPTER 1

INTRODUCTION

1.1 Introduction and motivations

According to the U.S. Environmental Protection Agency (EPA), about 250 million tons of municipal solid waste (MSW) was generated in the U.S. during 2010 [1, 2]. This represents an increase of about 20% in the last 10 years. Plastics represent 12.4% of the total MSW generated in 2010 [1, 2] with an increase of 81.8% with respect to 1990. In the products category, plastics represent 18.1% of the total containers and packaging which is 30.3% of the total MSW generated in 2010 [1, 2]. The amount of plastics used for containers and packaging increased by 98.2% compared to 1990.

Therefore, a rapid increase in the use and disposal of plastics can be noticed, especially in packaging, which has been considered in the last decade a major environmental issue [3] since most plastic packaging is non-biodegradable [4], and ends up accumulated in landfills at end of life [5].

Although, according to EPA, disposal of MSW to landfill has decreased from 145.3 million tons in 1990 to 135.5 million tons in 2010, landfill remains the primary method for handling MSW [6]. Landfill capacity is considered to be sufficient in the U.S. [2], but in some other countries that is not the case [7].

The inconvenience about disposing plastics into landfills lies in the fact that most of those materials do not degrade in a practical period of time under landfill conditions [6]. However, some plastics can be considered biodegradable and/or compostable.

Composting, which is designed to accelerate biodegradation, is an alternative to landfills [6]; it reduces the amount of MSW going to landfills, and produces compost which is a soil conditioner and fertilizer [5]. Composting is more predominant in Europe than in the U.S. [7], but in the U.S. it has grown significantly [6] in the last decade. According to EPA, materials recovered by composting have increased from 4.2 million tons in 1990 to 20.2 million tons in 2010 [2].

Thus, the use of biodegradable plastics allows disposal through composting, reducing landfill issues [6]. Due to the rapid increase in petrochemical prices [7] and the societal pressures for environmental-friendly plastics [8], interest in developing biodegradable plastics, biobased plastics, and plastics with enhanced degradation has increased [5, 9].

According to the ASTM D6400 standard, a biodegradable plastic is one in which degradation results from the action of microorganisms (bacteria, fungi, and algae) [10]. Biobased plastics can be defined as those that are made from natural renewable resources rather than fossil fuels [6]. Activated plastics (mostly polyolefins) are those that have been modified with additives to enhance their oxidative degradation [5, 11]. Additionally, an environmentally acceptable biodegradable plastic has been defined as one that not only accomplishes some degree of biodegradation, but also does not impact the environment by means of their by-products [12].

Conventionally, petroleum-based plastics are not easily degradable since they are stable and hydrophobic [5]. According to Shah *et al*, polyethylene (PE) can be made biodegradable by increasing its hydrophilicity and/or reducing its chain length by oxidation in order for microorganisms to assimilate the chains [4]. On the other hand, some plastics such as starch-based polymers are easily degradable due to their high hydrophilicity and since microorganisms are able to attack their polymer chains [11].

In addition to the fact that composting of biodegradable materials has become an attractive alternative to deal with environmental issues [13], some companies have obtained significant marketing advantages by claiming that their products have a positive contribution to the environment [6].

For these reasons, it is important not only to evaluate the biodegradation of these novel materials, but also the efficiency of their biodegradation process [3, 13] and consequently rapid assessment methods must be developed [12]. Nowadays, standard composting tests are used to compare and understand the biodegradation processes of polymers [9], providing knowledge for the design of new polymers in the future [8].

1.2 Goal and Objectives

The goal of this project was to design and to build an equipment called a direct measurement respirometric (DMR) system capable of simulating typical aerobic composting conditions for assessing the degree and rate of biodegradation of polymers according to the ASTM D5338 and ISO 14855 standards, which focus on determining

the percentage biodegradation of plastic materials by analysis of evolved carbon dioxide [14, 15].

The objectives of this project were as follows:

1. Assemble and construct a DMR system capable of testing up to 30 materials simultaneously in triplicate, since a single biodegradation test can last up to 6 months and the systems found in the literature are relatively small, capable of testing only up to 8 materials in triplicate [8, 9, 16, 17].
2. Develop a computer application to automatically analyze the data since a large amount of data is collected from the DMR system every day.
3. Assess the effectiveness of the equipment and the computer application by determining the degree and rate of aerobic biodegradation of selected plastic materials.

1.3 Thesis outline

1.3.1 Design and construction of the apparatus

A direct measurement respirometric system was designed and constructed based not only on the ASTM D5338 and ISO 14855 standards, but also on other apparatus found in the literature and by doing some modifications so it automatically operates up to 95 bioreactors simultaneously, and simulates different testing conditions by varying temperature, relative humidity, and flow rate.

The following are some of the components that the DMR system contains: the scrubbing system, the environmental chamber, the relative humidity generator, the air flow control, and the measurement devices. Additionally, a computer application was developed for controlling the system, as well as for measuring and recording the test variables.

1.3.2 Calibration

Calibration was performed to establish the relationship between the carbon dioxide analyzer signal and the concentration of known amounts of pure carbon dioxide injected into each bioreactor. The calibration was performed at the same conditions of the test, and then a calibration curve was determined by plotting the peak response concentrations against the actual concentrations for all the bioreactors.

1.3.3 Development of a data analyzer

Another computer application was developed to automatically analyze the data of the tested materials collected from the DMR system. This analysis was performed according to ASTM D5338 and ISO 14855 standards where the cumulative amount of carbon dioxide released by the bioreactor containing the test material was calculated as a function of time. Then, the percentage of biodegradation (mineralization) of the test materials was determined by comparing the amount of carbon originally present in the test material with the amount of carbon evolved as carbon dioxide [14, 15].

1.3.4 Performance of the test and analysis of the data

Among all the materials tested using the DMR system, five were selected, in addition to the positive control, to show the efficacy and efficiency of the apparatus. The selected test materials were: cellulose powder (positive control), low density polyethylene/ linear low density polyethylene blend film and polyethylene terephthalate sheet, both without and with 5% (by weight) oxidation-promoting additive, and gamma irradiated cellophane film.

The preparation of the test materials as well as the compost inoculum was, in general, performed as described in the ASTM D5338 and ISO 14855 standards. The tests were performed in a dark environment wherein temperature, aeration and humidity were closely monitored and controlled [14, 15].

Finally, the cumulative amount of carbon dioxide released by each bioreactor containing the test material as well as the percentage of biodegradation of each test material as a function of time were obtained by using the data analyzer previously mentioned.

CHAPTER 2

LITERATURE REVIEW

2.1 Polymer Degradation

According to Kumar *et al*., “degradation is any change of the polymer desired properties which involves a whole set of physical and chemical processes by which structural changes become irreversible. By the end, the material totally loses its original functionality” [18].

Polymer degradation results in a cleavage of main-chain bonds producing shorter oligomers, monomers, and/or other low molecular weight degradation products [19]. It may occur when the polymer is affected by environmental factors, such as light, heat, moisture, chemical conditions, or biological activity [4, 20]. Table 2-1 summarizes the types of degradation and their causes.

Table 2-1. Types and causes of polymer degradation. Adapted from Kumar *et al*. [18]

Type of degradation	**Causes/Environmental Factors**
Oxidative degradation	Oxygen, ozone
Thermal degradation	Heat (especially during processing)
Photodegradation	Light (e.g. ultraviolet and visible light)
Chemical degradation (hydrolysis)	Water
High-energy degradation	X-ray, α-, β-, γ- rays
Mechanical degradation	Stress, fatigue (esp. during processing)
Biodegradation	Micro/macroorganisms, enzymes

2.1.1 Oxidative degradation

Oxidative degradation occurs in the presence of oxygen (O_2) or ozone (O_3), and it is usually facilitated by the presence of light or high temperatures. Oxidation of polymers usually happens during processing, producing chain scission reactions. The rate at which these reactions occur depends on the availability of oxygen [20].

Research for efficient pro-oxidant agents, which can be incorporated into plastic materials, has increased. These additives are generally based on transition metals, which help generate free radicals and consequently free radical chain reactions and/or auto-oxidation [21].

2.1.2 Thermal degradation

Thermal degradation is related to the weakness of chemical structures by the effect of heat and temperature [22]. The basic principle is that all chemical bonds can be broken at high enough temperature [20].

This process can be due to random chain scission reaction, as well as depolymerization [22]. The difference is that depolymerization results in a high production of monomers generating weight loss, while random chain scission results in a rapid decrease in the polymer molecular weight [20].

2.1.3 Photodegradation

Photodegradation is related to the reaction of chemical chains of the polymers when they are exposed to light, generally ultraviolet (UV) radiation [23]. Generally, UV light activates their electrons to higher reactivity and causes oxidation, cleavage, and other degradation [4].

For photodegradation to occur, the radiation must be absorbed by the molecule, and it must be sufficiently energetic to result in changes in chemical bonds [20]. Therefore, a chromophore group (*e.g.*, carbonyl group), which is responsible for absorbing UV radiation and leading to degradation, has to be present in the polymer chain [24].

Photodegradation occurs almost entirely through a free radical process due to the absorbed radiation [20]. Once radicals are introduced into the system, chain degradation occurs independently of the light by auto-oxidation mechanisms [24, 25].

2.1.4 Hydrolytic degradation

Degradation by hydrolysis involves chemical reactions of the polymer chain with water [6]. The end result of hydrolysis is usually depolymerization in which monomers are produced. Hydrolysis primarily occurs in the amorphous regions of the polymer, and it is generally limited by the rate of diffusion of water into the bulk polymer [20].

In some cases, depending on the polymer, hydrolysis can be followed by thermal degradation or photodegradation, due to carbonyl group formation [20], or even by biodegradation in which the molecular weight of the polymer has been significantly reduced, allowing microbial attack [6].

2.1.5 Biodegradation

According to D. Briassoulis *et al*., biodegradation is chemical degradation caused by the enzymatic action of microorganisms such as bacteria, fungi, and algae [26]. At the end, biodegradation leads to the formation of biomass, water (H_2O), and carbon dioxide (CO_2) or methane (CH_4) depending on whether the biodegradation is aerobic or anaerobic. When oxygen (O_2) is not present, anaerobic degradation takes place [12].

If chemical hydrolysis, a non-enzymatic process, simultaneously occurs then it tends to accelerate the biodegradation by breaking down the polymer chains as previously mentioned [26].

2.2 Polymer biodegradation

Biodegradation can occur in two different environments: aerobic and anaerobic, which are in turn subdivided in aquatic and solid environments [12]. The two main solid environments for biodegradation to occur are soil and compost [26]. Polymers can be exposed to different environments in order to evaluate the rate of microbial activity during the degradation process. Each environment contains different microorganism populations and different physical-chemical parameters [27].

According to the ASTM D6400 standard, a biodegradable plastic is that in which the degradation results from the action of naturally occurring microorganisms, while a compostable plastic is that in which the degradation is caused by biological processes during composting to yield carbon dioxide, water, and biomass, meeting a particular time rate comparable to natural materials such as leaves, grass, and paper [10]. Thus, some compostable materials may or may not biodegrade in soil. Likewise, some biobased materials may or may not be biodegradable or compostable [26].

There are three indispensable factors for polymer biodegradation to take place: substrate, environment and microorganisms. The polymer characteristics such as its mobility, tacticity, crystallinity, molecular weight, functional groups, plasticizers, and additives highly influence the biodegradability of the substrate. Similarly, the

environment under which the polymer is expected to biodegrade and conditions such as temperature, oxygen and moisture play an important role in the biodegradation process. In addition to that, the microorganisms present in the environment should have the proper metabolic pathways to synthesize enzymes, specific for the target polymer, required to initiate depolymerization and subsequent mineralization of the monomers and oligomers previously formed [4, 12].

2.2.1 Biodegradation mechanism

Polymer biodegradation takes place in two main steps: primary degradation and ultimate biodegradation (see Figure 2-1) [5]. During primary degradation, fragmentation occurs due to oxidation or hydrolysis initialized by chemical or biological compounds (microbial enzymes) [4, 12]. These processes involve either chain scission or depolymerization resulting in the formation of low molecular weight chains that can be easily assimilated by the microorganisms [5, 12].

Once sufficiently small-size chains are formed, ultimate biodegradation occurs by microorganisms assimilating the polymer chains. This process leads to the formation of biomass, water, carbon dioxide (aerobic) or methane (anaerobic), salts and minerals [5, 12]. Then, this final step is also called mineralization [4, 12].

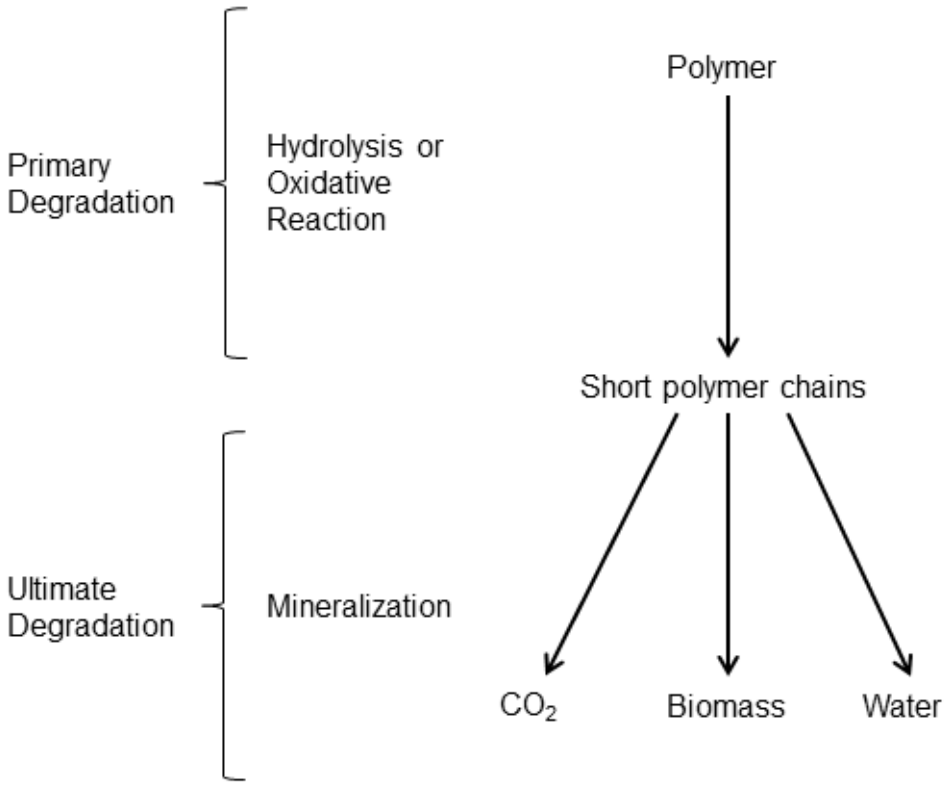

Figure 2-1. Schematic of polymer biodegradation mechanism. Adapted from T. Leejarkpai *et al.* [5]

2.2.2 Evaluation of Biodegradation

Biodegradation can be evaluated by different analytical techniques, either in a direct or an indirect approach [27]. Visual observations, weight loss measurements, and change in mechanical properties are the most basic techniques to evaluate degradation, but they do not necessarily prove the occurrence of a biodegradation process [4].

Visual observations involve changes in the polymer surface such as changes in color, and formation of holes or cracks. Weight loss measurements, as the name implies, are based on the reduction of polymer weight due to disintegration. Change in mechanical properties involves reduction in tensile elongation and tensile strength, which is associated with changes in molecular weight, which in turn is an indicator of degradation [4].

Other laboratory techniques can also be used to help assess the biodegradability of polymers, such as infrared (IR) and UV-visible spectroscopy, nuclear magnetic resonance (NMR), gel permeation chromatography (GPC), and scanning electron microscopy (SEM), but respirometric methods are generally preferred to evaluate biodegradation of polymers in laboratory settings [4].

In the respirometric methods, the consumption of oxygen or the evolution of carbon dioxide are directly measured [27]. Under aerobic conditions, microorganisms use oxygen to oxidize carbon (C) and form carbon dioxide as the end product [4, 12]. Studies have shown that degradation of solid carbon to carbon dioxide can mainly be described as a first order reaction (see equation 2-1) [5].

$$C_{polymer} + O_2 \rightarrow CO_2 + H_2O + C_{residue} + C_{biomass} + salts \qquad \text{(Eq. 2-1)}$$

For this method, the measurement can be performed in a discrete or a continuous way, and the residual samples from the test can also be evaluated using other characterization techniques [27]. Measurement of evolved carbon dioxide is the most used method since it is more precise and simpler to implement than other test methods [12].

2.3 Determination of aerobic biodegradation of polymers under composting conditions

A number of standards have been developed around the world to define the requirements and the methodologies to assess the biodegradability of plastic materials. Most of the methodologies adopted in various standards follow degradation through the evolution of carbon dioxide [26].

2.3.1 Requirements

Among the standards developed for determining the percentage biodegradation of plastic materials by analysis of evolved carbon dioxide, ASTM D5338 and ISO 14855 are the ones with major relevance for assessing the aerobic biodegradation of polymers under composting conditions [14, 15]. A comparison between these two standards is shown in Table 2-2.

Table 2-2. Requirements comparison between ASTM D5338 and ISO 14855 standards [14, 15]

Requirement		ASTM D5338	ISO 14855
Apparatus	Number of bioreactors	At least 12	At least 9
	Volume of bioreactors	2 to 5 L (sufficient headspace)	2 L or higher (sufficient headspace)
	Aeration	Water saturated Carbon-dioxide-free Accurate flow rate	Dry or water saturated Carbon-dioxide-free At pre-set flow rate
	Sensor	Specific sensors or appropriate gas chromatographs	Infrared analyzer Gas chromatograph
Compost Inoculum	Age	2-4 months old	2-4 months old

Table 2-2 (cont'd)

	Homogeneity	Sieved on a screen <10 mm Allows addition of structural material	Sieved on a screen of about 0.5 to 1 cm Allows addition of structural material
	Dry solids	Between 50 and 55%	Between 50 and 55%
	Volatile solids	Ash content less than 70%	No more than 15% of the wet or 30% of the dry solids
	pH	Between 7 and 8.2	Between 7 and 9
	Production of carbon dioxide	Between 50 and 150 mg of CO_2 per gram of volatile solids over the first 10 days	Between 50 and 150 mg of CO_2 per gram of volatile solids over the first 10 days
	C/N ratio	Between 10 and 40	Between 10 and 40
Substrate	Shape	Granules, powder, film, simple shapes	Granules, powder, film, simple shapes
	Surface Area	2X2 cm max.	2X2 cm max.
	Positive Control	Cellulose (particle size <20 µm)	Cellulose (particle size <20 µm)
	Negative Control	Polyethylene	Not required
Other	Temperature	58 ± 2°C	58 ± 2°C
	Water content	About 50%	About 50%
	Ratio of mixture	6:1 sample (dry solids)	6:1 sample (dry solids)
	Frequency of measurement	At least daily	At least twice per day
	Test Period	At least 45 days	Not exceeding 6 months
	Incubation	Dark or diffused light	Dark or diffused light
	Oxygen concentration	6% or higher	6% or higher

Note: General information is shown in the table. For more details refer to the appropriate standard

These test methods determine the degree and rate of aerobic biodegradation of plastic materials where temperature, aeration and humidity are closely monitored and controlled [14]. They are designed to simulate typical aerobic composting conditions for the organic fraction of solids mixed with the compost [15].

2.3.2 Methodology

According to the ASTM D6400 standard, composting is the aerobic mesophilic and thermophilic degradation of organic matter to make compost [10], which is an organic soil conditioner with a limited mineral content [15].

