MODELLING OF THE EARTHWORM *EUDRILUS EUGENIAE* AS A PLUG FLOW REACTOR

KATHEEM KIYASUDEEN SEENI MOHAIDEEN

UNIVERSITI SAINS MALAYSIA
2018

MODELLING OF THE EARTHWORM *EUDRILUS EUGENIAE* AS A PLUG FLOW REACTOR

by

KATHEEM KIYASUDEEN SEENI MOHAIDEEN

Thesis submitted in fulfilment of the requirements
for the degree of
Doctorate of Philosophy

August 2018

Dedicated to Azhar

ACKNOWLEDGEMENT

الله

محمد

خواجا محبي علي شاه

"This comparative study of earthworm gut to an established chemical reactor design is based on the motivation that although earthworms play a vital role in organic waste management, their potential have not been properly adopted or utilized. Particularly in comparison with current trends of advanced chemical fertilizers, worm-based fertilizer products are challenged in their efficiency as well as commercialization. My inquisitive nature and readings paved the way for an inspiration, and motivated me to come to an understanding that exploring more on earthworm digestion would be feasible through a chemical reactor approach. Hence, I started a multi-disciplinary research combining mathematics, biology and engineering perspectives on looking at earthworms as plug flow reactor".

I would like to express my sincere gratitude to my main supervisor Prof. Dr. Mahamad Hakimi Bin Ibrahim for his guidance, support, and I can't fit his contributions in simple words. Besides my main supervisor, I would like to thank my co-supervisors, Dr. Sultan Ahmed Ismail, Dr. Syahidah Muhammad Akmal, and Dr. Fadzil Noor Gonowan for their insightful comments, encouragement, and guidance to widen my research. Without their support, it would not be possible to conduct this research.

This acknowledgement would be incomplete without thanking my family in India and Malaysia. I would like to dedicate this work to them all for believing in me patiently throughout this journey of Ph.D. My simple thanks here would not be

sufficient for all their roles and contributions, yet, it gives me an immense pleasure to record some of their names here. I would like to express my gratitude starting from my flesh and blood, my parents, Seeni Mohaideen/Rabiyathul Basariya and my brothers, Ahamed Sirajudeen, Thahir Thajudeen, and Shameer Halideen for their constant support and faith. Secondly, my 'Qurrota aini' wife, Sofiyah binti Mohd Idrees, and daughter, Zoha az-zahra. These two are the main reason for pushing myself day after day towards betterment. A special thanks and respect to my mother-in-law, Mrs. Hussaina for her timely help, understanding, and patience.

At this juncture, I would to thank my friend, Sarjoon Ameen, for his faith, trust, and positivism. At the same time, I would also like to offer my profound gratitude to my fellow research scholars, Dr. Asha Embrandiri, Dr. Hafeez Nidaullah, and Ms. Shlrene Quaik. Their constant support and encouragement helped me in various ways towards completing this study.

Finally, I acknowledge the research funding received under the RUI grant (Grant No: 1001/PTEKIND/811254) and the academic support from USM via USM-Fellowship award (2015-2018). Furthermore, I acknowledge the financial support by Consumer Association of Penang (CAP) and I would like to extend my gratitude to Mr. Muhyiddin (Vice-President of CAP) and Mr. Subbarow for their kindness and timely help.

August 2018

Katheem Kiyasudeen

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

n	Number of tank reactors-in-series on a tubular reactor
C	Concentration of the reacting substance
t	Time
k	First-order rate constant
C_0	Concentration at time, t = 0
C_t	Concentration at time t
L	Total length of the reactor
T	Total gut transit time
l	Length of the individual sections
$F_{A\|_V}$	Flow rate of the substrate A
$F_{P\|_V}$	Flow rate of breakdown products P
$F_{A\|_{V+\Delta V}}$	Flow rate of substrate A
$F_{P\|_{V+\Delta V}}$	Flow rate of breakdown products P
F_A	Flow rate of the substrate A
F_P	Flow rate of the products P
C_A	Concentration of substrate A
C_P	Concentration of breakdown products P
N_A	Concentration of substrate A
N_P	Concentration of breakdown products P
V	Volume
ΔV	Change in volume

r_A	Rate of breakdown
A	Area
x	Length
V_0	Volumetric flow rate
α	Stoichiometric conversion factor
V_{max}	Maximal digestive rate (maximum velocity)
K_m	Michaelis-Menten constant
K_{ab}	First-order absorption constant
C_{A-1}	Initial concentration of the substrate
C_{P-1}	Initial concentration of the breakdown products
ΔC_A	Change in substrate concentration
ΔC_P	Change in breakdown products concentration
ΔL	Change in length of the gut
$C_{A\|_{n-1}}$	Initial concentration of the substrate
$C_{P\|_{n-1}}$	Initial concentration of the breakdown products
$C_{A\|_n}$	Substrate concentration
$C_{P\|_n}$	Breakdown products concentration
r	Ratio of rate constants
υ	Reaction rate
t	Time taken by the particles for transport across the gut sections
E	Maximum enzyme activity
ES	Enzyme-substrate complex

P	Products
K_p	Catalytic rate for the formation of the products
V_{max} initial	Maximum rate achieved at saturated substrate concentration at the pre-intestine region
β	Deactivation rate constant
L	Length of the gut segments

LIST OF ABBREVIATIONS

PFR	Plug flow reactor
BR	Batch reactor
CSTR	Continuous-flow stirred tank reactor
PFR-CSTR	Plug flow reactor and continuous-flow stirred tank reactor in series
CSTR-PFR	Continuous-flow stirred tank reactor and plug flow reactor in series
nCSTR	n number of CSTR reactor in series
G	Gates
TC	Total carbon
C	Carbon
TN	Total nitrogen
N	Nitrogen
^{13}C	Stable isotope of carbon
^{15}N	Stable isotope of nitrogen
C:N	Carbon/Nitrogen ratio
P	Phosphorous
E. eugeniae	*Eudrilus eugeniae*
dV	Differential volume element
M. sexta	*Manduca sexta*
SIM	Small intestine model
F	Foregut
M	Midgut
AM	Anterior midgut

PM	Posterior midgut
Midgut A	Anterior midgut region
Midgut B	Posterior midgut region
H	Hindgut
AH	Anterior hindgut
PH	Posterior hindgut
L	Length
D	Diameter
O_2	Oxygen
CO_2	Carbon di oxide
N_2O	Nitrous oxide
DOC	Dissolved organic carbon
C3 plants	Plants that use C3 cycle (Calvin cycle) for photosynthesis
C4 plants	Plants that use C3 cycle and C4 cycle for photosynthesis
SS	Sum of squares of residuals
SSE	Sum of squared errors
R^2	Determination of coefficient
*Ef*P-II	Protease enzyme isolated from *Eisenia fetida*
*Ef*P-III-1	Protease enzyme isolated from *Eisenia fetida*
*Lr*PI-0	Protease isolated from *Lumbricus rubellus*
*Lr*PI-I-1	Protease isolated from *Lumbricus rubellus*
*Lr*PI-I-2	Protease isolated from *Lumbricus rubellus*
*Lr*P-II	Protease isolated from *Lumbricus rubellus*

*Lr*P-III-1	Protease isolated from *Lumbricus rubellus*
*Lr*P-III-2	Protease isolated from *Lumbricus rubellus*
BAEE	N-α-benzoyl-l-arginine ethyl ester
SBTI	Soybean trypsin inhibitor
TLCK	N-α-*p*-tosyl-l-lysine chloromethyl ketone
TPCK	N-α-*p*-tosyl-l-phenylalanine chloromethyl ketone
Rusitec	A simulation technique developed to investigate the effect of enzymes on ruminants.
GTT	Gut transit time
R_{sample}	Isotopic ratio of the sample
$R_{standard}$	Isotopic ratio of the international reference
USGS-40	A reference material developed by United States Geological Survey for the calibration of stable carbon and nitrogen.
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
ARG	Arginine
ASN	Asparagine
HIS	Histidine
ILE	Isoleucine
LEU	Leucine
LYS	Lysine
MET	Methionine
PHE	Phenylalanine
THR	Threonine
TRP	Tryptophan

VAL	Valine
ALA	Alanine
ASP	Aspartic acid
GLU	Glutamic acid
GLY	Glycine
SER	Serine
TYR	Tyrosine
ANOVA	Analysis of variance
AOAC	Association of official analytical chemists

PEMODELAN CACING TANAH *EUDRILUS EUGENIAE* SEBAGAI REAKTOR ALIRAN PALAM

ABSTRAK

Cacing tanah dirujuk sebagai jurutera ekologi dan ususnya sering dibandingkan dengan reaktor kimia, walau bagaimanapun, model percubaan untuk membuktikannya adalah kurang. Tujuan kajian ini adalah untuk mengaplikasikan model reaktor yang lazim ke atas usus cacing tanah *Eudrilus eugeniae* bagi memahami dengan lebih baik kinetik pencernaannya. Pertama sekali, model matematik berdasarkan kinetik turutan pertama telah digunakan untuk menentukan pola kadar indikator pencernaan, iaitu jumlah karbon (%), jumlah nitrogen (%), nisbah C/N, ^{13}C (‰), dan ^{15}N (‰) pada 5 bahagian (*pra-usus, foregut, midgut A, midgut B,* dan *hindgut*) di sepanjang usus *E. eugeniae*. Kemudian, model enzim pencernaan dan penyerapan telah digunakan untuk menguji dua kebarangkalian hipotesis, (i) had pencernaan, dan (ii) had penyerapan untuk mengenalpasti mod operasi usus di dalam *E. eugeniae*. Bagi mencapai hipotesis berikut, profil kepekatan protein mentah (%) dan 17 asid amino (%) telah ditentukan dalam eksperimen sebagai substrat dan produk pecahan dalam 5 bahagian di sepanjang usus *E. eugeniae*. Data tersebut kemudiannya digunakan untuk menentukan kadar penghadaman dan penyerapan dengan menggunakan kaedah lengkung-padan. Akhir sekali, satu model berdasarkan kinetik penyahaktifan telah digunakan untuk memahami bagaimana tiga kadar pengaktifan (β = 0.05, 0.1, dan 0.15) mempengaruhi kadar pencernaan pada 5 bahagian di sepanjang usus. Hasil eksperimen berdasarkan model tertib pertama menunjukkan bahawa semua indikator menunjukkan corak linear pencernaan di sepanjang usus, sementara model enzim

mencerminkan bahawa kadar pencernaan lebih tinggi daripada penyerapan. Model penyahaktifan menunjukkan peningkatan kadar pengaktifan menyebabkan pengurangan kadar pencernaan. Model tertib pertama menunjukkan bahawa indikator-indikator yang digunakan menunjukkan pola pencernaan linear, yang menyokong model reaktor aliran palam. Model enzim menunjukkan bahawa terdapat kadar penghadaman yang tinggi bagi *E. eugeniae*, tetapi kadar penyerapan sangat rendah dibandingkan dengan penghadaman, oleh itu, menyokong had penyerapan. Kesan kadar penyahaktifan (β) mendedahkan bahawa aktiviti proteolitik semakin mengurang pada seluruh usus yang juga menyokong kelakuan PFR. Oleh itu, kajian ini menyimpulkan bahawa usus *E. eugeniae* mematuhi perilaku PFR.