In general, the test method is as follows [14, 15]:

1. Preparation of the compost inoculum
    a. Obtain the compost inoculum from the proper facility
    b. Sieve the compost inoculum to remove large materials
    c. Determine dry solids, volatile solids, carbon-nitrogen ratio, and pH
    d. Pre-condition the compost inoculum
2. Preparation of the test materials
    a. Get the test materials with the proper shape and size
    b. Determine dry solids, volatile solids, and carbon content
3. Preparation of the bioreactors
    a. Mix compost inoculum and test materials with the appropriate ratio
    b. Fill the bioreactors with the mixture allowing sufficient headspace
    c. Close bioreactors tightly to prevent leakage

4. Expose bioreactors to a controlled aerobic composting process
    a. Initiate aeration of the bioreactors with the proper temperature and relative humidity
    b. Measure carbon dioxide concentration with a minimum time interval of 6 hours
    c. Shake bioreactors weekly to prevent channeling
    d. Adjust moisture content to prevent excessively dry conditions by adding water
5. Assess the degree of biodegradability
    a. Determine the cumulative carbon dioxide production from the test materials
    b. Calculate the percent of biodegradation of the test materials

These standards also suggest and describe in general terms the main components that the apparatus should contain to successfully assess the biodegradation of plastic materials. A detailed description of the direct measurement respirometric system and its components is provided in the next chapter.

CHAPTER 3

DEVELOPMENT OF A DIRECT MEASUREMENT RESPIROMETRIC SYSTEM

3.1. Introduction

A modified and new direct measurement respirometric (DMR) system was designed and built at the School of Packaging (SoP) in Michigan State University (MSU), East Lansing, MI (see Figure 3-1). This DMR system was designed to operate simultaneously with up to 95 bioreactors, and it is able to simulate different testing conditions by varying temperature and relative humidity (RH). Temperature can be set from -23°C (-10°F) to 60°C (140°F), and RH can be modified by supplying either dry or water-saturated air. A computer application was developed for selecting automatically the bioreactors to test based on a predetermined sequence, as well as for measuring and recording test variables such as relative humidity, temperature, air flow rate, and carbon dioxide (CO_2) concentration.

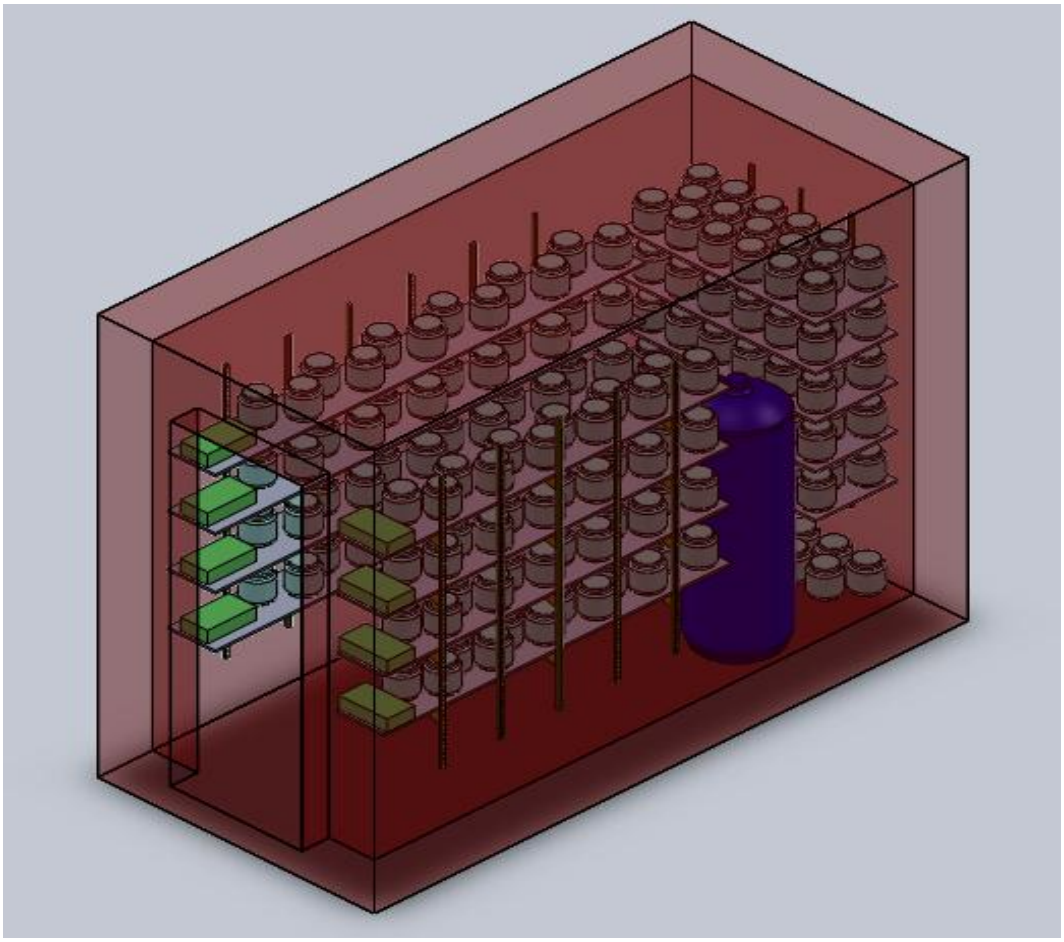

Figure 3-1. 3D model showing the general arrangement of the bioreactors inside the environmental chamber. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

The major components of the DMR system include a scrubbing system, an environmental chamber, a relative humidity generator, an air flow rate control, 95 bioreactors, a switching system, measurement devices, and control software (see Figure 3-2) [16]. These components are explained in more detail in further sections.

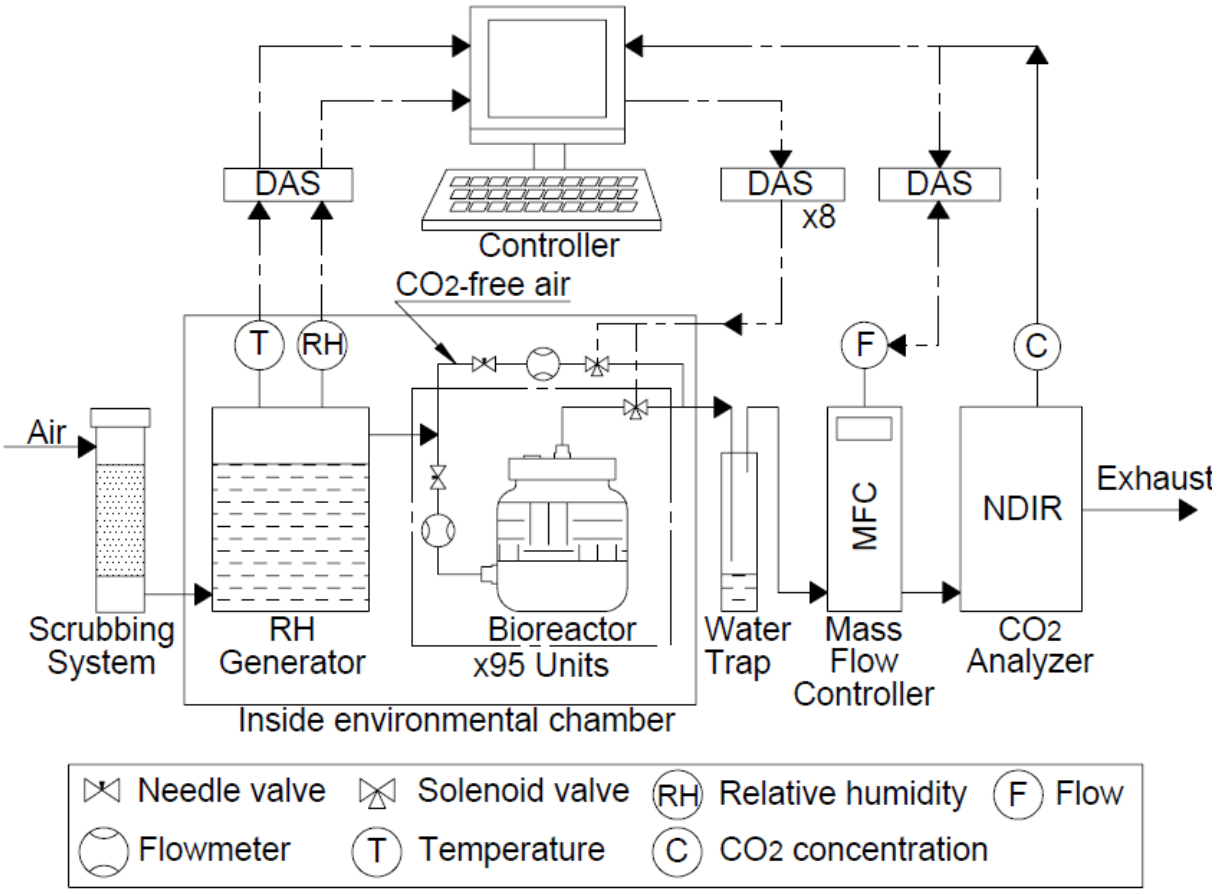

Figure 3-2. Schematic of the direct measurement respirometric system, adapted from C. Way *et al.* [8]

3.2. Scrubbing system

Carbon dioxide present in the air and coming from the air compressor is around 400 parts per million (ppm), and it was reduced to approximately 30 ppm [16] by using a series of cylinders containing a CO_2 absorbent called SODASORB® (W. R. Grace & Co. – Conn., Cambridge, MA, USA), more commonly known as soda lime, which consists primarily of hydrated lime ($Ca(OH)_2$) blended with a small quantity of sodium hydroxide (NaOH), and a fractional content of ethyl violet dye as an indicator of

exhaustion [28]. This scrubbing system was designed so the exhausted soda lime can be replaced every two weeks. It consists of 2 sets of parallel series of 8 refillable polycarbonate cylinders containing about 0.4536 kg (1lb) of soda lime each (Figure 3-3). Although ambient air can be used directly, the scrubbing system helps improve the accuracy of the measurements since CO_2 free air is used to purge the air lines as well as the CO_2 analyzer in every measuring sequence [15, 16].

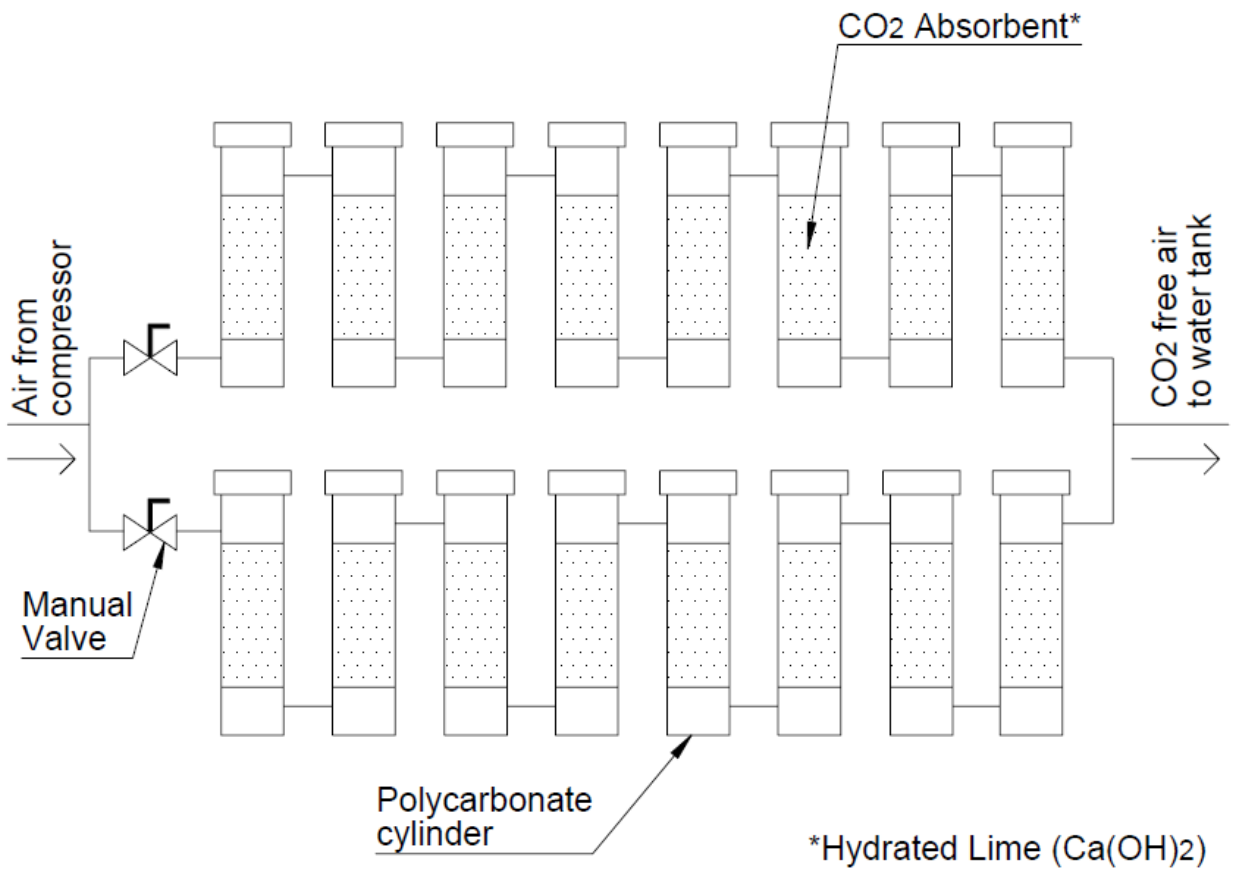

Figure 3-3. Schematic of the scrubbing system

3.3. Environmental chamber

A controlled environmental room (chamber) from Lab-Line Instruments, Inc. Melrose Park, IL, USA, model 750 ADX, was used to control the temperature of the bioreactors. The chamber operating temperature range is -23°C (-10°F) to 60°C (140°F), and therefore it is capable of generating the required temperature for simulating composting conditions.

3.4. Relative humidity generator

The relative humidity generator consists of dry and water-saturated air mixed together in order to obtain the desired relative humidity for the system. After the air passes through the scrubbing system, the CO_2-free air is divided into two lines. The air in the first line enters a 159-liter (42-gallon) water tank (Dayton Electric Mfg. Co., Niles, IL, USA) located inside the environmental chamber through a bubble diffuser in order to get humidified. The second line containing CO_2-free dry air passes directly to the mixing area. The desired humidity can be set by adjusting two high precision flowmeters with needle valves (Figure 3-4); one of them with a capacity of 1-10 LPM (liter per minute) air, and the other one with capacity of 0-5 LPM air, both from Cole-Parmer, Vernon Hills, IL, USA. Then, the temperature and RH of the conditioned air is measured by using a humidity and temperature probe (RH/Temp sensor), model HMP110 from Vaisala Oyj, Helsinki, Finland. A data acquisition system (DAS), model USB 6501,from

National Instruments Corporation, Austin, TX, USA, was used as an interface between the RH/Temp sensor and the control software.

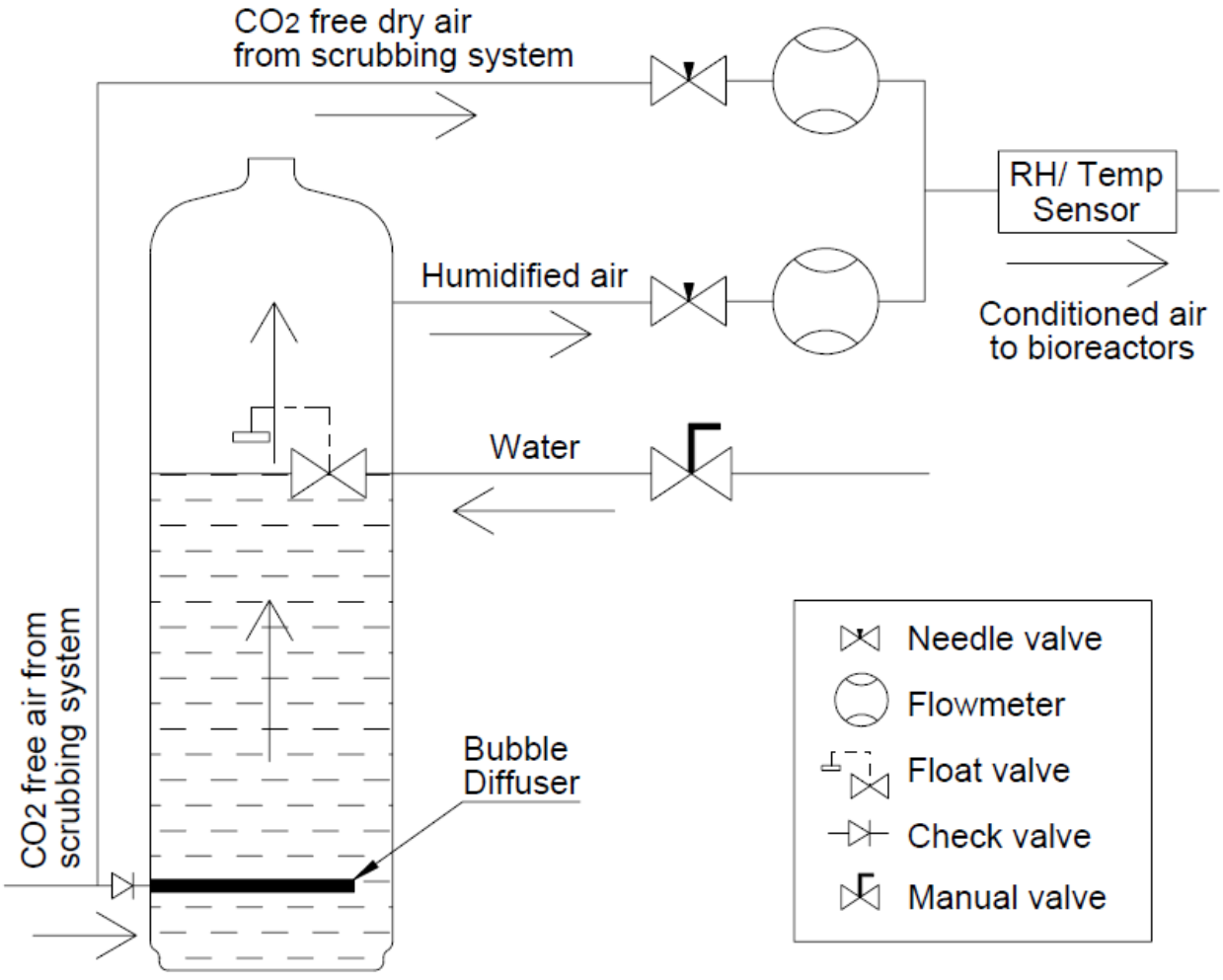

Figure 3-4. Schematic of the relative humidity generator

3.5. Air supply and flow control system

After the conditioned air exits the water tank and passes through the RH/Temp sensor, the air is divided into 12 lines. At the end of each line, a manifold containing 8 outlets is located. High precision flowmeters (96) with needle valves having a capacity of 0-100 ml/min (milliliters per minute) (air), model 022-14-N, from Aalborg, Orangeburg, NY, USA, were installed to control the flow of the air passing through the bioreactor. Tygon® flexible tubing (Saint-Gobain Performance Plastics, Akron, OH, USA) was used for the connections (Figure 3-5). Notice that one of these ports does not pass through any of the bioreactors, but rather is directly connected to a solenoid valve of one of the electronic manifolds; model EMC-12-06-40 from Clippard Instrument Laboratory, Inc., Cincinnati, OH, USA. The purpose of this line is to purge the flexible tubing and the carbon dioxide analyzer between measurements.