MODELLING OF THE EARTHWORM *EUDRILUS EUGENIAE* AS A PLUG FLOW REACTOR

ABSTRACT

Earthworms are referred as ecological engineers and their guts are often compared to chemical reactors, however, modeling experiments to substantiate it are lacking. The aim of this study was to apply established reactor models on the gut of the composting earthworm *Eudrilus eugeniae* to better understand its digestive kinetics. Firstly, a mathematical model based on first-order kinetics was applied to determine the pattern of digestion rates of indicators, namely total carbon (%), total nitrogen (%), C/N ratio, ^{13}C (‰), and ^{15}N (‰) at 5 intersections (*pre-intestine, foregut, midgut A, midgut B, and hindgut*) along the gut of *E. eugeniae*. Secondly, an enzyme model of digestion and absorption was used to test two probable hypotheses, (i) digestion limitation, and (ii) absorption limitation to identify the mode of gut operation in *E. eugeniae*. To achieve that, the concentration profiles of crude protein (%) and 17 amino acids (%) were experimentally determined as substrate and breakdown products at the 5 intersections along the guts of *E. eugeniae*. The data then were used to determine the rates of digestion and absorption using the curve-fit simulation analysis. Lastly, a model based on deactivation kinetics was used to understand how three deactivation rates (β=0.05, 0.1, and 0.15) affect the rates of digestion at those 5 intersections along the gut. The experimental results based on the first-order model revealed that all the indicators exhibited a linear pattern of digestion along the gut, while, the enzyme model reflected that the rate of digestion to be higher than that of absorption. The deactivation model revealed that an increase in deactivation rates result in the reduction of digestion rates. The first-order model

showed that the indicators exhibited a linear pattern of digestion, which supports the plug flow reactor model. The enzyme model showed that there exists a high rate of digestion in *E. eugeniae*, but the rate of absorption is extremely low compared to digestion, thus, supporting absorption limitation. The effect of deactivation rates (β) revealed that the proteolytic activity reduces across the gut which also supports PFR behaviour. Thus, the present study concludes that the guts of *E. eugeniae* adhere to PFR behaviour.

CHAPTER 1

INTRODUCTION

The first chapter of the thesis deals with a brief background on the previous research conducted on animals as chemical reactors and also discusses the perspective of earthworm as a plug flow reactor. The subsections discuss the problem statement, research objectives, research questions, hypotheses, scope and the limitations involved in the current study.

1.1 Research Background

Bio-chemical reactor theory can be successfully applied to an animal's digestive tract to explore more on the digestive mechanism since digestion in the gut is considered homologous with a reactor operation. Many models on the basis of *in vivo*, *in vitro,* and *in silico* conditions have been developed for various animals. Some of the important animals include polychaete annelids, foregut and hindgut fermenters (Penry and Jumars, 1987), vertebrate herbivores (Alexander, 1991), herbivore fishes (Horn and Messer, 1992; German, 2009), caterpillars (Woods and Kingsolver, 1999), grasshoppers (Wolesensky et al., 2005), ruminant animals (Krishnamoorthy et al., 1983; Van Bentum and Nelson, 2011), and small intestine of humans (Fonseca, 2012). Generally three types of ideal reactors are taken into account when modeling of an animal's digestive performance is required and such reactors are batch, plug-flow and continuously-stirred tank reactors. The advantage of using these models is that these industrial reactor configurations have already been modeled and tested successfully (Fonseca, 2012). Thus, comparing animal's digestive structure with these reactor frameworks can benefit in terms of

understanding digestive strategies because the reactor designs are best suited for maximizing production rates of the desired animal.

Earthworms are referred as ecological engineers and their guts are often compared to chemical reactors, however, modeling studies based on the reactor approach are lacking. Addressing earthworm's gut as a reactor can yield more understanding on its digestion and its associated processes and the opportunity to generate new research, more incentives, designer products and so on (Penry and Jumars, 1987). The waste materials excreted by earthworms are known as 'vermicasts' while the bio-product produced by the earthworm as a result of composting of organic wastes are commercially referred 'vermicompost' (Ansari and Ismail, 2012), which are rich in enhanced nutrients for plants like vitamins, enzymes, antibiotics, hormones, and immobilized microorganisms (Thampan, 1993). Moreover, the unit operation aspect pertaining to the vermicomposting technology is least cited or addressed in literature. This work aims to benefit various fields like animal physiology, feeding ecology, enzymology, agriculture, and can be a useful addition to animal response model studies due to its multi-disciplinary facet.

1.2 Statement of the problem

Several researchers model animal guts as reactors, although not many discuss earthworms specifically (Penry and Jumars, 1987; Woods and Kingsolver, 1999; Horn and Messer, 1992; Fonseca, 2012). Many researchers have referred earthworms as "ecological engineers" and often compared their gut to a reactor (Thampan, 1993; Pathma and Sakthivel, 2012), but, there is as yet research that correlates the earthworm gut to concepts of a plug flow reactor. Additionally, most studies dealing with the role of the earthworms in waste management have focused on the changes

before and after vermicomposting process rather than those occurring throughout the process (Lazcano et al., 2008; Vivas et al., 2009). Moreover, research on various feed sources include biosolids (Ndegwa et al., 2000), fruit and vegetable waste (Pattnaik and Reddy, 2010), newspaper and cafeteria waste (Jais and Hasnuri, 2008), as well as dung (Garg et al., 2006) have not cited on worm digestion kinetics. A poor understanding of digestive kinetics may lead to poor cast management; earthworms have been shown to drive greenhouse gases via vermicasts, particularly nitrous oxide (N_2O) (Majeed et al., 2013).

Moreover, the modeling studies on vertebrate guts (mammals to be particular) are predominantly found in literature while the invertebrate groups are often ignored or not shown interest (Karasov and Douglas, 2013). These arguments expresses an availability of knowledge gap in the field of comparative physiology, especially for invertebrates. Therefore, a thorough comprehension of the worm itself as a reactor and aspects pertaining to the kinetics need addressing to properly assess their effectiveness. A qualitative modeling strategy of considering earthworm guts as plug flow reactors (PFR) based on first-order, enzymatic and deactivation kinetics could possibly serve as a biologically meaningful study on earthworms. At the same time, a quantitative nutrient profiling along the gut section using related experimental approach and its application to the qualitative framework like mathematical modeling and computer-based simulation studies, i.e., an *in vitro-in silico* model approach would offer more understanding on the earthworm's digestion. More understanding on the digestion can be useful to generate new and unexplored studies on earthworms, and more opportunities to develop designer products using earthworms.

1.2.1 Research objectives

The research objectives of the present study are as follows:

i. To apply mathematical models based on first-order and enzyme kinetics to demonstrate the adherence of PFR kinetics on the gut of the vermicomposting earthworm *Eudrilus eugeniae*.

ii. To determine the concentration profile of total carbon, total nitrogen, C/N ratio, ^{13}C, and ^{15}N in understanding the rates and pattern of digestion along the gut of the vermicomposting earthworm *Eudrilus eugeniae*.

iii. To determine the concentration profile of crude protein and 17 amino acids in the gut of the vermicomposting earthworm *Eudrilus eugeniae* to demonstrate the mode of operation expressed during digestive process.

iv. To apply a mathematical model based on deactivation kinetics and to demonstrate the effects of deactivation on proteolytic activity, crude protein and amino acids concentration along the gut of the vermicomposting earthworm, *Eudrilus eugeniae*.

1.2.2 Research questions and hypotheses

This study aims to address the following questions:

i. How applicable is the chemical reactor kinetics to the gut of earthworm *Eudrilus eugeniae*?

ii. What are the fates of different nutrients that enters the digestive tract of the earthworm *Eudrilus eugeniae*?

iii. How does chemical reactor approach help in understanding digestion and its associated processes in detail in earthworms?

iv. To what extent, different deactivation rates affect enzyme activity, digestion of proteins and the absorption of amino acids during gut transit?

This study includes the following hypotheses:

i. Earthworm gut adheres to chemical reactor kinetics during digestion.

ii. Different nutrients undergo different rates of digestion but exhibit a linear pattern of digestion during gut transit.

iii. Protein digestion and amino acid absorption are mediated by enzymes and follow simple Michaelis-Menten kinetics.

iv. Proteolytic activity is affected by the presence of inhibitor compounds in the food.

1.2.3 Research scope, limitations and contributions

The scope of the present study are listed as follows:

i. The study would highlight the possibility of comparing earthworm gut to a plug flow reactor design to understand more on its digestion.

ii. The study would provide mathematical models (i.e. first-order, enzyme and deactivation), which will be simple to apply and a useful template for any animal showing structural and functional similarities with the earthworm *E. eugeniae*.

iii. The study would benefit modellers, researchers, students and can be a basis for post-doctoral research work for those who intend to work on animal response studies, chemical reactor approach, pharmacokinetics, and better agricultural practices, especially vermicomposting and organic waste management.

Although the study was intended to minimize all limitations, there were some constraints as follows:

i. Limited data along the gut of *E. eugeniae* – Only 5 sections (pre-intestine, foregut, midgut A, midgut B, and hindgut) along the gut of *E. eugeniae* was taken into consideration. However, this compartmentalization was based on the fact that enzymes in earthworms are regionally specified. Thus, this consideration may not affect the main objective.

ii. The 4 assumptions made on the gut of *E. eugeniae* for the PFR modeling purpose includes, (1) No axial mixing but perfect radial mixing occurs in the earthworm gut, (2) Substrate digestion and the absorption of breakdown products are mediated by enzymes that are distributed homogenously along the gut length, (3) The digestion and absorption occurs in a single step and follows Michaelis-Menten kinetics, and (4) Food content passes through the gut at a constant rate. These assumptions may or may not be closely met in earthworm gut, however, the adoption of these assumptions is to simplify the model. Moreover, it is recommended that considering qualitative assumptions may not affect the qualitative observations and findings to be obtained from the present type of study (Woods and Kingsolver, 1999).

iii. Using crude protein concentration as substrate in enzyme kinetics – The usage of a single protein concentration was not adequate to establish a substrate curve along the gut of invertebrates. For example, Woods and Kingsolver (1999) used a protein, Azocaesin to demonstrate the fate of substrate concentration along the midgut of the caterpillar, *Manduca sexta*. Contrarily, the azocaesin concentration was observed to be completely digested in the anterior-most section (before entering midgut). As a result, no substrate concentration was detected along the midgut. Hence, the usage of crude protein concentration as substrate could rectify this problem and can establish a more justifiable substrate concentration along the gut of *E. eugeniae*.

1.2.4 Research contributions

The present study offers simple and comprehensible mathematical models and their testing methods to the area of animal response studies. The main advantage of these kinetic models is that their simplicity and easy applicability as a 'template' to any animal that possesses simple tubular gut structure resembling earthworms or higher animals. Moreover, the demonstration of the mode of operation on the gut of *E. eugeniae* is a valuable extension in earthworm studies. More understanding of earthworm digestion helps in better agricultural practices, for instance, by manipulating the food ingredients, the vermicast potential could be enhanced. For example, the slow-release capacity of nutrients as seen in the vermicasts can be enhanced to develop controlled-release products that in turn can enhance the viability of earthworm-based agricultural products.

CHAPTER 2

LITERATURE REVIEW

This chapter presents a detailed literature overview in seven sections. Section 2.1 discusses the general overview on chemical reactors and their types, reactor models and their types, and the advantages and limitations involved in considering animal gut as reactors. Section 2.2 presents a critical review on selected animal models related to this study. Section 2.3 explains the need for considering earthworm as a chemical reactor. A general account on earthworms, their biology, and the processes involved in digestion are discussed in the section 2.4. Section 2.5 explores the possibility of adapting earthworm as plug flow reactor (PFR) by discussing the similarities and differences among earthworms and an ideal PFR. Kinetic approaches (first-order, enzyme, deactivation kinetics) and the simulation techniques involved are discussed in section 2.6. Section 2.7 deals with the vermicomposting earthworm *Eudrilus eugeniae* as the suitable species for the present study.

2.1 Modeling animal gut as reactor

In this part, the possibility of applying suitable model representing earthworm digestion and its associated processes using kinetic models are briefly described. Despite the availability of numerous models and simulation tools, the review is limited to model studies that are simple, easy to understand, requiring simple assumptions and mathematical equations, and most importantly that which better suits the objective of this study.

Digestive reactions in an animal's gut can be better explained using chemical reactor theory, most commonly with a plug flow reactors (Jumars, 2000). Penry and

Jumars (1987) emphasized that by applying chemical reactor approach, an animal's digestion can be analyzed deriving necessary variables initially, then develop a model that shall be biologically valid and mathematically powerful in addressing the possible relationship among the obtained variables, thus, characterizing the digestive strategy (Van Bentum and Nelson, 2011). This characterization paves way for a capable framework or concept by which different strategies can be tested, analyzed and compared. A potential animal model should focus on both kinetic and thermodynamic viewpoint (Campbell et al. 2005) and use the mathematical equations to simulate the digestive processes (Dumas et al., 2008).