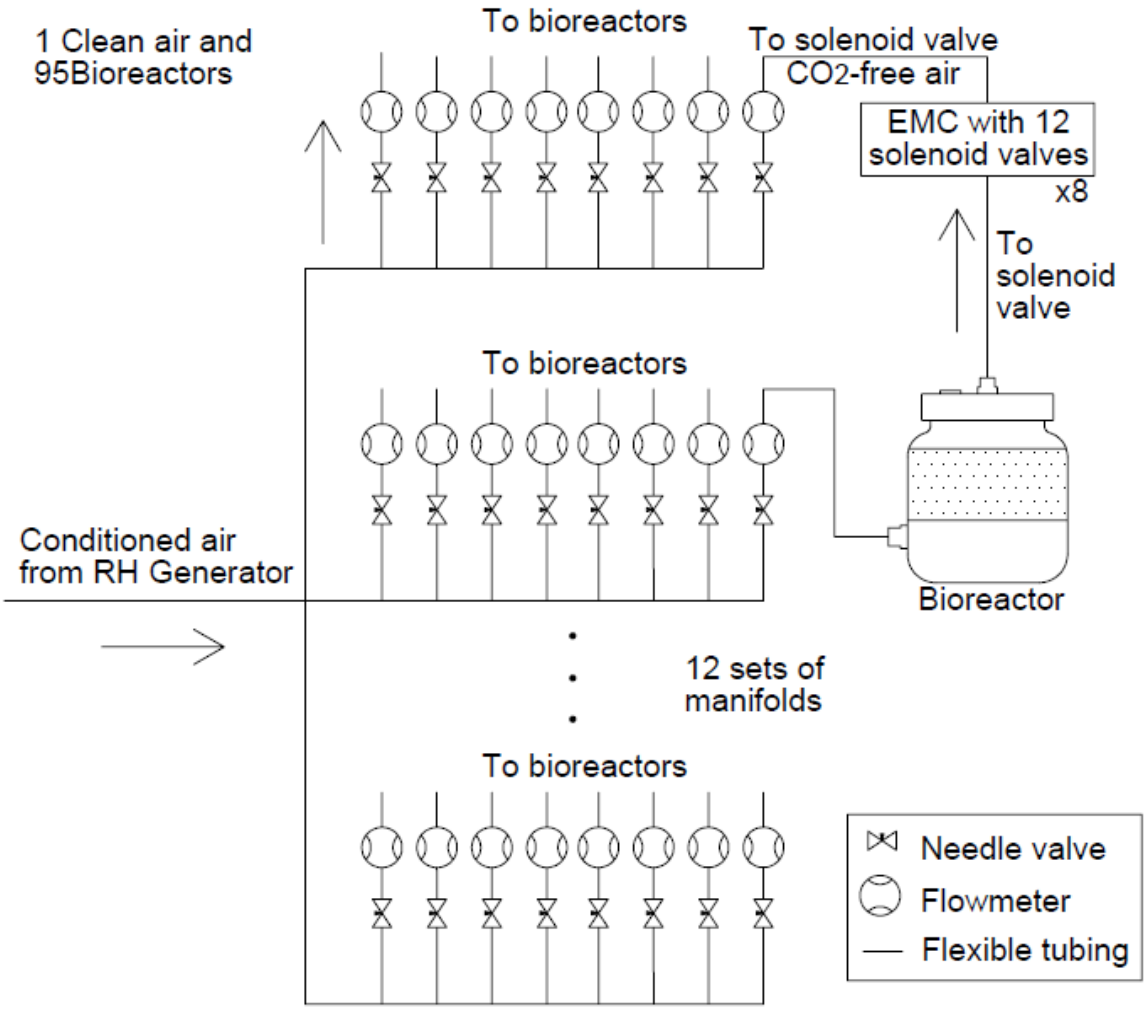

Figure 3-5. Schematic of the air supply and flow control system

3.6. Bioreactors

Figure 3-6 shows a bioreactor made from a glass jar size of 1.9 L (0.5 gallon) with an air-tight closure. A hole was drilled at about 2.54 cm (1 in) from the bottom of the jar, and a plastic quick connector was inserted to create an inlet port. Likewise, two holes were drilled in the aluminum lid, a plastic quick connector was inserted in one of

these holes to create an outlet port, and a rubber septum was inserted in the other hole to inject pure CO_2 during the calibration process and water when necessary during the duration of the experiment. Additionally, a copper-steel alloy grid supported a double screen formed by two aluminum screens in order to form a plate type bioreactor [8, 16].

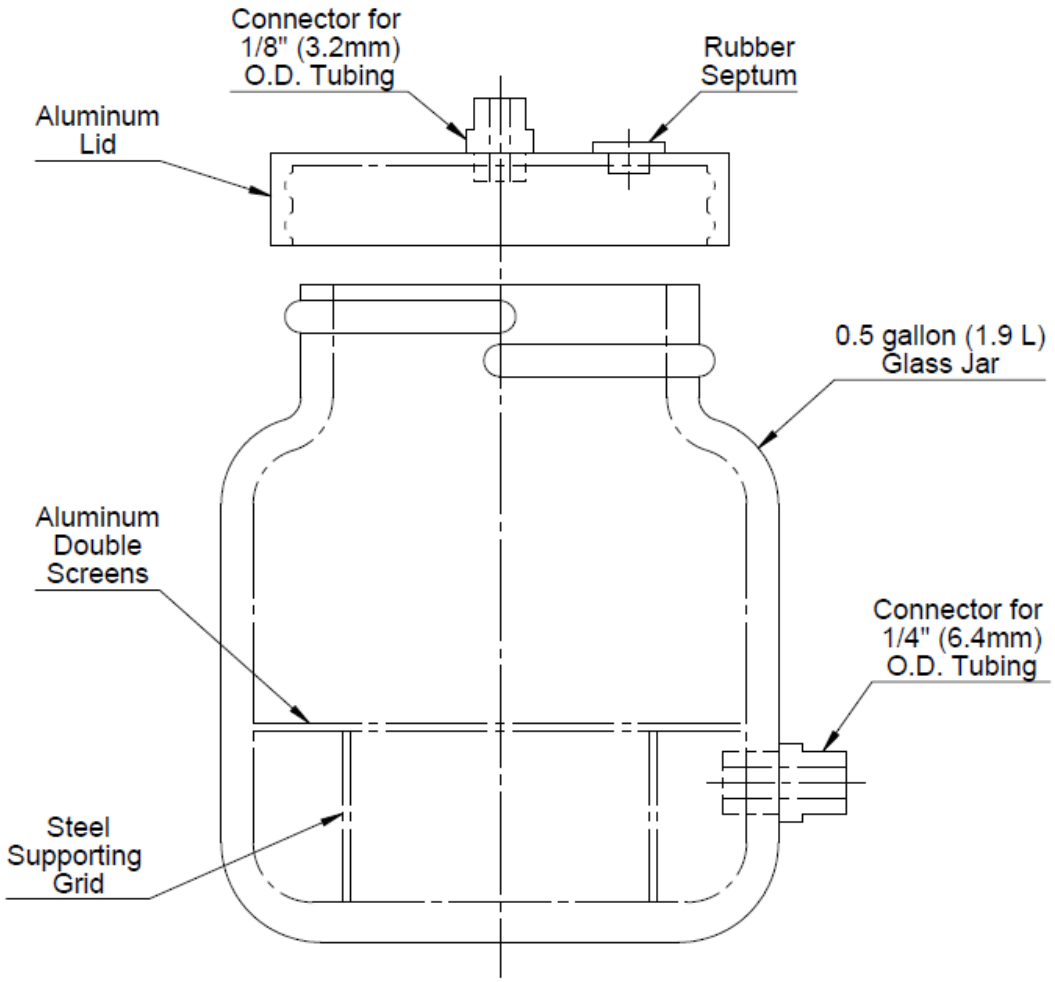

Figure 3-6. Drawing of the bioreactor, adapted from T. Kijchavengkul *et al.* [16]

3.7. Switching system

The outlet ports of the 95 bioreactors and the clean air line were then connected through flexible tubing (Tygon®, Saint-Gobain Performance Plastics, Akron, OH, USA) to a set of 8 electronic manifolds (model EMC-12-06-40) containing 12 solenoid valves each, from Clippard Instrument Laboratory, Inc., Cincinnati, OH, USA. The solenoid valves switch and direct the exhaust air from the selected bioreactor to the CO_2 analyzer for measuring concentration [16].

The electronic manifolds are controlled by using an in-house developed computer application, called control software, and a series of data acquisition systems (DAS), model USB 6501, from National Instruments Corporation, Austin, TX, USA. The DAS act as an interface between the control software and the solenoid valves; each DAS contains 24 channels which in turn control 24 three-way solenoid valves by using digital input/output (I/O) signals. Thus, 4 DAS are needed in order to control the valves of the 95 bioreactors and 1 clean air line. The output signals from each channel of the DAS (5 V and 8.5 mA) do not have sufficient power to operate the solenoid valve since it requires a voltage of 6 V and power of 0.67 W [16]. Therefore, an additional circuit was introduced after each channel (Figure 3-7). A total of 95 circuits were needed for this instrument. Thus, each solenoid valve is connected to a 6 V external power supply and to a collector of a NPN (Negative-Positive-Negative) transistor. The output signal from the DAS channel is connected to the base of the NPN transistor via a 2200 Ω resistor. Finally, an emitter of the NPN transistor was connected to ground (GND) [16].

The solenoid valves in the EMCs are 3-way normally open valves. Therefore, a solenoid valve is closed whenever a “1” signal (5 V DC signal) is sent from the corresponding channel of the DAS to the NPN transistor triggering it and allowing the current from the external power supply to pass through the solenoid valve to complete or close the circuit. Thus, a solenoid valve is open whenever the circuit remains incomplete or open during the “0” signal, or when no signal was sent from the DAS device to the NPN transistor [16].

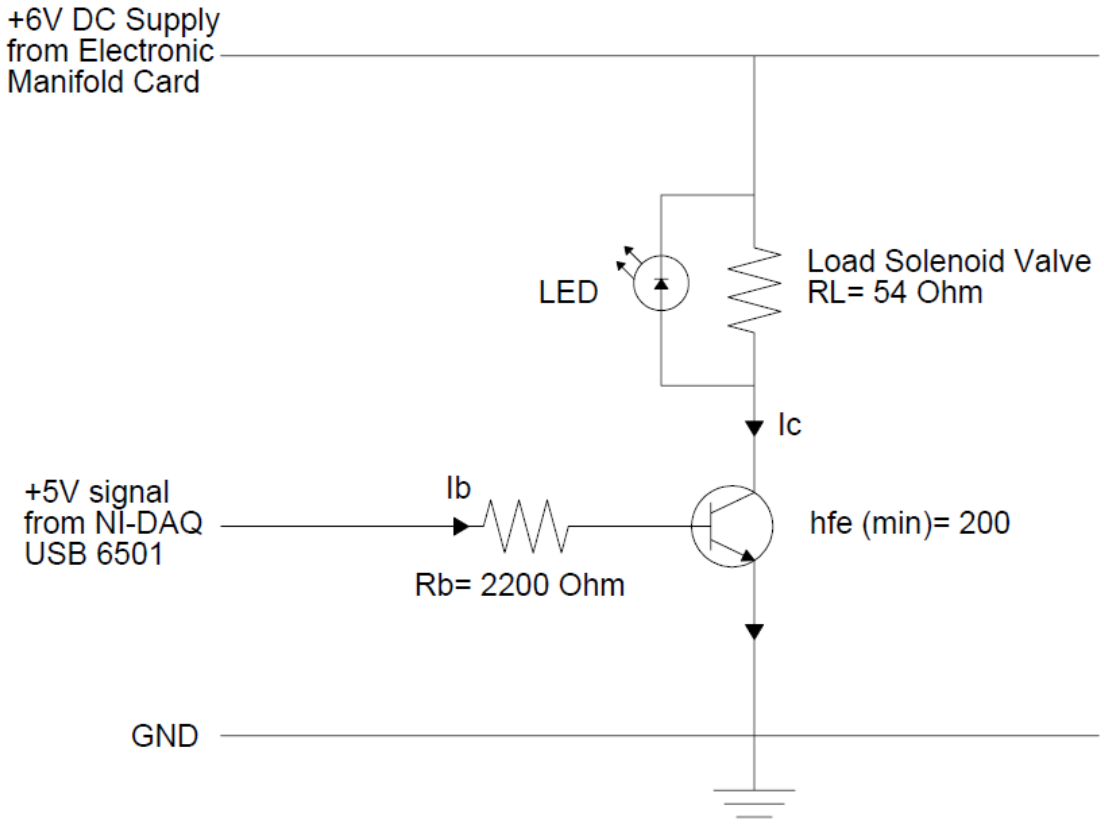

Figure 3-7. Switching circuit, adapted from T. Kijchavengkul *et al.* [16]

3.8. Measurement devices

A non-dispersive infrared gas analyzer (NDIR), model LI-820 from LI-COR Inc., Lincoln, NE, USA, with a measurement range from 0 to 20,000 ppm, was used to measure the concentration of carbon dioxide present in the exhaust air coming from the selected bioreactor. The NDIR was connected to the computer for the control software to record the concentration of carbon dioxide in parts per million. Since water condensed after exiting the environmental chamber (due to changes in the temperature), which can damage the NDIR, a water trap was installed by using an oil-bubbler device over a water bath, model RTE-100 from Neslab Instruments Inc., Newington, NH, USA, operated at 15°C [16].

Likewise, since an accurate and precise flow rate was needed for calculating the amount of carbon dioxide evolved from the bioreactors, a mass flow controller (MFC), model GFC17, from Aalborg, Orangeburg, NY, USA, was installed after the water trap in order to control and record the exact flow passing through the NDIR. This MFC was operated by the control software using a DAS (USB 6501, National Instruments Corporation, Austin, TX, USA) as an interface.

3.9. Control software

LabVIEW™ (version 7.1) from National Instruments Corporation, Austin, TX, USA, was used for developing an application (referred to as control software) to control the switching system (solenoid valves) as well as the measurement devices such as the RH/Temp sensor, the MFC, and the NDIR [16].

The main purpose of the control software is to select the bioreactors to test based on a predetermined sequence (set by the user) which is basically divided into three periods. The first is for purging the tubing and the CO_2 analyzer, the second period is for reaching a steady state, and the last period is for measuring the concentration of CO_2 present in the exhaust air coming from the selected bioreactor. This will be explained in more detail in a further section.

The other purpose of the control software is to record, during the measuring period, the relative humidity (%) and the temperature (°C) from the RH/Temp sensor, the air flow rate (standard cubic centimeters per minute or sccm) from the MFC, and finally the CO_2 concentration (ppm) from the NDIR. The air flow rate in the mass flow controller can be set at the desired level.

As additional features, the application displays a plot of “time vs. concentration” in real time, as well as the actual time during the sequence, the bioreactor that is being measured at that moment, and the cycle number (a cycle is considered finished when all 95 bioreactors have been measured).

CHAPTER 4

DMR SYSTEM CALIBRATION AND OPERATION

4.1 System Calibration

Calibration is - in this case - the process of establishing the relationship between the carbon dioxide analyzer signal (measured CO_2 concentration) and the known injected concentration of pure CO_2. Thus, when a measurement is made by the CO_2 analyzer, the signal measurement is multiplied by the calibration factor (k) to yield the actual concentration of carbon dioxide evolved from a sample [29].

To calibrate the DMR system, known amounts of pure CO_2 gas (1, 2, 4, 8 cc) were injected through a septum into empty bioreactors. The actual concentration of CO_2 in the bioreactor depended on the injected volume of CO_2 since it was calculated by dividing the injection volume by the total volume of the bioreactor (Equation 4-1) [16]. The calibration of all the bioreactors was performed at 58 ± 2°C and 55 ± 5% RH, the same conditions of the test.

$$Actual\ Concentration\ of\ CO_2 = \frac{Injected\ volume\ of\ CO_2}{Total\ volume\ of\ the\ bioreactor} \qquad \text{(Eq. 4-1)}$$

After purging the entire system by passing CO_2-free air through all the components, the solenoid valves were closed by the control software. Then, the predetermined amount of CO_2 gas was injected into the bioreactor through the septum, and no air was flowing at that moment. During the next few seconds, the injected CO_2 gas was mixed with CO_2-free air inside the bioreactor. Then the solenoid valve of the selected bioreactor was opened by the control software allowing the air to flow through the carbon dioxide analyzer at a flow rate of 40 sccm (using the mass flow controller). The CO_2 concentration and time were recorded every 2 seconds automatically by the control software. After the maximum concentration of CO_2 was reached, the clean air valve was opened until the CO_2 concentration was reduced to about 30 ppm, purging the CO_2 analyzer and preparing the system for the next injection [16]. This procedure was done for all 95 bioreactors and repeated for each injection volume (1, 2, 4, 8 cc of pure CO_2). Figure 4-1 describes the calibration procedure in more detail.

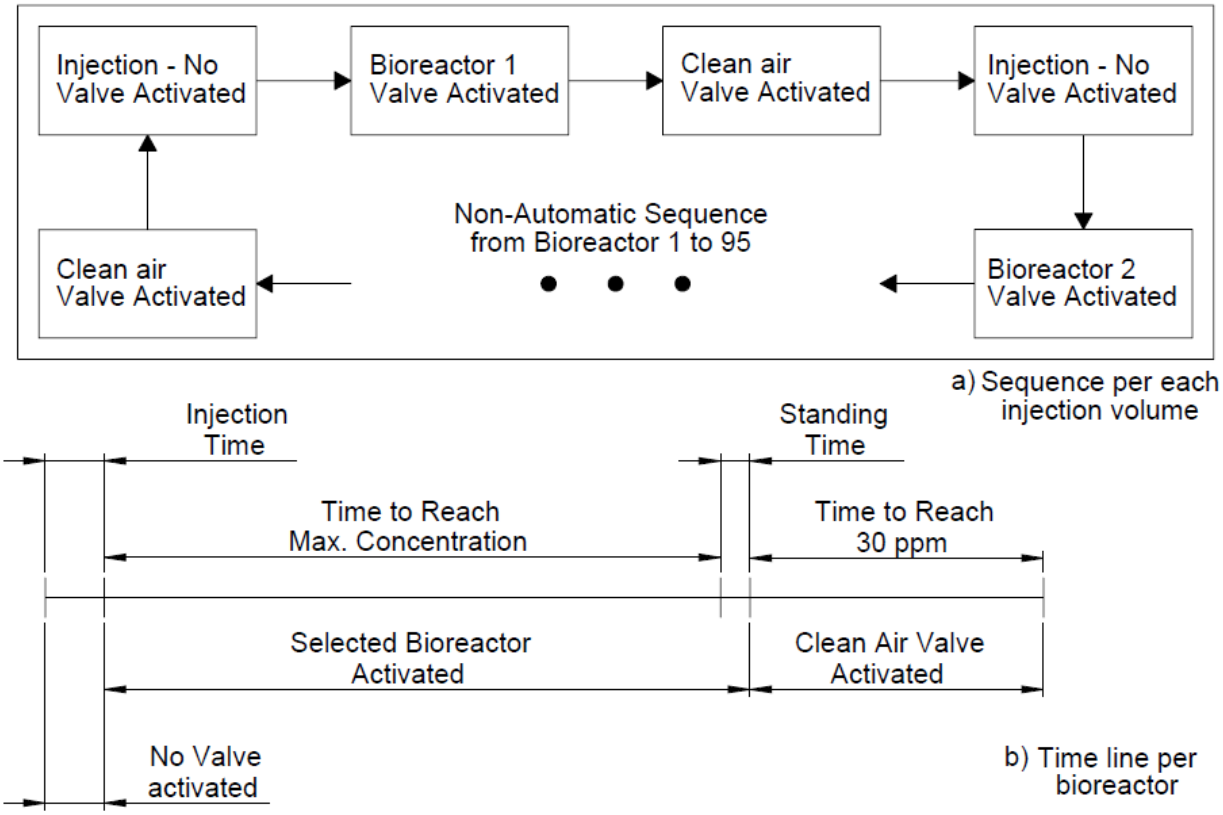

Figure 4-1. Schematic of the calibration procedure

The calibration curve was determined by plotting the peak response concentrations against the actual concentrations for all the bioreactors [29]. The results of the calibration are shown in Table 4-1 and Figure 4-2.