A meaningful modeling of digestion is achievable with better knowledge of biochemical aspect of the incoming food, the process of food selection and preference, and physiology of digestion (German, 2009). With this information, the predicted model can provide variety of answers on the factors affecting digestion, factors determining food quality and so on, thus, defining animal feeding, digestion, and its ecological role. In an animal gut, if temperature is maintained at a constant condition, it is recommended to consider only the conservation of mass (mass-balance) while developing a model (Penry and Jumars, 1986). Hence, the use of mass-balance laws and chemical kinetics should be reliable in developing and testing models to understand digestive reactions in earthworms. Karasov and Douglas (2013) also suggest considering some important factors while modeling, such as (1) Reaction rate of substrate breakdown, (2) Mean retention time (MRT-measured using inert tracers), (3) Volume of the gut, and (4) Flow rate of the digesta.

2.1.1 Types of chemical reactor

This section discusses the types of feasible chemical reactors to which an animal gut can be compared. Most of the discussions provided here are adopted from the works of Levenspiel (1972) and Penry and Jumars (1986).

Generally, three types of ideal chemical reactors are conventionally modeled: (1) batch reactors (BRs), (2) plug flow reactors (PFRs), and (3) continuous flow, stirred-tank reactor (CSTRs). These three reactors serve as the basis for all the chemical reactor designs. There are fundamental differences that exist among these three classes of reactors regarding the fate of reactants inside the reactor and these reactors can serve separately or in combination depending on the digestive structure of the desired animal that needs to be modeled.

Batch reactor models better suit for animals with a behaviour of eating discrete meals. In a batch reactor, materials are loaded first and then thoroughly mixed. The reactants are allowed to undergo chemical reaction and at the end, all the products and non-reacted materials are completely removed. All the properties inside the batch reactor are assumed to be uniform and changes in the reactant concentration takes place only against time. The main characteristic feature of a batch reactor is neither input nor output exist, thus, the mass balance is expressed as "the concentration of any reactant that disappears in the system or reactor is a function of the reaction rate, volume of reactants in the reactor and the holding time of the reactor". Some of the examples include hydras, jellyfish, sea anemones, and starfish. Deposit feeders, other detrivores, herbivores, and folivores cannot be modelled as batch reactors because of their more or less continuous feeding

behaviour (Penry and Jumars, 1986), which contradicts with the characteristics of a batch reactor.

In a plug-flow reactor, materials enter and experience a continuous-flow in a tubular medium with an orderly pattern and exit in the same sequence as during entry. Perfect radial mixing exists but no axial mixing or diffusion occur along the reactor or considered negligible. Residence time is usually identical for all the materials entering and leaving the tubular vessel. Under steady-state conditions, changes occur with respect to axial position and the mass balance is expressed over differential volume element (dV). Animals having the behaviour of consuming more or less continuously can be modeled as a plug-flow reactor. Some important examples of animals with simple tubular gut morphology that express plug flow reactor behaviour include geese, corophid amphipods, and deposit-feeding polychaetes (Annelida) (Penry and Jumars, 1986).

In a CSTR, the entering materials experience a constant flow and complete mixing inside the reaction vessel. Under steady-state condition, the composition of the entering material is uniform throughout the reactor and does not change over time. The concentration of reactants disappearing in the reactor is a function of rate of reaction and volume. Penry and Jumars (1986) argued that no animal possesses a gut structure that can be entirely modeled as a CSTR, however, a portion or a single structure may operate as a CSTR, i.e. a well-organized stomach or hindgut caecum have been modeled as a CSTR. A gut of an animal that hosts a structure resembling CSTR-like behaviour can be modeled as a series of reactors, for example, a ruminant gut can be expressed as a CSTR followed by a PFR.

2.1.2 Advantages and limitations of animal digestion models

Some of the advantages and benefits of applying chemical reactor models for an animal gut are as follows:

i. Generates new studies in an animal (Penry and Jumars, 1986).

ii. Maximizes production rates or efficiency of an organism (Jumars and Penry, 1989).

iii. Extracts more information and facilitates future research (Dumas et al., 2008).

iv. Facilitates research that links digestive physiology and animal nutrition in developing designer products in vertebrates and invertebrates via production, agriculture and aquaculture (Karasov and Douglas, 2013).

v. Contributes to the understanding of the impacts of temperature change on animals in predicting the effect of climate change and animal responses (Allison, 2012; Karasov and Douglas, 2013).

vi. The development of new tools and methods will enhance the understanding of the differences in mechanistic basis of digestive function and absorption that exist between various species (Karasov and Douglas, 2013).

Some of the limitations are briefly discussed as follows:

i. The mathematical modeling of animal gut requires many assumptions that may or may not suit or meet in the desired animal. For example, while modeling caterpillar midgut (*Manduca sexta*) as a plug flow reactor, Woods and Kingsolver (1999) made few assumptions which were not suitable or

applicable in caterpillar's midgut. Contrarily, they stated that 'gut contents in caterpillar midgut flow with no axial mixing but with perfect radial mixing - a typical PFR characteristic', but at the same time agreed that this assumption was unrealistic. They further emphasized that the assumption was to simplify the mathematics. Although unrealistic, Woods and Kingsolver's (1999) assumption favors modeling and becomes extremely useful if the rate of axial and radial mixing in animal guts are unknown or difficult to determine due to relatively smaller or thinner gut structures as seen in earthworms.

ii. Despite the importance in bridging the gap between the biological communities and climate change, mathematical tools are scarce to represent the biological diversity to the global level (Allison, 2012). This indicates the possibility of building more model based tools in linking micro-level to the global scale.

iii. Modeling studies on vertebrate guts (mammals to be particular) are predominantly found in literature while the invertebrate groups are often ignored or not shown interest. It was Karasov and Douglas (2013), who brought this gap out to the literature via their review article, entitled '*Comparative digestive physiology*' which was published in 2013. They commented on the preference given to the vertebrates by pointing out their biomedical importance but emphasized the need of modeling studies in invertebrates by stating that "the field of comparative digestive physiology is constrained by our ignorance of most of the invertebrate groups". This expresses the tremendous opportunity in the field of comparative physiology in terms of creating novel models and products from invertebrate science.