Table 4-1. Actual and peak response concentrations of each injection volume of CO_2 used to calibrate the DMR system [16]

Injected volume (cc) [a]	**Actual concentration, ppm**	**Response concentration, ppm** [b]
1 ± 0.05	507 ± 25	567 ± 32
2 ± 0.05	1014 ± 25	1109 ± 62
4 ± 0.05	2028 ± 25	2160 ± 88
8 ± 0.05	4056 ± 25	4213 ± 129

[a] Pure CO_2 was used for injection

[b] Tolerances based on standard deviation

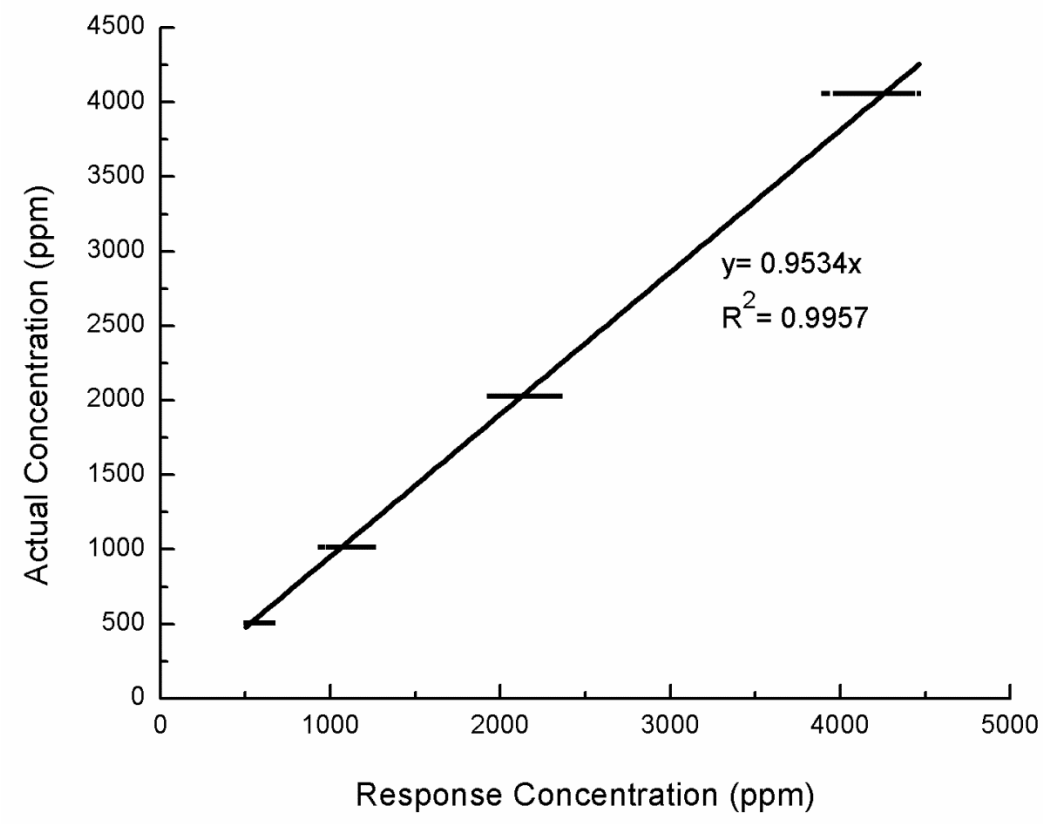

Figure 4-2. Calibration curve at 58 ± 2°C and 55 ± 5% RH, and a flow rate of 40 sccm

A best-fit equation using linear least squares regression was obtained using Microsoft® Excel® 2010. In this case, the calibration curve was found to be a linear relationship with an R-square (R^2) value of 0.9957, represented by the equation: y= 0.9534x, where “x” is the response concentration, “y” is the actual concentration, and 0.9534 is the calibration factor (k).

4.2 System operation

An application to operate the DMR system was developed using LabVIEW™ software and data acquisition systems from National Instruments Corporation, Austin, TX, USA, as mentioned in section 3.9. Basically, the control software opens and closes the solenoid valves attached to the bioreactors based on a predetermined time sequence set by the user, and in an ascending order according to the bioreactor number.

The time sequence for each bioreactor is divided into three periods: purging time, time to reach steady state, and measuring time (Figure 4-3a). A cycle is defined as the time required for all 95 bioreactors to complete this time sequence (Figure 4-3b).

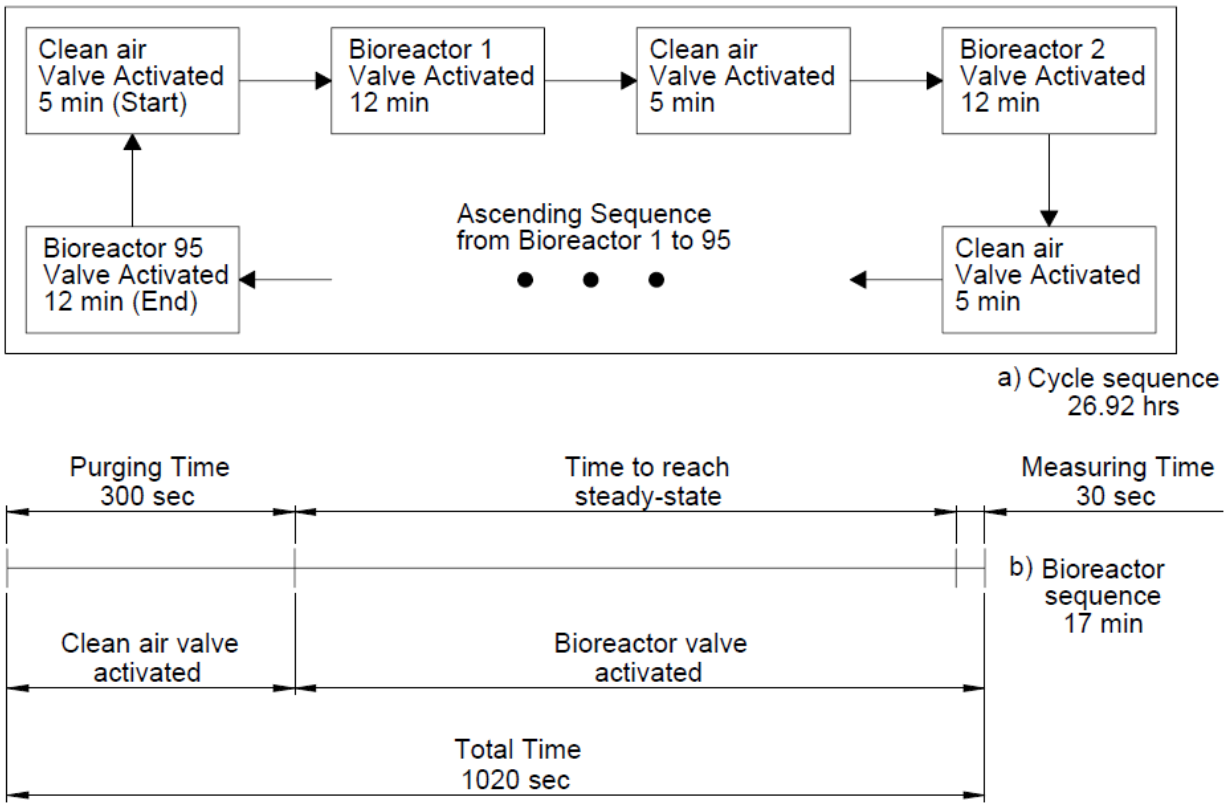

Figure 4-3. Schematic of the system operation

The time periods shown in Figure 4-3 were determined during the calibration procedure. A carbon dioxide injection volume of 8 cc required about 600 seconds to reach steady state, as shown in Figures 4-4 and 4-5. This value was used as a reference for setting the time to reach steady state in the cycle sequence. Ninety seconds were added (690 seconds total) as a safety factor when there is a higher evolution of CO_2 at the beginning of the test. The purging time, which is the time required to reduce the concentration to about 30 ppm, was also determined during calibration, and was found to be 300 seconds. Finally, the measurement time was set

as 30 seconds, during which the control software records the relative humidity (%) and the temperature (°C) from the RH/Temp sensor, the air flow rate (sccm) from the MFC, and the CO_2 concentration (ppm) from the NDIR every 2 seconds.

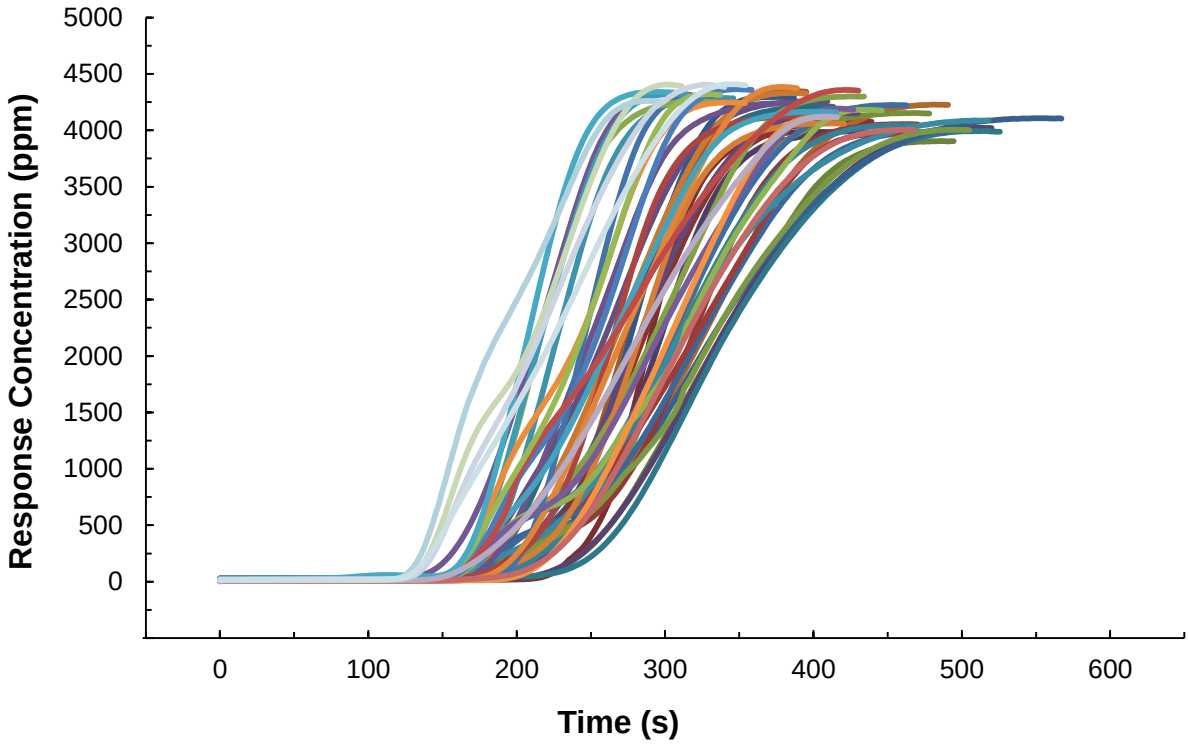

Figure 4-4. Response concentration and time required for selected bioreactors to reach the peak concentration for an injection volume of 8 cc of CO_2

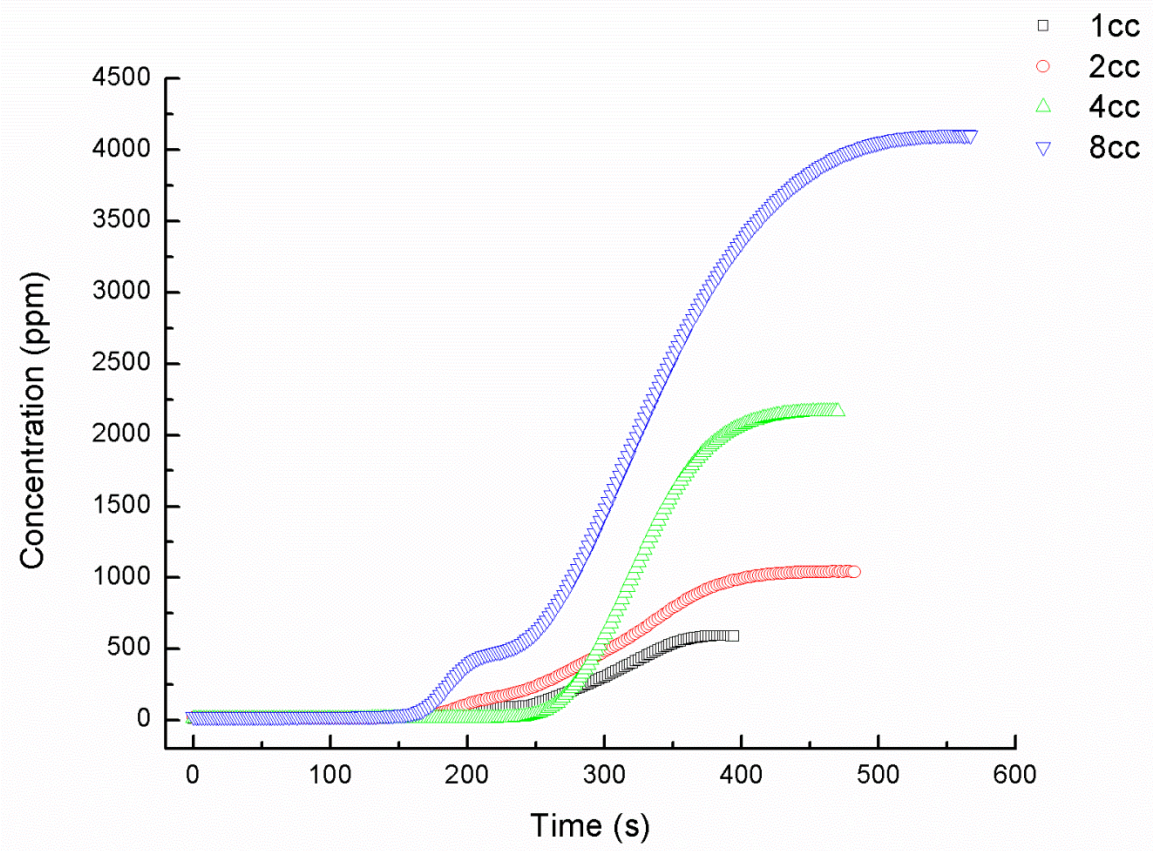

Figure 4-5. Response concentration and time required for a selected bioreactor to reach the peak concentration for each injection volume [16]

Another computer application was developed using Microsoft® Visual Basic® for Excel® to calculate the carbon dioxide evolution and the mineralization of the samples according to ASTM D5338 [ASTM] and ISO 14855 [ISO] standards. This is explained in more detail in the following chapter.

CHAPTER 5

DATA ANALYSIS

The data analysis of the tested materials was performed according to ASTM D5338 [14] and ISO 14855 [15] standards for determination of aerobic biodegradation of plastic materials under controlled composting conditions. The method of analysis of evolved carbon dioxide, in which the amount of CO_2 evolved in each bioreactor and the percentage mineralization of each test material are calculated, was used. According to ASTM D6400 Standard Specification for Compostable Materials [10], a plastic material consisting of a single polymer can be identified as biodegradable when at least 60% of the carbon content thereof is converted to CO_2 by the end of the test when compared with the positive control [10].

5.1 DMR data analyzer

The analysis of the data collected by the DMR was conducted on another computer application called DMR Data Analyzer developed using Microsoft® Visual Basic® for Excel® 2010. A general schematic diagram of the developed application is shown in Figure 5-1. The DMR Data Analyzer code is shown in Appendix A.

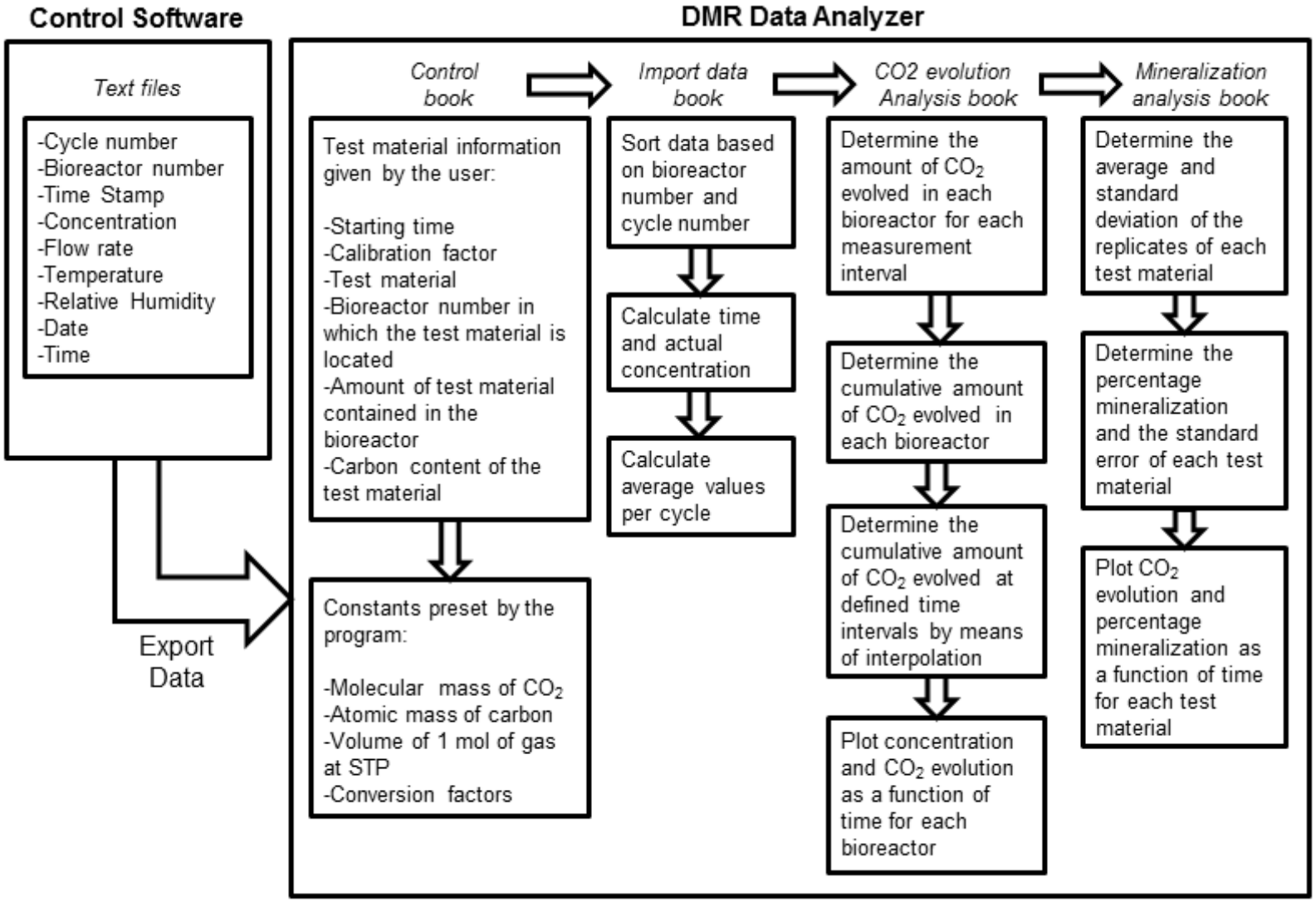

Figure 5-1. Schematic diagram of the DMR Data Analyzer

The amount of CO_2 evolved from the exhaust air of each bioreactor was measured directly using an infrared analyzer about every 27 hours, as stated in section 4.2. Since the CO_2 concentration was measured directly with an NDIR analyzer, it was also required to control and measure the flow rate of the air passing through the infrared analyzer [15].

The control software records the bioreactor number, the time stamp, the CO_2 concentration (ppm), the standard air flow rate (sccm), the temperature (°C), the relative humidity (%), the date (mm/dd/yyyy), and the time (hh:mm:ss), every 2 seconds during the last 30 seconds of a 720-second measurement period; therefore, 15 measurements of each variable are recorded in text files every cycle.