2.1.4 Gut models and their types

The pioneering works on animal modeling using the chemical reactor theory can be found in the works of Penry and Jumars (1987), Jumars (2000), Dumas et al (2008), and Van Bentum and Nelson (2011) from which the evolution, history, and trends of modeling are taken. Other reference works are mentioned appropriately after relevant usage of data or statement. This section has a flow of reviewing the general introduction on animal models and their types followed by relevant literature on suitable animal models.

Application of chemical reactor aspect to an animal gut involves the simulation of nutrient intake and the fate of breakdown products while flowing through the digestive tract (Dijkstra et al. 2007). Most of the available research articles on animal models have focused on the flow of materials in a digestive tract and its effect on animal nutrition and physiology. Classic models usually consider the physical properties (morphology) of the gut contents and the physico-chemical characteristics of the digestive tract and develop multi-compartmental models with the aim to simulate the mechanistic view of a substrate and its breakdown at various stages within compartments into products and the absorption process along the intestine. Notably, compartmental models have been successfully used in animal nutrition studies to understand digestion (animals like sheep, pigs, dairy cows, etc.), to determine the rate of amino acids intake and assimilation, to explain the fate of food materials in the rumen, and to predict voluntary food intake. The usage of a non-reactive tracer is usually employed to estimate the mean retention time of food materials as the rate of passage determines the intake of nutrients and consuming strategy of an animal. However, the application of compartment model to whole

animal or special tissue is least addressed or rarely studied (Dumas et al., 2008). Moreover, compartmentalizing animals with smaller or simple gut structures may pose difficulty in modeling or may result in inaccurate predictions. On the contrary, non-compartmental approach also has been in use i.e. generally applied in pharmacokinetic studies (Moxon et al., 2016), which involves fitting of mathematical model to *in vivo* data that provides accurate results for the fitted parameters for the analysed system or animal. This approach seems to be appropriate in addressing animals with simple or relatively thin tubular guts or animals having not so well-organized digestive structures.

Generally, the digestive processes that occur in an animal gut are reproduced using either *in vitro* or *in silico* models. Models based on *in vitro* are highly applicable in biomedical field and particularly preferred to demonstrate the effect of food or drug in the digestive tract using either static (time is invariant or steady-state) or dynamic approach (time dependent changes occur in the system). Static *in vitro* models are also referred as biochemical models which help in the study of drug release, absorption, and bioavailability of active molecules, for example, Membrane-based model referred as Parallel artificial membrane permeability assay is basically used for studying passive diffusion in guts. Dynamic models simulate both physical and physiological aspects of digestion in animals. For instance, Krishnamoorthy et al (1983) developed a dynamic *in vitro* model based on rate equations to simulate rumen proteolysis in order to estimate the amount of dietary N undegraded in the rumen of cows. Similarly, Fonseca (2012) applied a dynamic *in vitro* small intestine model (SIM) to explain digestion via starch hydrolysis and glucose absorption occurring in the small intestine of humans. The *in silico* models are computer based

approaches introduced into animal nutrition studies during 1970s. The application of *in silico* models have been reported to determine mean residence time distribution, release, and inactivation of probiotics, drugs and food (Stoll et al., 2000). Additionally, computational models have been developed to predict and assess the absorption process in the gastro-intestinal tract and small intestine of humans (Fonseca, 2012; Moxon et al., 2016).

The efficiency of models can be accurate if simulation is based on *in vivo* feeding methods using animals than *in vitro* or *in silico* models (Hur et al., 2011). The *in vivo* studies in higher vertebrates like humans are considered to be expensive and a time consuming effort, while the usage of animals *in vivo* is seen as an alternative (Wickham et al., 2006). As discussed earlier, the modeling approach in vertebrates are encouraged highly due to their biomedical importance, for example, a considerable amount of literature is available on modeling pertaining to drug delivery system in humans. These models are usually based on *in vitro* in combination with *in silico* studies (Fonseca, 2012) because the *in vivo* modeling requires usage of highly expensive non-invasive imaging techniques like Scintigraphy, Ultrasonography, Computer Tomography, Magnetic Resonance Imaging, and Echoplanar Magnetic Resonance Imaging (Kong and Singh, 2008). For this reason, even modellers who work on human studies avoid *in vivo* method and prefer *in vitro* in combination with *in silico*. On the contrary, the *in vivo* method seems to be quite workable in invertebrates without the requirement of the aforementioned highly expensive monitoring systems. Moreover, invertebrates with wide applications like earthworms can be easily cultured and monitored, thus, a combination of *in vivo* data with *in*

silico approach can be successfully tested. Figure 2.1 shows the general classification of animal response models.

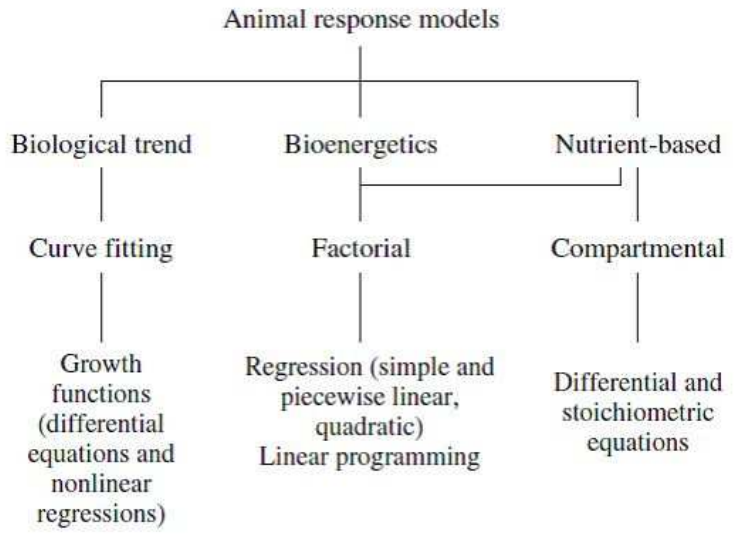

Figure 2.1: A classification of animal response models (adopted from Dumas et al., 2008).

Dumas et al. (2008) classified the animal response models suggested the following key points while considering animal modeling:

i. The modellers should include multi-disciplinary aspect while modeling because many developed models have not been utilized to their full potential especially in physiology-related studies.

ii. Animal models should be designed with concerns on improving product quality, traceability of food composition and animals, and environmental sustainability.

iii. Powerful simulation models can improve agriculture by predicting growth, nutritional requirements, body compositions, and production costs.

Hence, modeling an animal digestion needs careful adoption of assumptions and appropriate kinetic model with a feasible aim to enhance feeding preference, to improve product value, to extract newer studies and to resolve unanswered questions.

2.2 Critical review on selected research works

It would be appropriate to begin the review with the pioneer research articles published by Penry DL and Jumars PA (Penry and Jumars, 1986; 1987; and 1990) on the comparison of the digestive process with a chemical reactor since their framework and predictions have kindled many successive researches and development in field of animal response studies.

It was through the article published in 1986 (Penry and Jumars, 1986), they made the popular statement that "Modeling the digestive process is a problem in chemical engineering" and proposed to solve it by initially describing animal guts as a single or in series and used reactor design to identify the variables using reactor-specific mass balance equations that reflects digestion. They identified that the gut of ruminants (stomach followed by the intestine) express a configuration of CSTR-PFR in series based on the data generated using a tracer to evaluate retention time distribution. The pattern of the tracer concentration was observed to be an exponential output curve for a single ideal CSTR whereas a single ideal PFR had a step function. On the evaluation of the study presented by Penry and Jumars (1986), it is clear that their perspective on digestion is simple and applicable to any animal provided their gut morphology and tracer pattern is known.

The follow-up work was published in the year 1987. In this highly cited article, Penry and Jumars (1987) developed mathematical models of digestion and

proposed various conditions and pre-requisites for the process design of a reactor. They proposed that while modeling, at first, reactions of interest (catalytic or autocatalytic) need to be identified from which kinetic modeling (batch or PFR or CSTR) can be developed. With the reaction and its model specified, the ideal reactor configuration and operating strategy of digestion can be evaluated. Based on this outline, the authors tested marine deposit feeders i.e., polychaete annelids, mammalian foregut fermenters, i.e., kangaroos, cows, and sheep), and hindgut fermenters (e.g., horses, rabbits). They highlighted that an animal which possesses the capacity to catalyze digestive reactions with its own enzymatic system (catalytic) will have the reaction rate as a function of the concentration of the reactants and the throughput/gut transit time study revealed that the gut functions as an ideal PFR (deposit feeders, e.g., *Neries succinea*, *Corophium* spp., and *Pseudopolydora kempi japonica*) because it maintains a gradient in reactant concentration. On the other hand, an animal showing microbial fermentation are categorized as autocatalytic in which the reaction rate is a function of the concentration of not only reactants but also microbes. In this case, a gut should function as either CSTR-PFR (e.g. ruminant gut) or PFR-CSTR (e.g. hindgut fermenters) in series. In animals with simple and tubular guts, fermentation process also may occur, which is a property of a CSTR. At this condition, an animal cannot function as an ideal PFR except in a case where the gut transit time is low and it makes the fermentation negligible. This specific character may vary with animal type and can be identified by measuring nutrients produced as a result of fermentation process. The overall examination of this research reveals that an animal with a simple and tubular gut (similar to deposit feeders) showing a reduction pattern in reactant concentration can be modelled as PFR.

In 1990, Penry and Jumars published another important article on 42 species of marine deposit feeders as PFR in which they introduced compartmentalization in to the tubular deposit feeder guts i.e. foregut represented as F, anterior midgut as AM, midgut as M, posterior midgut as PM, anterior hindgut as AH, and posterior hindgut as PH with an aim to understand the interrelationships among the gut morphologies. To achieve the objective, they categorized the 42 species into four groups based on the compartments and their respective symbolism as: (i) carnivores with simple tubular guts were represented as H, (ii) deposit feeders with simple tubular guts were also referred as H, (iii) deposit feeders with 3 gut compartments were classified as F-M-H, and (iv) deposit feeders with 4 or 5 gut compartment were assigned either F-AM-PM-H or F-AM-PM-AH-PH. This particular study highlights the possibility of introducing regional specification or compartmentalization in the gut of animals for which a modeling is desired. For instance, earthworms are often addressed in terms of regional specificity, such as foregut, midgut and hindgut (Horn et al. 2003).

Horn and Messer (1992) acknowledged the proposals and the outline provided by Penry and Jumars (1986 & 1987) as "consistent theoretical framework" and successfully applied on four marine herbivorous fishes. They incorporated an additional feature to the model called gates (G) referring to special structures like gill rakers, and pharyngeal mills. They identified the appropriate gut configuration for all the four fishes using mass balance and Michaelis-Menten enzyme kinetics as follows: (i) *Scarus rubroviolaceus* with a simple tubular structure i.e. pharyngeal mill followed by an intestine identified as PFR, (ii) *Cebidichthya violaceus* with a stomach (acidic pH - 2.2 to 2.5) and intestine as CSTR-PFR, (iii) *Mugil cephalus*

with a stomach (acidic pH – lower than 3.5), gill rakers, and intestine as CSTR-G-PFR, and (iv) *Kyphosus sydneyanus* with a stomach (acidic pH – 2.8 to 3.0), intestine and hindgut caecum as CSTR-PFR-CSTR. From the work of Horn and Messer (1992), the case of *S. rubroviolaceus* as PFR seems to be applicable and suits other species of similar gut structure. The absence of CSTR in this fish was explained with two reasons: (i) above a threshold throughput rate, a gut can no longer function as a CSTR (Alexander, 1991). The gut throughput time in *S. rubroviolaceus* was evaluated to be very low (2.5 hours) than the other fishes with very high reaction rate. (ii) Gut pH was expressed as weakly acidic or slightly alkaline. Hence, an animal with relatively low gut transit time and near alkaline pH conditions inside the gut cannot be considered as a CSTR, but PFR. Both of this conditions suit earthworms since there are reports on low gut transit time, 3 hours in *Eisenia fetida* (Hartenstein and Hartenstein, 1981), 6 hours in *E. eugeniae* (Mba, 1989) and the existence of near neutral pH conditions inside their gut (Horn et al., 2003).