First, the user provides the necessary information for the analysis such as the starting time, the calibration factor obtained from the calibration procedure (see section 4.1), the test materials, the bioreactor number in which each sample of the test material was located, the amount of test material in each bioreactor, and the carbon content of each test material in an Excel® book, which is in turn the control for the application to start the analysis.

5.2 Start-up of the analysis

The data provided by the control software is then exported to another Excel® book, called Import Data, and sorted to different sheets based on bioreactor and cycle

numbers. After that, the actual CO_2 concentration of each measurement is determined by multiplying the response CO_2 concentration by the calibration factor (Equation 5-1).

$$[C] = c * k \quad \text{(Eq. 5-1)}$$

where *[C]* is the actual concentration of CO_2 of each sample (ppm), *c* the response concentration of CO_2 as measured by the NDIR analyzer (ppm), and *k* the calibration factor described in section 4.1.

Then, the time (min) at which each measurement was done, relative to the starting time, is determined by Equation 5-2.

$$t_n = \frac{TS_n - TS_o}{60} \quad \text{(Eq. 5-2)}$$

where t_n is the time at which each measurement was done (min), TS_n is the time stamp at time t_n, and TS_o is the time stamp at time t_o corresponding to the time at which the experiment started.

Next, the average time (min), average concentration (ppm), average flow rate (sccm), average temperature (°C), and average RH (%) are calculated since 15 measurements of each variable are recorded every cycle and only a representative value per cycle is used for the CO_2 evolution calculation . These averaged values are exported to another Excel® book called CO_2 Evolution Analysis.

5.3 Determination of the evolved carbon dioxide

The concentration of CO_2 (ppm) is converted to mass of CO_2 (g) evolved from each bioreactor in the period of time between measurements (measurement interval) as follows:

$$E(CO_2) = \frac{F \times C \times T \times 44}{22414 \times 10^6} \qquad \text{(Eq. 5-3)}$$

where *E(CO2)* is the mass of evolved carbon dioxide (g), *F* the flow rate (sccm), *T* the measurement interval, *C* the concentration of CO_2 evolved during the measurement interval, *22414* the volume of 1 mol of gas in cc at STP, *44* the molecular weight of CO_2 (g/mol), and 10^6 the ppm conversion factor [16].

If the time is plotted against the concentration, as shown in Figure 5-2, then the area under the curve for a specific measurement interval represents the product *C* X *T* in the previous equation (Eq. 5-3) and it is determined by Equation 5-4.

$$A = \frac{([C]_n + [C]_{n-1}) \times (t_n - t_{n-1})}{2} \qquad \text{(Eq. 5-4)}$$

where *A* is the area under the curve (ppm•min), t_n the time in which the measurement was done (min), t_{n-1} the time in which the previous measurement was done (min), $[C]_n$ the concentration of CO_2 (ppm) at time t_n, and $[C]_{n-1}$ is the concentration of CO_2 (ppm) at time t_{n-1}.

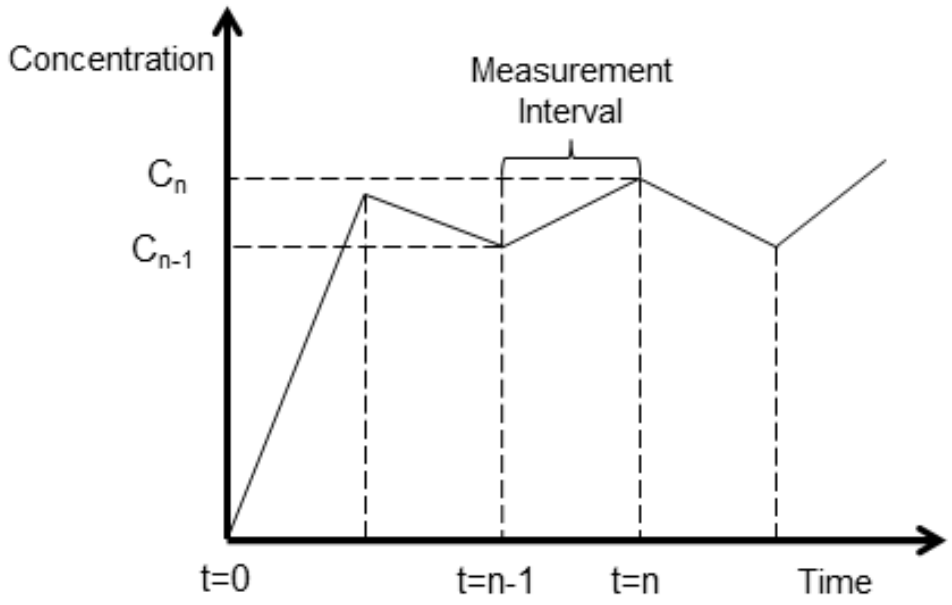

Figure 5-2. Time vs. Concentration Plot

Then, the cumulative amount of CO_2 evolved in each bioreactor for each measurement interval is calculated using Equation 5-5.

$$C(CO_2) = E(CO_2)_n + C(CO_2)_{n-1} \quad \text{(Eq. 5-5)}$$

where $C(CO_2)$ is the cumulative mass of CO_2 (g), $E(CO_2)_n$ is the mass of CO_2 (g) evolved from the sample at time t_n, and $C(CO_2)_{n-1}$ is the cumulative mass of CO_2 (g) until the previous measurement (at time t_{n-1}).

After that, the time is converted from minutes to days, and an interpolation of values is performed since the cumulative mass of CO_2 of the blank has to be subtracted

from the cumulative mass of CO_2 of each sample at the same time interval for further calculating the percentage mineralization (Equation 5-6).

$$I(CO_2) = C(CO_2)_L + (C(CO_2)_H - C(CO_2)_L)\frac{t_I - t_L}{t_H - t_L} \qquad \text{(Eq. 5-6)}$$

where t_I (d) is the time interval, t_L (d) is the immediate lower value of the time interval, t_H (d) is the immediate higher value of the time interval, $I(CO_2)$ (g) is the interpolated cumulative mass of CO_2 at time t_I, $C(CO_2)_L$ (g) is the cumulative mass of CO_2 at time t_L, and $C(CO_2)_H$ (g) is the cumulative mass of CO_2 at time t_H.

5.4 Determination of the percentage biodegradation (mineralization)

Once the cumulative mass of CO_2 of each bioreactor is obtained, the average and standard deviation of the 3 replicates of the blank is calculated as well as those for the samples in another Excel® book called Mineralization Analysis. Thus, the percentage mineralization is calculated using Equation 5-7.

$$\%\, Mineralization = \frac{A(CO_2)_T - A(CO_2)_B}{M_{TOT} \times C_{TOT} \times \frac{44}{12}} \times 100 \qquad \text{(Eq. 5-7)}$$

where *% Mineralization* is the percent carbon molecules converted to CO_2, $A(CO_2)_T$ is the average cumulative mass of CO_2 (g) evolved from the sample, $A(CO_2)_B$ the average cumulative mass of CO_2 (g) evolved from the blank, M_{TOT} the mass of test material (g), C_{TOT} the proportion of total organic carbon in the total mass of test material (g/g) , *44* the molecular weight of carbon dioxide, and *12* the atomic weight of carbon [14-16]. The denominator in equation 5-7 is known as the theoretical amount of carbon dioxide $Th(CO_2)$ that can be produced by the test material, i.e. when 100% of the carbon molecules present in the test material were converted to CO_2 [15].

In addition to the calculations, the concentration and CO_2 evolution are plotted as a function of time for each bioreactor, and the CO_2 evolution and percentage mineralization are plotted as a function of time for each test material.

Some plastic materials were selected and tested using the DMR system. The collected data were calculated using the developed DMR Data Analyzer to determine their biodegradability. The materials and methods, as well as the results and their interpretation are discussed in the next chapter.

CHAPTER 6

RESULTS AND VALIDATION

Two tests for determining the aerobic biodegradation of several polymers under composting conditions were performed to show the efficacy and efficiency of the DMR system and the DMR Data Analyzer. The tests were performed following the requirements and methodology described in section 2.3. The differences between the two tests were the compost inoculum used and the water availability. Further details are described in the following sections.

6.1. Preparation of the compost inoculum

For the first test (run 1), Earthgro® organic humus and manure from Scotts Miracle-Gro (Marysville, OH, USA) was obtained. For the second test (run 2), a 12-month-old mature compost inoculum was obtained from MSU Composting Facility (East Lansing, MI). In both cases, the compost was sieved on a 10-mm screen to remove large debris and inert material as recommended by the ASTM D5338 and ISO 14855 standards. Then, the compost inoculum was pre-conditioned for a period of 3 days in an environmental chamber at 58°C for microorganisms to acclimatize to the test conditions [30].

The compost moisture content was determined by using a moisture analyzer, model MX-50, from A&D Company, Tokyo, Japan, and it was found to be 35.92% and

37.76% (wet) for run 1 and 2, respectively. Distilled water was added to increase the moisture content up to about 50%.

Vermiculite premium grade from Sun Gro Horticulture Distribution Inc., Bellevue, Washington, was saturated with distilled water (1:5 parts water), and added in both cases to the compost (1:4 parts dry weight compost) to provide better aeration and to prevent clogging [13].

A thermogravimetric analysis of the compost was performed using a thermogravimetric analyzer model TGA Q50 (TA Instruments, New Castle, DE) to determine the ash content, dry solids, and volatile solids thereof (see Figures 6-1 and 6-2). The heating rate was set at 10°C/min, and the samples were run from room temperature to 560°C.

According to the ISO 14855 Standard, the total dry solids are obtained after drying the compost sample to about 105°C, while the volatile solids are obtained by subtracting the residues after incineration at about 550°C from the total dry solids of the same sample [15].

Additionally, samples of the compost were sent to the Soil and Plant Nutrient Laboratory at Michigan State University (East Lansing, MI, USA) for determination of pH and carbon-nitrogen (C/N) ratio. The pH of the compost inoculum was found to be 7.6 and 8.3, for run 1 and 2, respectively. The C/N ratio of the compost for both tests was found to be 12.5. A summary of physical-chemical parameters of the compost is shown in Table 6-1.

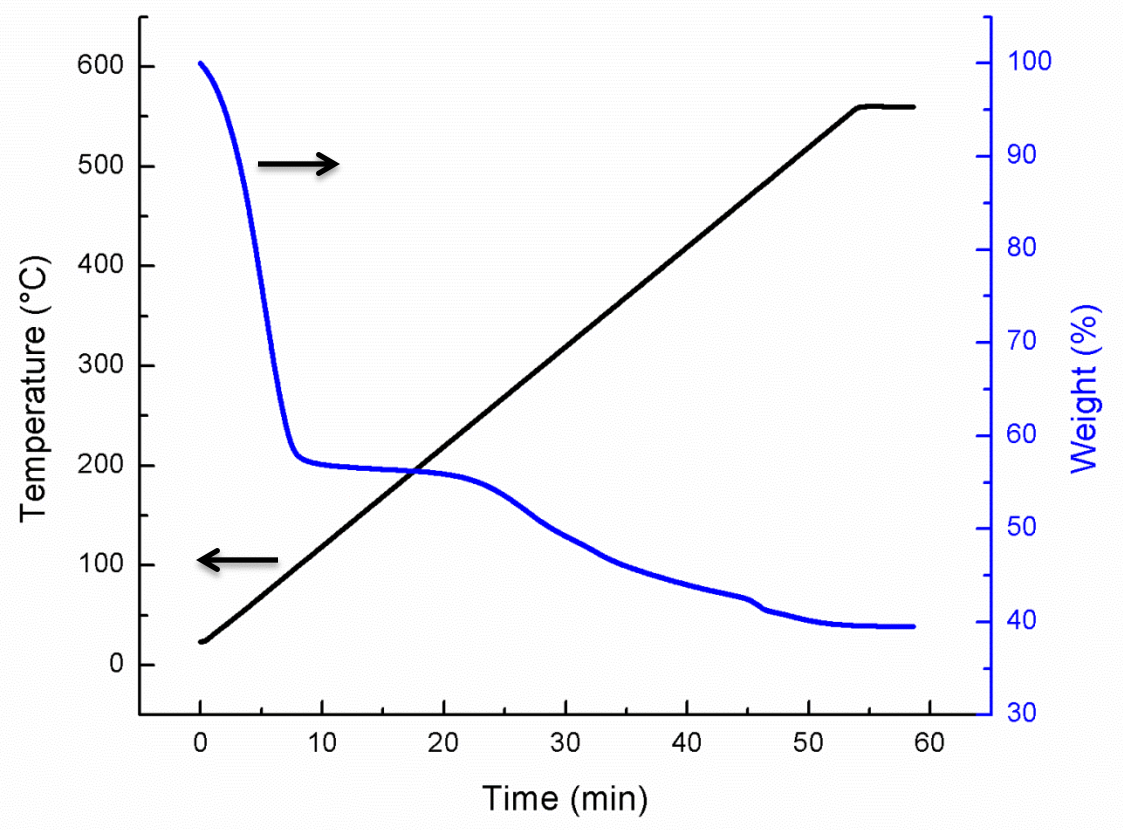

Figure 6-1. Thermogravimetric analysis of the compost in run 1

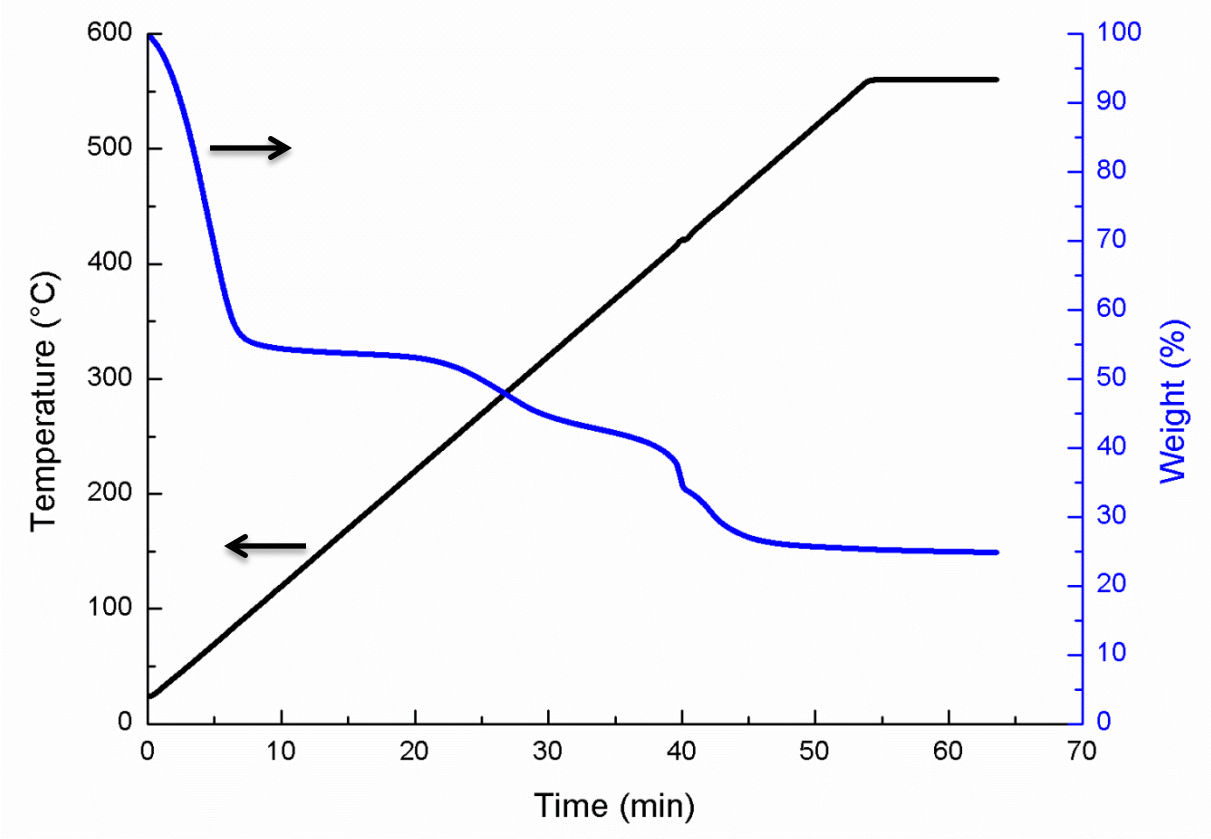

Figure 6-2. Thermogravimetric analysis of the compost in run 2

Table 6-1. Physical-chemical parameters of the compost inoculum used on the biodegradation test

Parameter	Range	Run 1	Run 2
pH	7 – 9 [b]	7.6	8.3
C/N ratio	10 – 40 [ab]	12.5	12.5
Ash content	< 70% [a]	39.7	25.4%
Dry solids	50 – 55% [ab]	57.4	54.8%

[a] Values based on ASTM D5338-11
[b] Values based on ISO 14855-1:2005

The ASTM D5338 and ISO 14855 standards also require the compost inoculum to produce between 50 and 150 mg of CO_2 per gram of volatile solids over the first 10 days. Figures 6-3 and 6-4 show the production of CO_2 (mg) per g of volatile solids for run 1 and 2, respectively. Compost used in run 1 and 2 produced 35 and 51 mg of CO_2 per g of volatile solids at day 10, respectively. The production of CO_2 over the first 10 days indicates the microbial activity of the compost.

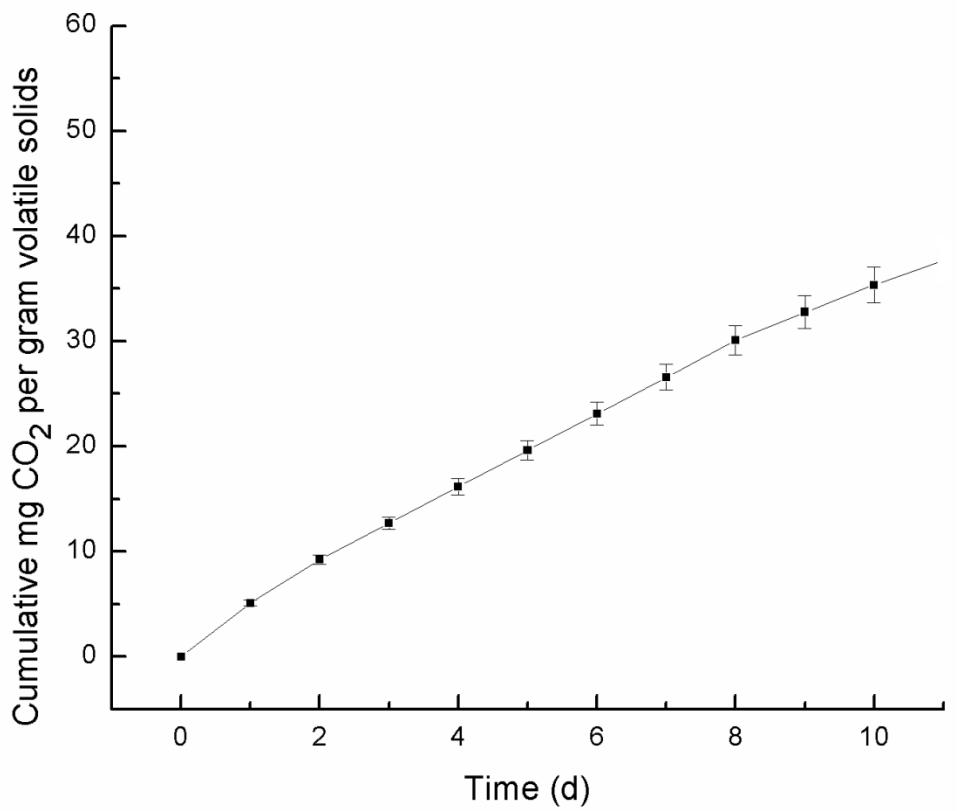

Figure 6-3. Production of CO_2 per gram of volatile solids in run 1 as a function of time

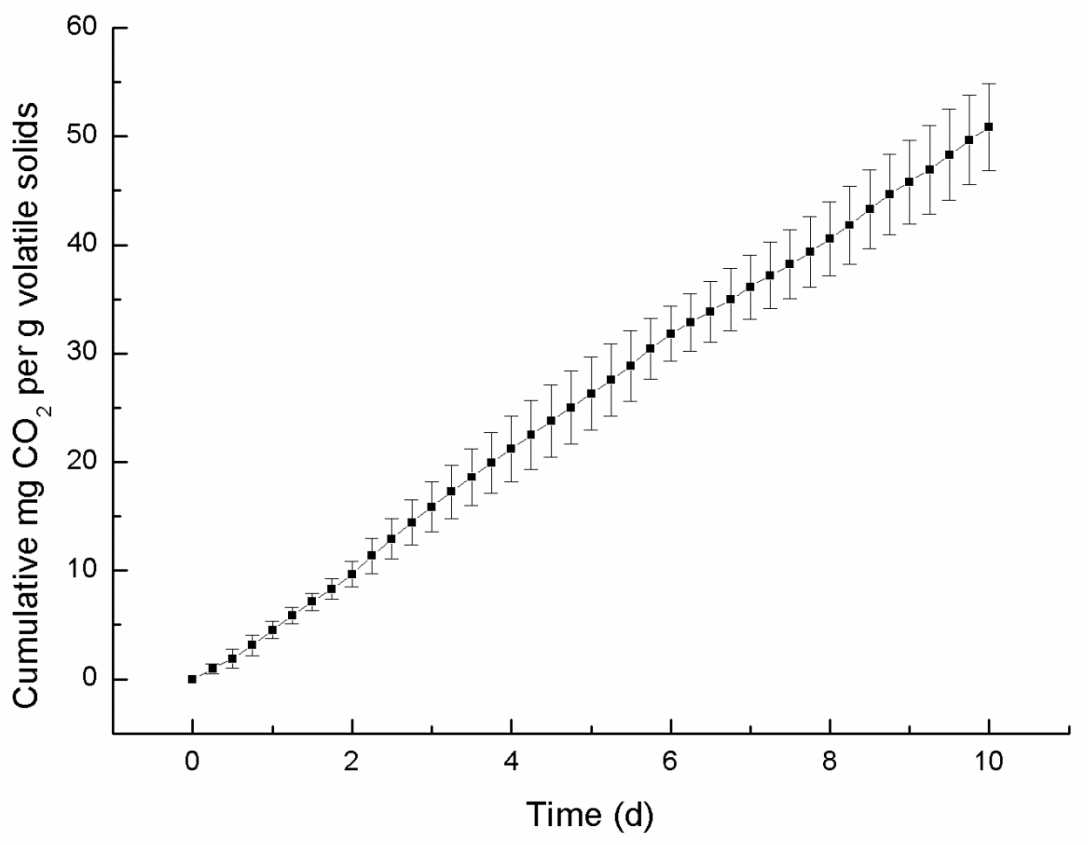

Figure 6-4. Production of CO_2 per gram of volatile solids in run 2 as a function of time

6.2 Preparation of the test materials

Five test materials were selected in each case among all materials tested using the DMR system. Triplicates of each test material were analyzed. Additionally, triplicates of cellulose powder (positive control), and 3 replicates of blank bioreactors (with compost inoculum only) were evaluated.

The materials selected in both cases were as follows: low density polyethylene/ linear low density polyethylene (LDPE/LLDPE) blend film and polyethylene

terephthalate (PET) sheet, both without and with 5% (by weight) oxidation-promoting additive; and gamma irradiated cellophane (GMCP) film. All test materials were cut to 1cm X 1cm pieces.

Cellulose powder (particle size ~20 μm) was obtained from Sigma-Aldrich (St. Louis, MO, USA). The cellulose moisture content was determined by using a moisture analyzer, model MX-50, from A&D Company (Tokyo, Japan), and it was found to be 5.29% (wet).

LDPE and LLDPE were blended at a ratio of 3:7 parts LDPE by weight for both without and with five percent (by weight) of Reverte® degradation-promoting additive, obtained from Wells Plastics Ltd (Stone, Staffordshire, UK). The films were obtained by blown extrusion. The overall thickness of the LDPE/LLDPE without and with 5% additive film was 0.0229 mm (0.9 mil), and 0.0279 mm (1.1 mil), respectively.

PET sheet was manufactured by cast extrusion, without and with 5% (by weight) of Reverte® degradation-promoting additive from Wells Plastics Ltd (Stone, Staffordshire, UK). The overall thickness of the PET without and with 5% additive sheet was 0.2337 mm (9.2 mil) and 0.2286 mm (9.0 mil) respectively.

Cellophane films, thickness of 0.0229 mm (0.9 mil), were obtained from Innovia Films Inc. (Atlanta, GA, USA). These films were gamma irradiated (^{60}Co source 1.3 million curies at Food Technology Service, Inc, FL, USA), and stored at 25°C and 60% RH during a period of 3 months for run 1 and 6 month for run 2.

The percentage carbon content of all the test samples was determined by using a PerkinElmer 2400 Series II CHNS/O Elemental Analyzer (Shelton, CT, USA). The carbon content of the selected test materials is provided in Table 6-2.

Table 6-2. Carbon content of selected test materials

Test material	**Carbon content** [a]
Cellulose powder	43.5
LDPE/LLDPE film w/o additive	84.8
LDPE/LLDPE film 5% additive	85.5
PET sheet w/o additive	62.1
PET sheet 5% additive	61.5
Cellophane gamma irradiated film	40.2

[a] percentage by weight

6.3 Determination of the aerobic biodegradation

Conditioned compost inoculum (550 g and 400 g for run 1 and 2, respectively) was weighed and mixed with 8 grams of the test material in a container in order to get a homogeneous mixture. Then, the mixture was transferred to the bioreactor, which was tightly closed to prevent leakage. Bioreactors were filled to about three-quarters of their volume with the mixture leaving sufficient headspace to allow further manual shaking.

Subsequently, bioreactors were placed into the environmental chamber which was preconditioned at a constant temperature of 58°C. Aeration was initiated using water-saturated carbon-dioxide-free air, and the flow rate through each bioreactor was

set at 40 sccm. Thus, bioreactors were connected to the air supply system as described in sections 3.5 and 3.6.

Finally, the control software was activated using the parameters described in section 4.2 for both cases. A summary of these parameters is shown in Table 6-3.

Table 6-3. Parameters for setting-up control software

Parameter	**Setting**
Air humidity	55 ± 5%
Air flow rate	40 ± 2 sccm
Temperature	58 ± 2°C
Duration	1020 s [a]
Purging time	300 s [a]
Measuring time	30 s [a]

[a] per bioreactor per cycle

The tests were carried out in the dark for a period of 140 days and 45 days for run 1 and 2, respectively. Throughout the testing period, water was added to avoid drying conditions and bioreactors were shaken to prevent clogging. The results and further details are shown in the next section.

6.4 Results and Discussion

The analysis of the data collected by the DMR control software was conducted every week by using the DMR data analyzer as described in section 5.1. First, the amount of CO_2 evolved from each bioreactor during the measurement interval was calculated using equation 5-3.

Subsequently, the average cumulative amount of CO_2 evolved from each test material was calculated using equations 5-4 to 5-6. Figures 6-5 and 6-6 show the cumulative evolved CO_2 of the selected test materials as a function of time for run1 and 2, respectively.

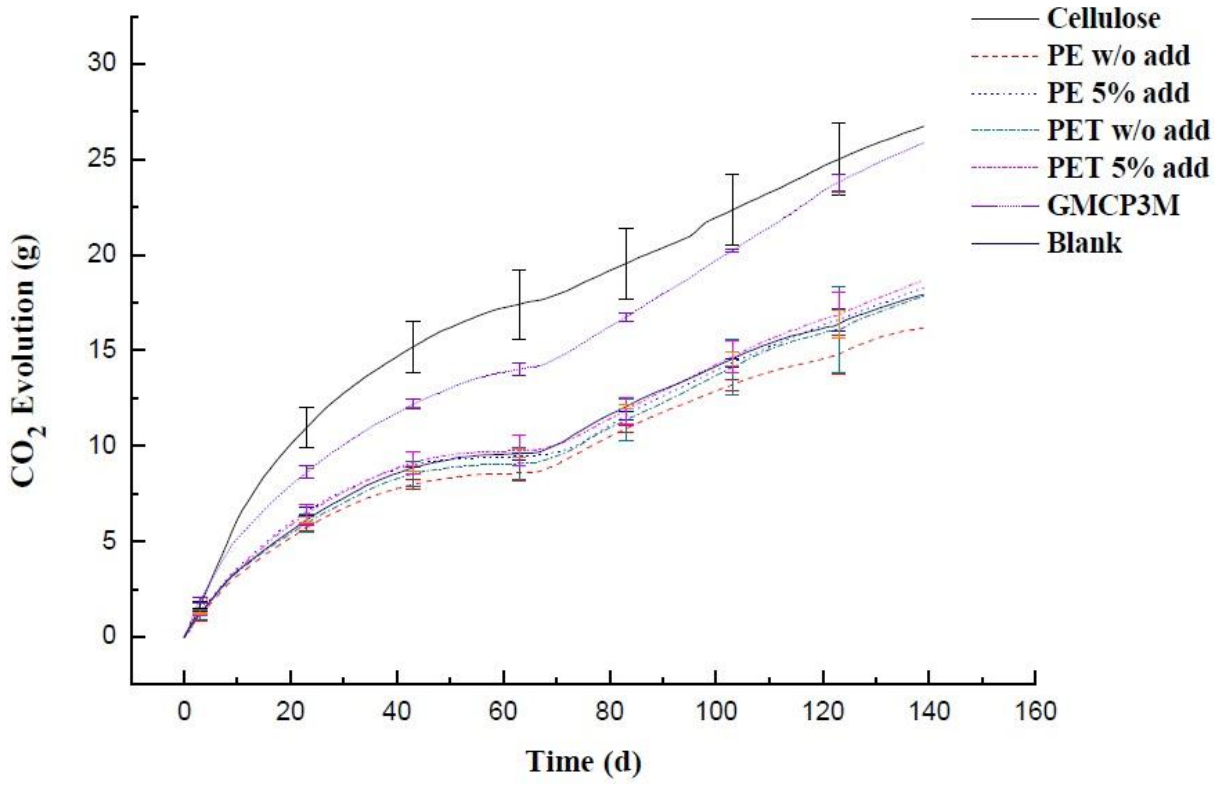

Figure 6.5. Evolution of CO_2 of selected test materials in run 1 as function of time

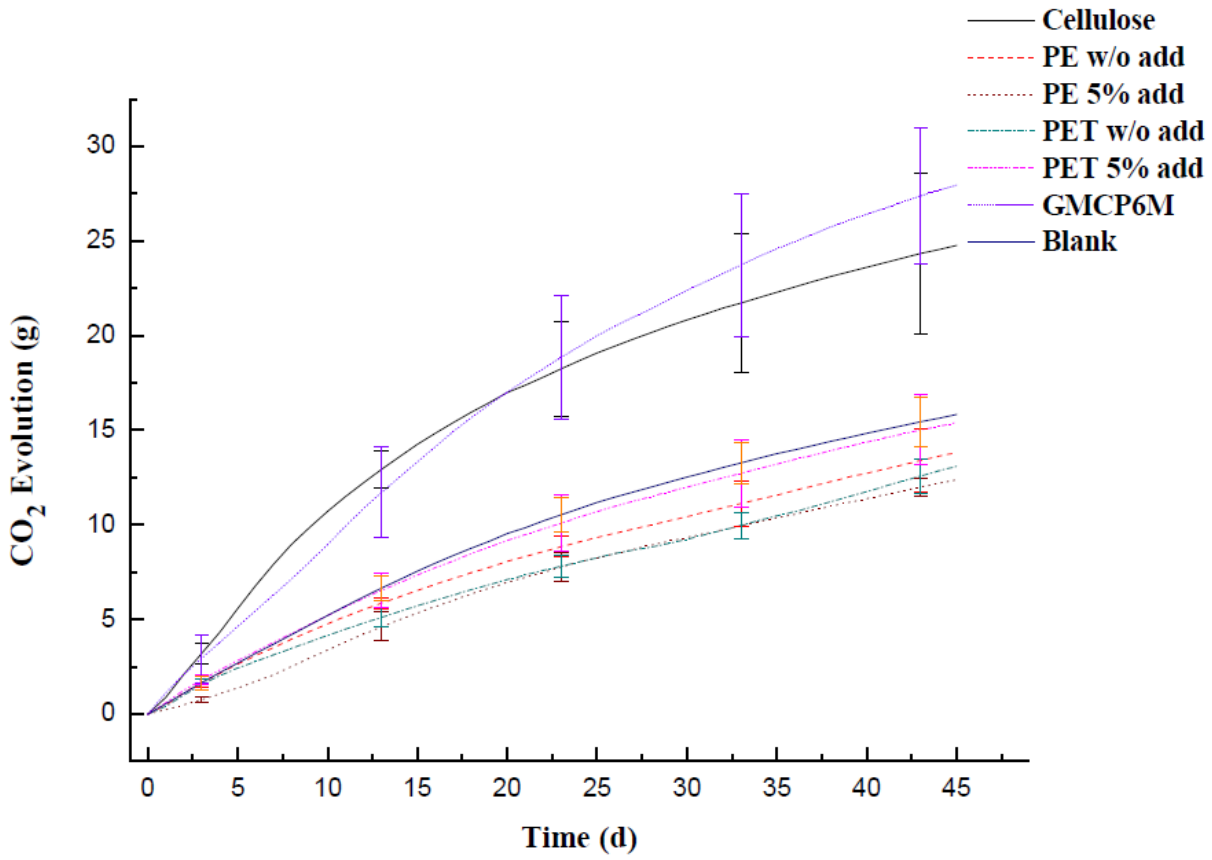

Figure 6.6. Evolution of CO_2 of selected test materials in run 2 as function of time

Then, the percentage mineralization of each test material was calculated using equation 5-7, which is the relationship between the actual amount of carbon dioxide evolved from the test material and the theoretical amount of carbon dioxide that can be evolved from the same test material. Thus, for example, the *Th(CO_2)* of the cellulose powder, i.e. when 100% of the carbon molecules present in the cellulose were converted to $CO_2$is expected to be 12.1 and 12.7 g for run 1 and run 2, respectively.

Figures 6-7 and 6-8, show the percentage mineralization of the selected test materials as a function of time for run1 and 2, respectively.

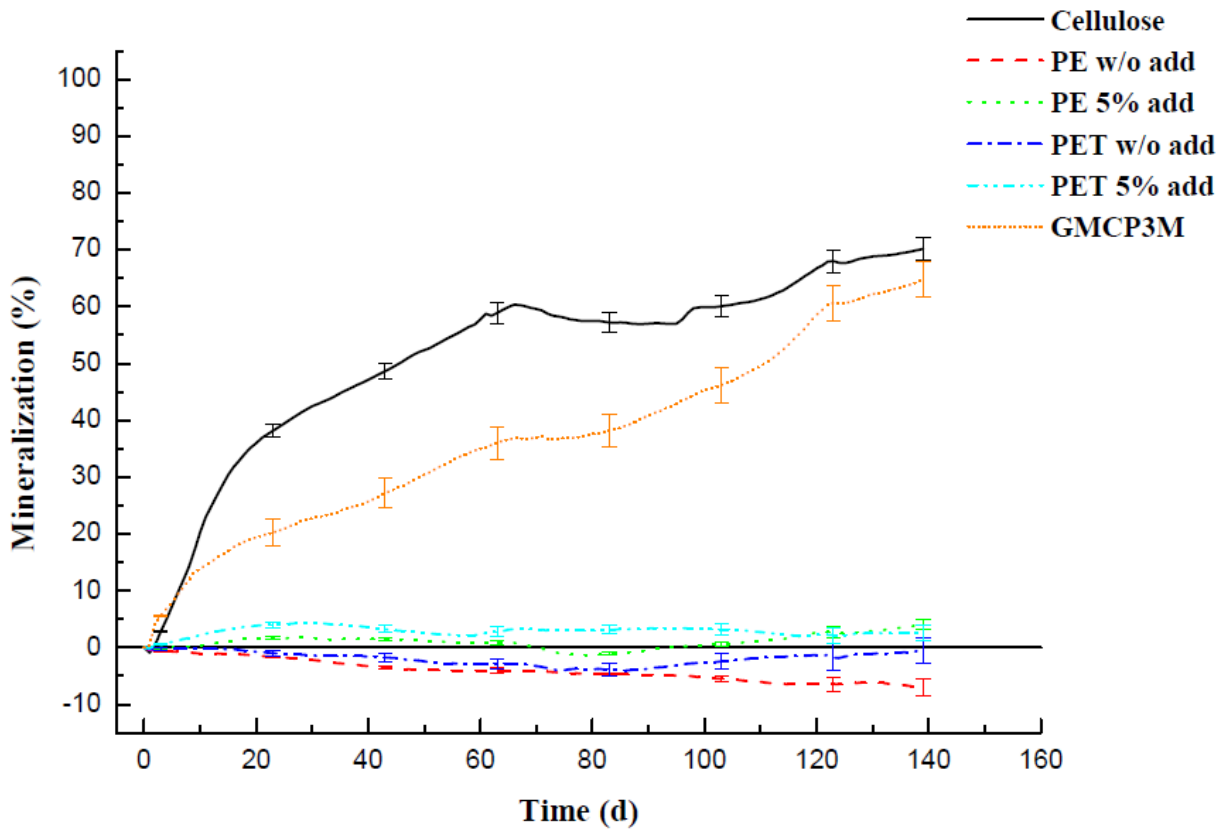

Figure 6-7. Percentage mineralization of selected test materials in run 1 as function of time

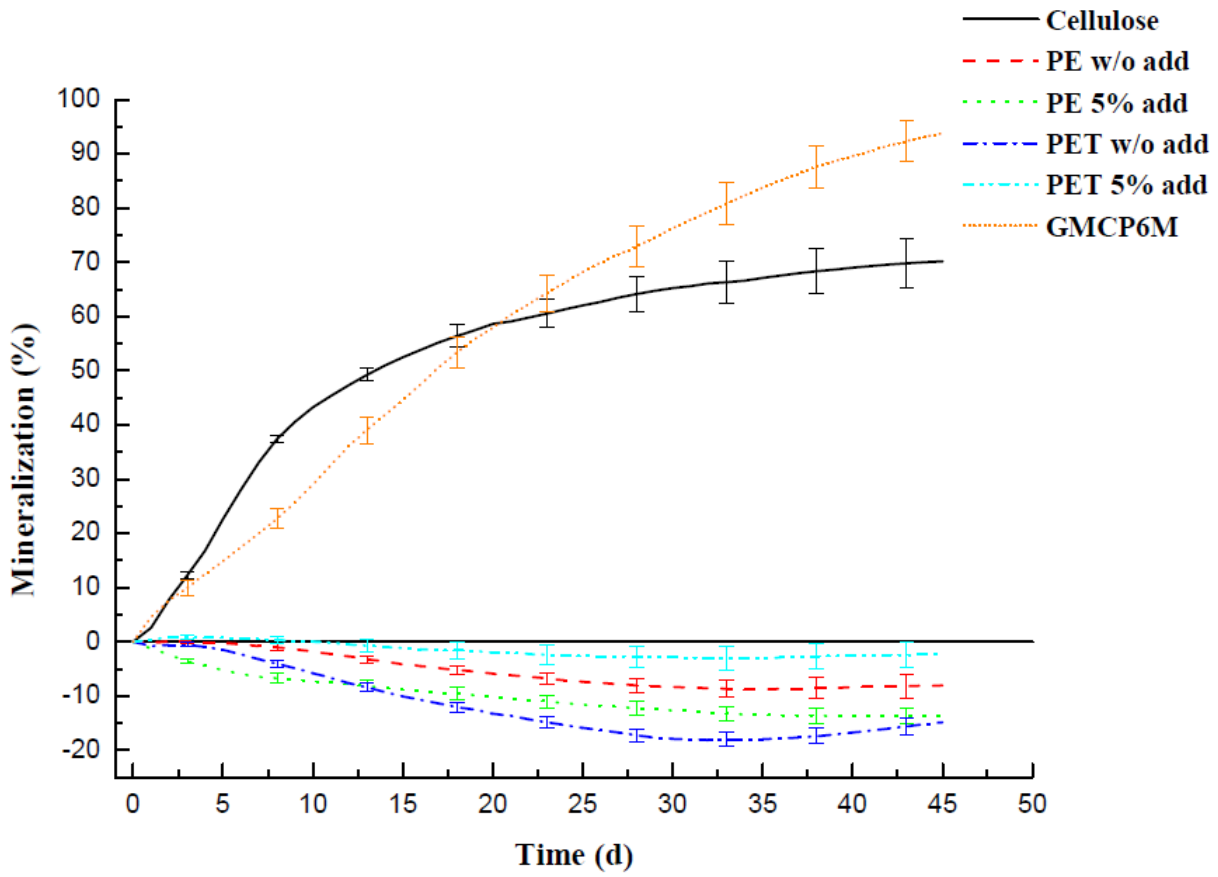

Figure 6-8. Percentage mineralization of selected test materials in run 1 as function of time

Tables 6-4 and 6-5, show a summary of the results at day 139 for run 1 and day 45 for run 2, respectively.