During 1999, a more biologically meaningful approach was put forward by Woods HA and Kingsolver JG (1999). They applied PFR model for the simple tubular midgut in caterpillar, *Manduca sexta*. The significance of their approach was in developing two model equations based on Michaelis-Menten kinetics for tracking protein degradation representing the substrate or reactant and amino acid production as breakdown products along the midgut. To carry out this more appropriate objective, they made four basic assumptions suiting PFR characteristics, (a) materials flow with no axial mixing but with perfect radial mixing, (b) digestion and absorption processes are mediated only by enzymes that are distributed homogenously throughout the gut, (c) breakdown and absorption occur in a single

step and follow Michaelis-Menten enzyme kinetics, and (d) the flow of contents inside the gut is constant. As these assumptions reflect an ideal PFR, the possible existence of these conditions in the midgut is questionable i.e. may or may not strictly be met in *M. sexta*. But at the same time, Woods and Kingsolver (1999) argued that these assumptions were considered for the purpose of mathematical simplicity and to understand the qualitative structure of the gut processes, thus, minor violations of the proposed assumptions may not affect the conclusions. Moreover, their study attempted to understand more on physiology by identifying the mode of gut operation using the profile of the substrate and breakdown products along the gut. To achieve that objective they proposed five hypotheses, (i) Matched process-rate of digestion and absorption are equal, (ii) Consumption-a limiting step, (iii) Digestion-a limiting step, (iv) Absorption- a limiting step, and (v) Post-absorptive process-a limiting step and elucidated the possible trends using simulation procedure. While the assumptions proposed by Woods and Kingsolver (1999) seem to be not appropriate or wrong, they were aiming to understand the gut processes in a qualitative aspect and it appears to provide a practicable template for various other animals with simple tubular gut structure like *M. sexta*. This particular perspective seems to suit earthworms. The selection of hypotheses depends on the number of consumption rates preferred since it can be assumed constant and as per the objective of the model. Although the work of Woods and Kingsolver (1999) provides a clear and easily workable template, their choice of kinetic parameter determination seems to be ineffective because they attempted to measure the maximum activity (V_{max}) and enzyme affinity (K_m) by using an artificial protein diet showing its rate of breakdown as a function of single protein (azocaesin) concentration. A high V_{max} and considerably lower K_m values resulted in complete digestion of proteins within the

first few millimeters of the midgut, thus, the prediction of the substrate profile was not properly illustrated or unable to establish a pattern successfully. This can be avoided by choosing an alternative way of determining kinetic parameters that can express a viable profile along the gut rather than a single protein, for example, a measure of crude protein could rectify this problem.

Modeling an animal gut as an ideal PFR require a condition that no significant axial mixing exists in the gut. Jumars (2000) attempted to relax this assumption by modeling a tubular gut as a series of CSTRs. The author proposed *n*CSTR model called as 'tank-in-series', which is an intermediate between a single ideal CSTR and PFR. In this case, *n* (number of tank-in-series) is calculated by dividing the length '*L*' of the tubular reactor with the diameter '*D*' of the gut lumen, i.e. ($n = L/D$). The study recommended that if the value of *n* is equal or greater than 10 ($n \geq 10$), then the gut of the desired animal should function as PFR. From the simulation experiment using an indigestible tracer, Jumars (2000) argued that an animal with relatively thinner and longer guts may effectively perform digestion and absorption by restricting axial mixing. In other words, the longer the gut, the lesser is the chance for significant axial mixing to occur. In vermicomposting earthworm *E. eugeniae*, the digestive tract is occupied mainly by the intestine (Blakemore, 2015), which may yield the condition $n \geq 10$ supporting PFR behaviour. Moreover, the application of the equation $n = L/D$ could serve as a confirmation of PFR behaviour in terms of dimension.

Logan et al. (2003) proposed a PFR model based on first-order and Michaelis-Menten kinetics to optimize efficiency of digestion and absorption in animals having simple digestive tract. Their study adopted two of the hypotheses:

digestion and absorption limitation from the work of Woods and Kingsolver (1999). The significance of their work is in the presentation of the ratio of digestion and absorption rate constants given as, $r=a/k$ (i.e. a-absorption rate constant and k-digestion rate constant) in determining which hypotheses an animal gut adheres to. They further explained that in the case $r<<1$, the process is absorption limited, while, $r>>1$ favors digestion limitation. If $r=1$, then both digestion and absorption rates are balanced. This aspect of testing hypotheses seems to be simple to apply and can provide accurate results with an advantage of applicability for almost all animals possessing a tubular gut structure.

In 2009, an interesting research article was published considering the guts of four herbivore minnows belonging to the genus, *Campostoma* as PFR devoid of mathematical models (German, 2009). The author showed that the PFR approach can be demonstrated in animals using *in vivo* data. This unique study adopted various predictions from already established works (Penry and Jumars, 1987; Horn and Messer, 1992; and Jumars, 2000) and presented in a guideline form to test the PFR behaviour. The statements and results of his study include: (i) an animal with a PFR gut should express steep gradients of nutrient concentration (i.e., a decrease or fall in protein or glucose or lipid concentration) and enzyme activity (for example, trypsin or amylase or lipase activity) along the gut with high concentration and activity in the anterior region followed by increased absorption occurring in the midgut to the posterior region, (ii) the concentrations of fermentation products should not possess a regional localization at any site of the digestive tract and the presence of short chain fatty acids should be in lower concentrations, (iii) the gut transit time should be short and rapid (In his work, *Campostoma* was highlighted to possess rapid gut transit