Table 6-4. Summary of results for run 1 at day 139

Test material	CO_2 production (g)	Mineralization (%)	Relative mineralization [a] (%)
Cellulose	26.43	70.15	100
LDPE w/o additive	16.19	-7.00	-9.98
LDPE w/ additive	18.26	1.33	1.90
PET w/o additive	17.84	-0.48	-0.68
PET w/ additive	18.67	4.08	5.82
GMCP3M	28.79	88.83	126.63
Blank	17.93	NA	NA

[a] Percentage mineralization relative to the cellulose

Table 6-5. Summary of results for run 2 at day 45

Test material	CO_2 production (g)	Mineralization (%)	Relative mineralization [a] (%)
Cellulose	24.76	70.18	100
LDPE w/o additive	13.82	-8.04	-11.46
LDPE w/ additive	12.38	-13.71	-19.54
PET w/o additive	13.10	-14.87	-21.19
PET w/ additive	15.40	-3.32	-4.73
GMCP6M	27.95	93.80	133.66
Blank	15.82	NA	NA

[a] Percentage mineralization relative to the cellulose

From Figure 6-9, it can be noticed that in both cases cellulose, which is the positive reference, was able to reach 70% mineralization but at different rates. As reviewed by Kijchavengkul and Auras, biodegradability and biodegradation rates are affected by two factors: polymer characteristics, depending on the polymer itself, and exposure conditions which are divided in abiotic and biotic factors. Temperature, pH, and moisture are among the abiotic factors while biotic factors depend on the enzymes produced by different microorganisms to assimilate the polymer chains [31].

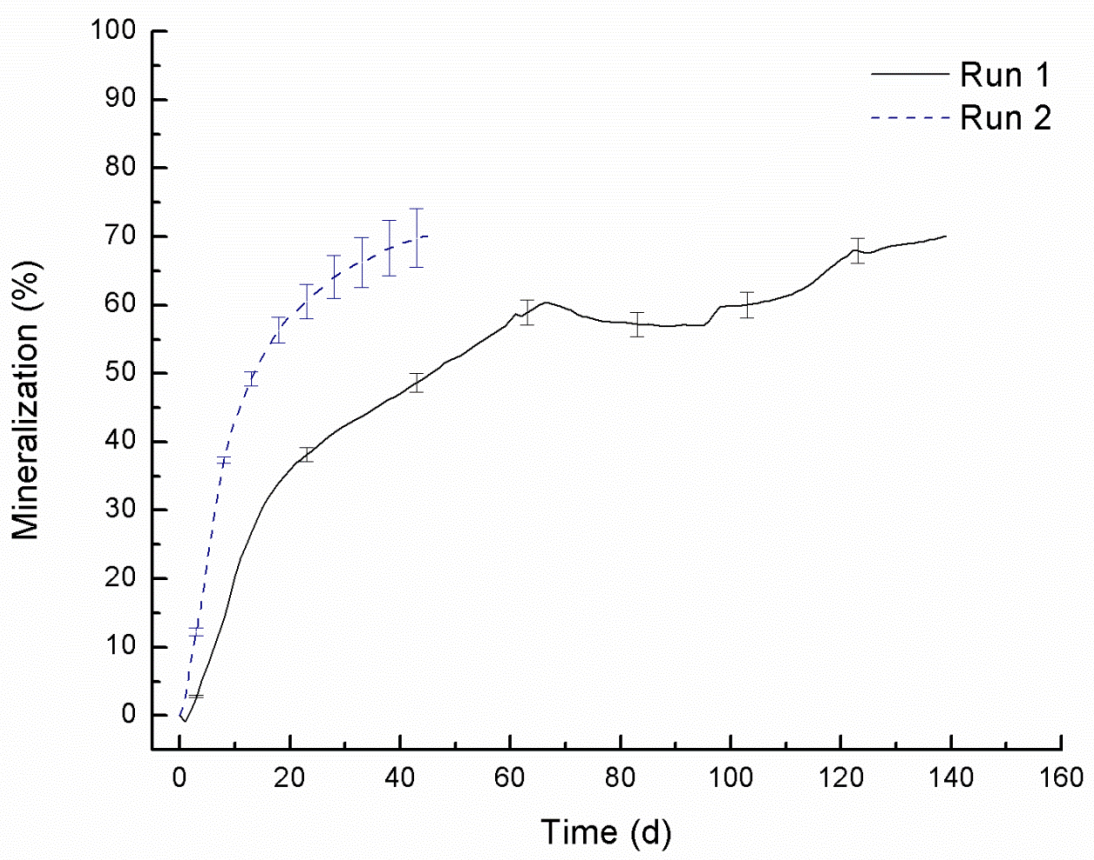

Figure 6-9. Comparison between run 1 and run 2 of cellulose mineralization

Usually, temperatures about 58°C are considered optimal for composting since compost microorganisms are thermophilic, while temperatures above 65°C would kill several microbial species limiting the biodegradation rate [12].

Likewise, a neutral or weakly basic pH allows the survival and full activity of the microorganisms, while an acidic pH can inhibit biodegradation [12]. Similarly, it is recommended to keep the moisture content at about 50% since lower values tend to slow down the biodegradation process. On the other hand, excess of moisture leads to anaerobiosis by compost packing down [12].

Moisture is one of the most important factors affecting the biodegradation rate since water is a distribution medium for microorganisms and nutrients, and it influences microbial development and metabolic activity [12].

In this case, it is thought that difference in the biodegradation rate between run 1 and 2 was due to water availability and initial microbial cosmos of the initial compost since other exposure conditions were kept as recommended in the literature and ASTM D5338 and ISO 14855 standards.

In the case of run 1, water was not added at the beginning of the test, i.e. the moisture content depended entirely on the water-saturated air supplied to the bioreactors. As reviewed by Way et al, the use of water-saturated air helps preventing excessive drying of the compost, but it is not enough to maintain the moisture content thereof at the level required [8]. Therefore, regular addition of water throughout the testing period is recommended [8, 9, 12]. Thus, after day 60, when the compost experienced excessive drying, about 12 ml of distilled water was injected into each bioreactor every three days clearly affecting the biodegradation rate as shown in Figures 6-5 and 6-7.

In the case of run 2, a measured volume of water was added to each bioreactor every three days in order to maintain the moisture content of the compost at about 50%. This was achieved by first measuring the moisture content of the compost in the control bioreactors which are two blank bioreactors, additional to the three required for the test, that were submitted to the test conditions for monitoring moisture content and pH of the

compost throughout the testing period. The measurement was performed by using the moisture analyzer.

Thus, the amount of water required for adjusting the moisture content at about 50% was calculated based on the dry weight of the compost contained in each bioreactor. Then, the measured volume of water was injected through the septum located on top of the bioreactor lid (see figure 3-6). After the water was injected, all the bioreactors were shaken in order to homogenize the contents and prevent clogging.

Even though this method did not keep the moisture content of the compost at a constant level, it did help preventing excessive drying of the compost, helping to get more rapid biodegradation in comparison to run 1, and in accordance with the period of time suggested by the ASTM D5338 and ISO 14855 standards.

On the other hand, the composition of the compost also plays an important role in the rate of biodegradation. Different raw materials such as manure, yard, and food waste produce different microbial activity [13], and contain different amounts of carbon and nitrogen. For example, manure is rich in nitrogen, but although it is required for microorganisms to grow, manure is usually mixed with other materials rich in carbon to obtain a proper C/N ratio [12, 31].

Kale *et al.* reported that the addition of vermiculite to compost might contribute to faster biodegradation by providing better aeration and retaining moisture [13]. Additionally, studies have shown that vermiculite is a good microbial carrier [12].

Additionally, from figures 6-7 and 6-8, it can be clearly noticed that regardless the time, two main groups of polymers are present. The first group, which includes PE and

PET polymers, does not show any meaningful mineralization while the second group, consisting of cellulose and cellophane materials, shows significant mineralization and according to ASTM D6400 can be considered as biodegradable materials since they reached more than 60% mineralization.

The difference in the behavior between groups 1 and 2 can be explained by the second factor affecting biodegradation, which depends entirely on the polymer characteristics such as the chemical structure, conformational flexibility, crystallinity, molecular weight, tacticity, hydrophobicity, and addition of additives [31].

On one hand, polymers in group 1 are synthetic polymers known for not being easily degradable because of their hydrophobic characteristics, and relatively high stability [5] which is provided by the presence of single bonds between carbon atoms in the polymer chain that are especially difficult to break [11]. Notice that the polymers containing degradation-promoting additives seem to follow the same behavior as those without the additive at least for the duration of the test.

On the other hand, cellulose and cellophane (group 2) are considered biodegradable since microorganisms have evolved enzymes to attack their polymer chains. Besides, they tend to interact strongly with water due to their hydrophilic characteristics which make them first degrade by hydrolysis accelerating even more the biodegradation process [11]. Cellulose reached a higher mineralization value than cellophane; this may have occurred since the cellulose was tested as a powder while the cellophane was tested as a film. Biodegradation is usually, but not always, a surface erosion mechanism. Therefore, materials in powder usually degrade more easily than

films since the area/volume ratio is maximized [12, 31]. From group 2, it can also be noticed that gamma radiation may have affected the biodegradation rate of the cellophane, but that discussion is beyond the scope of this work.

CHAPTER 7

CONCLUSIONS AND RECOMENDATIONS

7.1 Conclusions

A medium-scale automated direct measurement respirometric (DMR) system capable of simultaneously assessing the aerobic biodegradation of up to 30 materials in triplicate was designed and built with the following characteristics:

- A scrubbing system capable of reducing the ambient carbon dioxide concentration to values below 30 ppm, thereby increasing the signal-to-noise ratio;
- An environmental chamber and a relative humidity generator to simulate different testing conditions;
- An air supply and flow control system to ensure aerobic conditions for the test;
- Bioreactors with the proper characteristics to ensure optimal aeration, and to allow the performance of calibration and injection of water;
- A non-dispersive infrared gas analyzer capable of measuring the carbon dioxide concentration of the exhaust air;
- Two in-house developed computer applications, one for automatically controlling and measuring the test variables, and another one for rapidly and automatically analyzing the generated data.

In this context, it was demonstrated that the DMR system developed in this work accomplishes the characteristics required for assessing aerobic biodegradation of materials, regardless of whether they are biodegradable or not, as required by both ASTM D5338 and ISO 14855 standards. Additionally, the assessment can be done in a shorter period of time since the equipment is capable of testing several materials simultaneously and the analysis of the generated data can be performed in minutes by using the DMR data analyzer.

Further recommendations were drawn upon the completion of several tests. Besides running the biodegradation tests and collecting data, it also was very important to keep the exposure conditions, especially moisture which highly affects the biodegradation rate, under strict controlled conditions. Periodic injection of water helps preventing excessively drying conditions of the compost allowing faster biodegradation.

Likewise, the physical and chemical parameters of the compost used for testing were important to consider since different kinds of compost have different microbial activity which can directly affect the rate of biodegradation. Additionally, highly active compost may decrease the signal-to-noise ratio or simply fall beyond the limits of the sensor.

Finally, non-biodegradable polymers, which showed low evolution of CO_2, and biodegradable polymers, in which at least 60% of their organic carbon content was converted to CO_2 at the end of the test, were successfully tested concluding that the developed DMR system was capable of tracking the carbon dioxide evolution of the

tested samples. Finally, further calculations were performed to demonstrate the quality of the data obtained from the DMR.

7.2 Recommendations

In this case two different computer applications were developed, one for controlling the DMR system and one for analyzing the data. In the future, it would be good to join these two applications together, so that the DMR unit is able to operate and give results automatically in the period of time set by the user. Besides, with the inclusion of internet connection the data can be stored and back up automatically in a regular basis allowing the user to have remote monitoring. This system would also allow the equipment to send an alarm message whenever one of the variables involved fall outside the preset limits.

As previously stated, water availability is a very important factor affecting biodegradation rates. Two things can be done regarding this issue. First, the relative humidity can be improved in some way to allow better humidification of the incoming air. Second, an automatic system for injecting water into the bioreactors can be designed and installed, but a mechanism for monitoring the moisture content of the compost of each bioreactor is required for this purpose.

The use of distilled water or purified water may improve the performance of the relative humidity generator by avoiding sediment accumulation on the bubble diffuser. Maintaining the water level inside the tank is also important for this purpose.

There were some minor issues regarding the interfaces between the devices and the computer; hence it is recommended to use more recent data acquisition devices which have improved functionality. Likewise, updating the software and optimizing the developed computer application is recommended.

Due to the high temperature and moisture required for the test, solenoid valves rusted; hence locating the EMC's outside the environmental chamber, insertion of water filters, or obtaining solenoid valves made of a stainless material is recommended. The use of dielectric grease on the solenoid valves and regular maintenance helped prevent excessively rusty conditions.

Since water in the exhaust air is condensed once it is outside the environmental chamber, a water trap was required to avoid damage of the mass flow controller and the gas analyzer. However, this water trap needed to be emptied every two days due to a rapid accumulation of water; hence the use of a bigger water trap or another system would prevent the need to empty the water trap very often.

Finally, it is recommended to use either stronger lids or another system for tightly closing the bioreactors, such as air-tight metal clamp lids with rubber gaskets, since currently, the aluminum lids are screwed to the glass container, and after some time when the bioreactors are opened and closed several times the lids tend to warp, which may cause leakage through the lid.

APPENDIX

APPENDIX A

DMR DATA ANALYZER CODE

A.1 Import and Filter Data

```
Public Sub Import()
'Import data from especified folder
    Dim myFile As String
    Dim completeName As String
    Dim count As Integer
    Dim Location As String
    Dim sourceFolder As String
    Dim processedFolder As String
    Dim totalDataStart As Integer
    Dim totalDataEnd As Integer

 'Specify folder location
    Location = Workbooks("Control").Sheets("FileLocation").Range("C2").Value
    sourceFolder = Workbooks("Control").Sheets("FileLocation").Range("C3").Value
    processedFolder = Workbooks("Control").Sheets("FileLocation").Range("C4").Value
    myFile = Dir(Location & sourceFolder & "*"): If myFile = "" Then Exit Sub
     Workbooks("Import_Data").Activate
    Sheets("DataInput").Select
     'Select last cell and save position
    Range("B1048576").End(xlUp).Offset(1, 0).Select
```

```
totalDataStart = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
Do
'Import data
completeName = "TEXT;" & Location & sourceFolder & myFile
    With ActiveSheet.QueryTables.Add(Connection:= _
   completeName, Destination:=Range( _
   "$B$" & totalDataStart))
   .Name = myFile
   .FieldNames = True
   .RowNumbers = False
   .FillAdjacentFormulas = False
   .PreserveFormatting = True
   .RefreshOnFileOpen = False
   .RefreshStyle = xlInsertDeleteCells
   .SavePassword = False
   .SaveData = True
   .AdjustColumnWidth = False
   .RefreshPeriod = 0
   .TextFilePromptOnRefresh = False
   .TextFilePlatform = 850
   .TextFileStartRow = 1
   .TextFileParseType = xlDelimited
   .TextFileTextQualifier = xlTextQualifierDoubleQuote
   .TextFileConsecutiveDelimiter = False
   .TextFileTabDelimiter = True
   .TextFileSemicolonDelimiter = False
   .TextFileCommaDelimiter = False
```

```
        .TextFileSpaceDelimiter = False
        .TextFileColumnDataTypes = Array(1, 1, 1, 1, 1, 1)
        .TextFileTrailingMinusNumbers = True
        .Refresh BackgroundQuery:=False
            'Select last cell and save position
               Range("B1048576").End(xlUp).Select
               totalDataEnd = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

            'Print cycle number
            For i = totalDataStart To totalDataEnd
               Cells(i, 1) = Mid(myFile, 1, Len(myFile) - 3)
            Next i
            totalDataStart = totalDataEnd + 1

    End With

     'Move processed files to specified folder
    CreateObject("Scripting.FileSystemObject").GetFile(Location & sourceFolder &
myFile).Move Location   & processedFolder
    myFile = Dir
    Loop Until myFile = ""

    'Sort data based on bioreactor and cycle number respectively
    Range("A2:I" & totalDataEnd).Select
    Range("I" & totalDataEnd).Activate
    ActiveWorkbook.Worksheets("DataInput").Sort.SortFields.Clear
    ActiveWorkbook.Worksheets("DataInput").Sort.SortFields.Add Key:=Range( _
```

```
        "B2:B" & totalDataEnd), SortOn:=xlSortOnValues, Order:=xlAscending,
DataOption:= _
        xlSortNormal
    ActiveWorkbook.Worksheets("DataInput").Sort.SortFields.Add Key:=Range( _
        "A2:A" & totalDataEnd), SortOn:=xlSortOnValues, Order:=xlAscending,
DataOption:= _
        xlSortNormal
    With ActiveWorkbook.Worksheets("DataInput").Sort
        .SetRange Range("A2:I" & totalDataEnd)
        .Header = xlGuess
        .MatchCase = False
        .Orientation = xlTopToBottom
        .SortMethod = xlPinYin
        .Apply
    End With
Range("A2").Select
Workbooks("Control").Activate
Sheets("General").Select
Range("A1").Select
End Sub


Public Sub Filter()
'Filter data from the input data to each of the designated sheets based on bioreactor
number
Dim Bioreactor As Integer
Dim Cycle As Integer
Dim TimeStamp As Double
Dim Concentration As Double
```

```
Dim Flow As Double
Dim Temperature As Double
Dim RH As Double
Dim StartingTime As Double
Dim n As Integer
Dim m As Integer
Dim a As Integer
Dim b As Integer
Dim count As Integer
Dim totalData As Integer
Dim check As String
Dim FixedFlow As Integer

StartingTime = Workbooks("Control").Sheets("General").Range("C2").Value
check = Workbooks("Control").Sheets("Restrictions").Range("C8").Value
FixedFlow = Workbooks("Control").Sheets("Restrictions").Range("F8").Value

'Count is the row number
count = 2

'n is the position of the last sample
Workbooks("Control").Activate
Sheets("SampleInfo").Select
Range("A1048576").End(xlUp).Select
n = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
```

```
'm is the position of the last ignored cycle
Sheets("IgnoredCycles").Select
Range("A1048576").End(xlUp).Select
m = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address)) + 1
Workbooks("Import_Data").Activate

While Range("DataInput!B" & count).Value <> ""

    'Evaluate whether an specific bioreactor has to be analyzed or not, 1 meaning true, 0 meaning false
    a = Application.WorksheetFunction.CountIf(Workbooks("Control").Sheets("SampleInfo").Range("A2:A" & n), Workbooks("Import_Data").Sheets("DataInput").Range("B" & count).Value)

    'Evaluate whether an specific cycle has to be ignored or not, 1 meaning true, 0 meaning false
    b = Application.WorksheetFunction.CountIf(Workbooks("Control").Sheets("IgnoredCycles").Range("A2:A" & m), Workbooks("Import_Data").Sheets("DataInput").Range("A" & count).Value)

    If a = 1 And b = 0 Then

        Sheets("DataInput").Select

        'Copy data
        Cycle = Cells(count, 1)
        TimeStamp = Cells(count, 3)
        Concentration = Cells(count, 4)
        Flow = Cells(count, 5)
```

```
    Temperature = Cells(count, 6)
    RH = Cells(count, 7)
    'Select proper sheet
    Sheets(CStr(Range("DataInput!B" & count).Value)).Select
    Range("A1048576").End(xlUp).Offset(1, 0).Select
    totalData = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    'Paste data

    If check = "" Then
        Cells(totalData, 4) = Flow
    Else
        Cells(totalData, 4) = FixedFlow
    End If

    Cells(totalData, 1) = Cycle
    Cells(totalData, 2) = (TimeStamp - StartingTime) / 60
    Cells(totalData, 3) = Concentration
    Cells(totalData, 5) = Temperature
    Cells(totalData, 6) = RH

    totalData = totalData + 1

End If

    count = count + 1
```

```
Wend

Workbooks("Control").Activate
Sheets("General").Select
Range("A1").Select

End Sub

Public Sub Averages()
'Calculate the average of all the test variables
Dim ResponceConc As Double
Dim ActualConc As Double
Dim Time As Double
Dim Concentration As Double
Dim Flow As Double
Dim Temperature As Double
Dim RH As Double
Dim CorrectionFactor As Double
Dim cyclenumber As Integer
Dim totalDataPerSheet As Integer
Dim LastCycle As Integer
Dim FirstCell As Integer
Dim LastCell As Integer
Dim totalData As Integer
Dim n As Integer
Dim m As Integer
Dim MinFlow As Integer
```

```
Dim MinConc As Integer

CorrectionFactor = Workbooks("Control").Sheets("General").Range("C3").Value
MinFlow = Workbooks("Control").Sheets("Restrictions").Range("C2").Value
MinConc = Workbooks("Control").Sheets("Restrictions").Range("C4").Value

'Determine the position of the last sample
Workbooks("Control").Activate
Sheets("SampleInfo").Select
Range("A1048576").End(xlUp).Select
n = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

'i counts sheets based on bioreactor number
For i = 2 To n

    'm is the bioreactor number
    m = Workbooks("Control").Sheets("SampleInfo").Range("A" & i).Value

    'Select last cell and save position of the first available cell
    Workbooks("Import_Data").Activate
    Sheets(CStr(m)).Select
    Range("A1048576").End(xlUp).Select
    totalDataPerSheet = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
    LastCycle = ActiveCell.Value

        Do
            'Count cycles
```

```
Workbooks("Import_Data").Activate
Sheets(CStr(m)).Select
Range("G1048576").End(xlUp).Offset(1, 0).Select
FirstCell = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
Range("A" & FirstCell).Select
cyclenumber = ActiveCell.Value

Do
   'Select and save position of the last cell with the cycle number selected
   If ActiveCell.Value = cyclenumber Then
   ActiveCell.Offset(1, 0).Select
   Else
   ActiveCell.Offset(-1, 0).Select
   LastCell = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
   Exit Do
   End If

Loop

For j = FirstCell To LastCell

   RespConc = Cells(j, 3)

   ActualConc = RespConc * CorrectionFactor
   Cells(j, 7) = ActualConc

Next j
```

```
        Flow =
Application.WorksheetFunction.Average(Workbooks("Import_Data").Sheets(CStr(m)).R
ange("D" & FirstCell & ":D" & LastCell))

        Concentration =
Application.WorksheetFunction.Average(Workbooks("Import_Data").Sheets(CStr(m)).R
ange("G" & FirstCell & ":G" & LastCell))


        If Flow > MinFlow And Concentration > MinConc Then


        Workbooks("CO2_Evolution_Analysis").Activate

        Sheets(CStr(m)).Select

        Range("A1048576").End(xlUp).Offset(1, 0).Select

        totalData = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))


        Cells(totalData, 1) = cyclenumber

        Cells(totalData, 5) = Flow

        Cells(totalData, 4) = Concentration


        Time =
Application.WorksheetFunction.Average(Workbooks("Import_Data").Sheets(CStr(m)).R
ange("B" & FirstCell & ":B" & LastCell))

        Cells(totalData, 2) = Time

        Cells(totalData, 3) = Time / 1440

        Cells(totalData, 11) = Time / 1440


        Temperature =
Application.WorksheetFunction.Average(Workbooks("Import_Data").Sheets(CStr(m)).R
ange("E" & FirstCell & ":E" & LastCell))

        Cells(totalData, 6) = Temperature
```

```
        RH =
Application.WorksheetFunction.Average(Workbooks("Import_Data").Sheets(CStr(m)).R
ange("F" & FirstCell & ":F" & LastCell))

        Cells(totalData, 7) = RH

        End If


    Loop Until cyclenumber = LastCycle


Next i


   Workbooks("Control").Activate

   Sheets("General").Select

   Range("A1").Select


End Sub
```

A.2 CO_2 Evolution Analysis

```
Public Sub Evolution()

'Calculate the amount of CO2 evolved from each bioreactor
Dim Cycle As Integer
Dim Time As Double
Dim Concentration As Double
Dim Flow As Double
Dim Dtime As Double
Dim Area As Double
Dim EvolvedCO2 As Double
Dim CumulativeCO2 As Double
Dim FirstCycle As Integer
Dim MolWeight As Double
Dim Volume As Double
Dim ppm As Double
Dim count As Integer
Dim n As Integer
Dim m As Integer

MolWeight = Workbooks("Control").Sheets("General").Range("C4").Value
Volume = Workbooks("Control").Sheets("General").Range("C5").Value
ppm = Workbooks("Control").Sheets("General").Range("C6").Value

'Determine the position of the last sample
Workbooks("Control").Activate
Sheets("SampleInfo").Select
```

```
Range("A1048576").End(xlUp).Select
n = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

'i counts sheets based on bioreactor number
For i = 2 To n

    'm is the bioreactor number
    m = Workbooks("Control").Sheets("SampleInfo").Range("A" & i).Value

    'Select last cell and save position of the first available cell
    Workbooks("CO2_Evolution_Analysis").Activate
    Sheets(CStr(m)).Select
    FirstCycle = Range("A2").Value

    Range("H1048576").End(xlUp).Offset(1, 0).Select
    count = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    While Range("A" & count).Value <> ""

        Cycle = Cells(count, 1)
        Time = Cells(count, 2)
        Concentration = Cells(count, 4)
        Flow = Cells(count, 5)

        If Cycle = FirstCycle Then

            Dtime = Time
```

```
    Cells(count, 8) = Dtime
    Area = (Concentration * Dtime) / 2
    Cells(count, 9) = Area

    EvolvedCO2 = (Area * Flow * MolWeight) / (Volume * ppm)
    Cells(count, 10) = EvolvedCO2

    CumulativeCO2 = EvolvedCO2
    Cells(count, 12) = CumulativeCO2

Else

    Dtime = Time - Range("B" & count - 1).Value
    Cells(count, 8) = Dtime

    Area = ((Concentration + Range("D" & count - 1).Value) * Dtime) / 2
    Cells(count, 9) = Area

    EvolvedCO2 = (Area * Flow * MolWeight) / (Volume * ppm)
    Cells(count, 10) = EvolvedCO2

    CumulativeCO2 = EvolvedCO2 + Range("L" & count - 1).Value
    Cells(count, 12) = CumulativeCO2

End If

    count = count + 1
```

```
        Wend
Next i

    Workbooks("Control").Activate
    Sheets("General").Select
    Range("A1").Select

End Sub
```

Public Sub Interpolation()

'Interpolate the cumulative amount of CO_2 evolved from each bioreactor at specific time interval

```
Dim InterpolatedTime As Double
Dim InterpolatedCO2 As Double
Dim LastDay As Integer
Dim Day As Integer
Dim Lower As Integer
Dim Higher As Integer
Dim TimeInterval As Double
Dim FirstCycle As Integer
Dim n As Integer
Dim m As Integer
Dim t As Double

TimeInterval = Workbooks("Control").Sheets("Restrictions").Range("C6").Value
```

```
'Determine the position of the last sample
Workbooks("Control").Activate
Sheets("SampleInfo").Select
Range("A1048576").End(xlUp).Select
n = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

Workbooks("CO2_Evolution_Analysis").Activate
Sheets(CStr(Workbooks("Control").Sheets("SampleInfo").Range("A2").Value)).Select
Range("K1048576").End(xlUp).Select
LastDay = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
Day = Application.WorksheetFunction.RoundDown(Range("K" & LastDay).Value, 0)

'i counts sheets based on bioreactor number
For i = 2 To n

    'm is the bioreactor number
    m = Workbooks("Control").Sheets("SampleInfo").Range("A" & i).Value

    'Select last cell and save position of the first available cell
    Workbooks("CO2_Evolution_Analysis").Activate
    Sheets(CStr(m)).Select
    Range("M1048576").End(xlUp).Offset(1, 0).Select
    t = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
    InterpolatedTime = Range("M" & t - 1).Value + TimeInterval

    While InterpolatedTime <> Day + TimeInterval
```

```
        Cells(t, 13) = InterpolatedTime

        If Range("K2").Value < InterpolatedTime Then


        Lower = Application.WorksheetFunction.Match(InterpolatedTime, Range("K1:K" &
t), 1)


        Higher = Application.WorksheetFunction.Match(InterpolatedTime, Range("K1:K" &
t), 1) + 1


        InterpolatedCO2 = Range("L" & Lower).Value + (Range("M" & t).Value - Range("K"
& Lower).Value) * (Range("L" & Higher).Value - Range("L" & Lower).Value) /
(Range("K" & Higher).Value - Range("K" & Lower).Value)

        Cells(t, 14) = InterpolatedCO2


        Else


        InterpolatedCO2 = (Range("M" & t).Value) * (Range("L2").Value) /
(Range("K2").Value)

        Cells(t, 14) = InterpolatedCO2


        End If


        t = t + 1

        InterpolatedTime = InterpolatedTime + TimeInterval


    Wend


Next i
```

```
    Workbooks("Control").Activate
    Sheets("General").Select
    Range("A1").Select

End Sub
```

A.3 Mineralization Analysis

```
Public Sub MineralizationData()
'Filter calculated data based on test material
Dim CO2 As Double
Dim Time As Double
Dim TimeInterval As Double
Dim sample As String
Dim LastDay As Double
Dim n As Integer
Dim m As Integer
Dim j As Integer
Dim a As Integer
Dim b As Integer
Dim c As Integer
Dim k As Integer
Dim x As Integer
Dim countB As Integer
Dim countS As Integer

TimeInterval = Workbooks("Control").Sheets("Restrictions").Range("C6").Value

'n is the position of the last bioreactor
Workbooks("Control").Activate
Sheets("SampleInfo").Select
Range("A1048576").End(xlUp).Select
n = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
```

```
Sheets("SampleTable").Select
Range("A1048576").End(xlUp).Select
m = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

'i counts sheets based on bioreactor number
For i = 2 To n

    'j is the bioreactor number
    j = Workbooks("Control").Sheets("SampleInfo").Range("A" & i).Value

    'Evaluate whether an specific bioreactor is replicate 1
    a = Application.WorksheetFunction.CountIf(Workbooks("Control").Sheets("SampleTable").Range("C3:C" & m), j)

    'Evaluate whether an specific bioreactor is replicate 2
    b = Application.WorksheetFunction.CountIf(Workbooks("Control").Sheets("SampleTable").Range("D3:D" & m), j)

    'Evaluate whether an specific bioreactor is replicate 3
    c = Application.WorksheetFunction.CountIf(Workbooks("Control").Sheets("SampleTable").Range("E3:E" & m), j)

    If c = 1 Then

        Workbooks("Control").Activate
        Sheets("SampleTable").Select
```

```
    Range("E3:E" & m).Find(What:=j, LookAt:=xlWhole, SearchOrder:=xlByColumns,
SearchDirection:=xlNext, MatchCase:=False).Activate
    k = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
    sample = Range("A" & k).Value

    Workbooks("Mineralization_Analysis").Activate
    Sheets(CStr(sample)).Select
    Range("D1048576").End(xlUp).Offset(1, 0).Select
    countS = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    If countS = 2 Then
       LastDay = Range("A" & countS).Value
    Else
       LastDay = Range("A" & countS - 1).Value + TimeInterval
    End If

    Workbooks("CO2_Evolution_Analysis").Activate
    Sheets(CStr(j)).Select
    Range("M1048576").End(xlUp).Select
    x = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
    Range("M2:M" & x).Find(What:=LastDay, LookAt:=xlWhole,
SearchOrder:=xlByColumns, SearchDirection:=xlNext, MatchCase:=False).Activate
    countB = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    While Workbooks("CO2_Evolution_Analysis").Sheets(CStr(j)).Range("M" &
countB).Value <> ""

       Workbooks("CO2_Evolution_Analysis").Activate
```

```
        Sheets(CStr(j)).Select

        'Copy data
        Time = Cells(countB, 13)
        CO2 = Cells(countB, 14)

        Workbooks("Mineralization_Analysis").Activate
        Sheets(CStr(sample)).Select

        Cells(countS, 1) = Time
        Cells(countS, 5) = Time
        Cells(countS, 7) = Time
        Cells(countS, 4) = CO2

        countB = countB + 1
        countS = countS + 1

    Wend

ElseIf b = 1 Then

    Workbooks("Control").Activate
    Sheets("SampleTable").Select
    Range("D3:D" & m).Find(What:=j, LookAt:=xlWhole, SearchOrder:=xlByColumns,
SearchDirection:=xlNext, MatchCase:=False).Activate
    k = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
    sample = Range("A" & k).Value
```

```
    Workbooks("Mineralization_Analysis").Activate
    Sheets(CStr(sample)).Select
    Range("C1048576").End(xlUp).Offset(1, 0).Select
    countS = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    If countS = 2 Then
       LastDay = Range("A" & countS).Value
    Else
       LastDay = Range("A" & countS - 1).Value + TimeInterval
    End If

    Workbooks("CO2_Evolution_Analysis").Activate
    Sheets(CStr(j)).Select
    Range("M1048576").End(xlUp).Select
    x = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
    Range("M2:M" & x).Find(What:=LastDay, LookAt:=xlWhole,
SearchOrder:=xlByColumns, SearchDirection:=xlNext, MatchCase:=False).Activate
    countB = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    While Workbooks("CO2_Evolution_Analysis").Sheets(CStr(j)).Range("M" &
countB).Value <> ""

       Workbooks("CO2_Evolution_Analysis").Activate
       Sheets(CStr(j)).Select

       'Copy data
       CO2 = Cells(countB, 14)
```

```
        Workbooks("Mineralization_Analysis").Activate
        Sheets(CStr(sample)).Select

        Cells(countS, 3) = CO2

        countB = countB + 1
        countS = countS + 1

    Wend

ElseIf a = 1 Then

    Workbooks("Control").Activate
    Sheets("SampleTable").Select
    Range("C3:C" & m).Find(What:=j, LookAt:=xlWhole, SearchOrder:=xlByColumns,
SearchDirection:=xlNext, MatchCase:=False).Activate
    k = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
    sample = Range("A" & k).Value

    Workbooks("Mineralization_Analysis").Activate
    Sheets(CStr(sample)).Select
    Range("B1048576").End(xlUp).Offset(1, 0).Select
    countS = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    If countS = 2 Then
        LastDay = Range("A" & countS).Value
    Else
        LastDay = Range("A" & countS - 1).Value + TimeInterval
```

```
        End If

        Workbooks("CO2_Evolution_Analysis").Activate
        Sheets(CStr(j)).Select
        Range("M1048576").End(xlUp).Select
        x = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))
        Range("M2:M" & x).Find(What:=LastDay, LookAt:=xlWhole,
SearchOrder:=xlByColumns, SearchDirection:=xlNext, MatchCase:=False).Activate
        countB = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

        While Workbooks("CO2_Evolution_Analysis").Sheets(CStr(j)).Range("M" &
countB).Value <> ""

            Workbooks("CO2_Evolution_Analysis").Activate
            Sheets(CStr(j)).Select

            'Copy data
            CO2 = Cells(countB, 14)

            Workbooks("Mineralization_Analysis").Activate
            Sheets(CStr(sample)).Select

            Cells(countS, 2) = CO2

            countB = countB + 1
            countS = countS + 1

        Wend
```

```
    End If

Next i

Workbooks("Control").Activate
Sheets("General").Select
Range("A1").Select

End Sub

Public Sub MineralizationCalculation()
'Calculate the percentage mineralization of the test materials
Dim AverageCO2 As Double
Dim StandardDeviation As Double
Dim Weight As Double
Dim Carbon As Double
Dim CO2Blank As Double
Dim DevBlank As Double
Dim CO2Sample As Double
Dim Mineralization As Double
Dim DevSample As Double
Dim Error As Double
Dim FirstRow As Integer
Dim LastRow As Integer
Dim n As Integer
Dim sample As String
```

```
'Determine the position of the last sample
Workbooks("Control").Activate
Sheets("SampleTable").Select
Range("A1048576").End(xlUp).Select
n = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    'Blank
    Workbooks("Mineralization_Analysis").Activate
    Sheets("Blank").Select

    Range("F1048576").End(xlUp).Offset(1, 0).Select
    FirstRow = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    Range("D1048576").End(xlUp).Select
    LastRow = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    For j = FirstRow To LastRow

        AverageCO2 = Application.WorksheetFunction.Average(Range("B" & j).Value,
Range("C" & j).Value, Range("D" & j).Value)
        Cells(j, 6) = AverageCO2

        StandardDeviation = Application.WorksheetFunction.StDev(Range("B" & j).Value,
Range("C" & j).Value, Range("D" & j).Value)
        Cells(j, 9) = StandardDeviation

    Next j
```

```
'i counts sheets based on sample
For i = 4 To n

    'Sample information
    sample = Workbooks("Control").Sheets("SampleTable").Range("A" & i).Value

    Weight = Workbooks("Control").Sheets("SampleTable").Range("I" & i).Value

    Carbon = Workbooks("Control").Sheets("SampleTable").Range("B" & i).Value

    Workbooks("Mineralization_Analysis").Activate
    Sheets(CStr(sample)).Select

    Range("F1048576").End(xlUp).Offset(1, 0).Select
    FirstRow = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    Range("D1048576").End(xlUp).Select
    LastRow = Mid(ActiveCell.Address, 4, Len(ActiveCell.Address))

    For j = FirstRow To LastRow

        Sheets("Blank").Select

        CO2Blank = Cells(j, 6)
        DevBlank = Cells(j, 9)

        Sheets(CStr(sample)).Select
```

```
        CO2Sample = Application.WorksheetFunction.Average(Range("B" & j).Value,
Range("C" & j).Value, Range("D" & j).Value)

        Cells(j, 6) = CO2Sample


        Mineralization = ((CO2Sample - CO2Blank) / (Weight * Carbon * (44 / 12))) * 100

        Cells(j, 8) = Mineralization


        DevSample = Application.WorksheetFunction.StDev(Range("B" & j).Value,
Range("C" & j).Value, Range("D" & j).Value)

        Cells(j, 9) = DevSample


        End If


      Next j


   Next i


Workbooks("Control").Activate

Sheets("General").Select

Range("A1").Select


End Sub
```

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