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Comparison of the Kinetic Parameters of Escherichia coli 0157:H7, Listeria monocytogenes and Salmonella typhimurium Derived from the Baranyi and Huang Models in a Chemically Defined Minimal Medium

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COMPARISON OF THE KINETIC PARAMETERS OF *ESCHERICHIA COLI* O157:H7, *LISTERIA MONOCYTOGENES* AND *SALMONELLA TYPHIMURIUM* DERIVED FROM THE BARANYI AND HUANG MODELS IN A CHEMICALLY DEFINED MINIMAL MEDIUM

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
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in

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by
Jose Isidro Fuentes
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Abstract

Microbial growth can be characterized by parameters such as lag time, growth rate, and maximum population density at any specific point of time. Mathematical models that predict microbial growth of foodborne pathogens are increasingly used in the food industry as a viable alternative to traditional methods of microbial enumeration. The Baranyi model has been widely used as the primary model of choice by many authors because of its performance and accuracy. The most recently developed Huang model has been less implemented and few comparisons between the Baranyi and Huang models have been made when modeling pathogenic growth. For this research, pure cultures of *E.coli* O157:H7 ATCC 43895, *Salmonella Typhimurium* ATCC 14028 and *Listeria monocytogenes* V7 (serotype 1/2a) strains were sub-cultured overnight in Brain-Heart Infusion broth at 37 °C for 24 h. Bacteria were grown in a chemically defined media and sampled periodically at regular time intervals to estimate microbial growth. Three repetitions for the growth experiments were conducted. Kinetic parameters of both models from the growth curves were obtained using the USDA Integrated Pathogen Modeling Program. An analysis of variance was performed to determine whether there were any significant differences among means of parameter estimates at a 95.0% confidence level. Additionally, statistic indicators were used to validate the performance of the models based on the bias factor and the accuracy factor. Predictions made by the Baranyi and Huang models for each treatment were evaluated using the Acceptable Prediction Zone, Akaike's Information Criterion, the Mean Square Error, and the Root Mean Square Error. Graphically, pathogenic growth as a function of time was well described by both models. Bacteria grew faster at 10 mM of glucose compared to a higher (15 mM) or lower (5 mM) nutrient concentration. Both models performed well as indicated by the MSE, RMSE, and AIC. The Baranyi model consistently estimated longer lag

phases and higher growth rates than the Huang model. These results provide an insight into modeling growth of pathogens as a function of time and nutrient concentration and may help to choose between the Baranyi or Huang models when determining the best-fitting model.

Chapter 1. Introduction to Predictive Microbiology

1.1. Definition

Predictive microbiology is the integrated discipline of traditional microbiology with tools from mathematics, statistics and information systems and technology to describe and predict microbial behavior in order to prevent food spoilage and food-borne illnesses (Fakruddin et al. 2011). Predictive microbiology aims to develop models that may assist in food safety evaluation, estimation of the shelf-life of foods, identification of critical points during production and distribution processes, and fundamentally, to describe the relationship between the environment and the response of pathogenic or spoilage bacteria (Grijspeerdt and Vanrolleghem 1999).

Mathematical models are a set of assumptions formulated by differential equations, the biological processes are extremely complex, and models describing such process must inevitably include these simplifying idealizations (Baranyi an Roberts 1995). However, despite such assumptions, if a model can produce realistic predictions, significant reductions in costs and time associated with laboratory testing of foods can be achieved.

The basic premise of predictive microbiology is that responses of microorganisms to environmental factors are reproducible, and by defining those factors it is possible to predict the responses of microorganisms in similar environments (Fakruddin et al. 2011). The models implemented in predictive microbiology are first developed in laboratory media and then applied to food systems.

1.2. Brief History

One of the first predictive models to be widely implemented within the food industry was developed by Esty and Meyer (1922), describing the thermal death of *Clostridium botulinum* type A spores by a log-linear model in low-acid canned foods. The model states that the percentage of the cell population inactivated by heat (specific death rate) will be constant with time (Fakruddin et al. 2011). Nowadays, the most frequently assumed relationship in thermal inactivation is that the logarithm of the specific death rate decreases linearly as the temperature increases (Baranyi and Roberts, 2004).

A further development was achieved by Scott (1936), who studied the relationship between the specific death rate and water activity (Aw) using an unitless scale between 0 (dry) and 1 (wet). However, the term "Predictive microbiology" was first coined by Roberts and Jarvis (1983) who developed a predictive model of *Clostridium botulinum* in cured meats (Brul et al. 2007).

1.3. Microbial Growth

When microbial cells are placed into a suitable medium, the increase in numbers or bacterial mass can be measured as a function of time to obtain a growth curve, and several distinct phases of growth can be observed (Pepper et al. 2011). These include the lag phase, the exponential or log phase, the stationary phase, and the death phase.

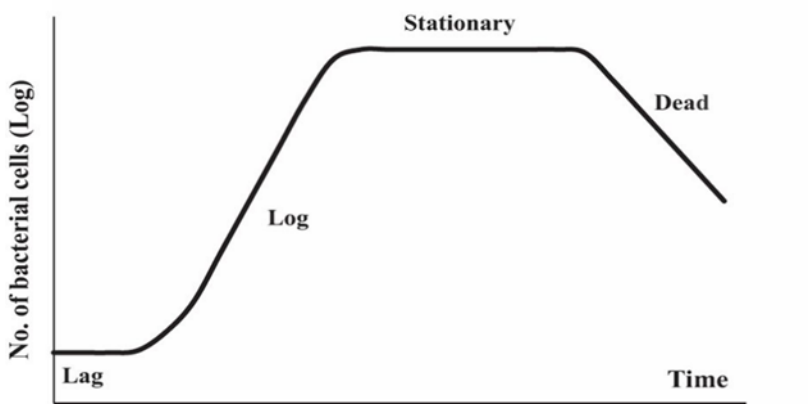

Figure 1. Typical growth curve observed in a batch system (Wang et al. 2015).

The growth rate associated with each phase varies significantly and it has been extensively mathematically characterized. The lag phase is defined as the transition to the exponential phase after the initial population has doubled. The lag phase occurs due to physiological adaptation of bacteria to the new culture conditions and involves processes such as protein synthesis and induction of specific messenger RNA (mRNA) (Yates and Smotzer, 2007). The lag phase can last from minutes to several hours, depending to some extent on the type of medium and the initial inoculum size.

The exponential phase is characterized by a period of rapid growth and the time it takes for a cell division to occur is called the generation time. If the initial cell number or cell mass is represented by X_0, the number of cells after n divisions can be expressed as $2^n X_0$, thus the exponential phase in a microbial growth curve can be represented by the Equation 1.1 (Maier and Pepper 2015):

$$\frac{dX}{dt} = \mu X \quad \text{(Eq. 1.1)}$$

Where X is the number or mass of cells, t is time, and μ is the specific growth rate constant (1/time). By rearranging Equation 1.1, the generation time and the specific growth rate using data generated from a growth curve can be calculated from Equation 1.2:

$$\frac{dX}{X} = \mu dt \quad \text{(Eq. 1.2)}$$

The stationary phase in a batch culture can be defined as a state of no net growth, and can be expressed by Equation 1.3 (Maier and Pepper 2015):

$$\frac{dX}{dt} = 0 \quad \text{(Eq. 1.3)}$$

Cells growth and division does not stop during the stationary phase, but an equal number of cells are dying balancing the net growth. Stationary phase occurs due to depletion of energy source and other essential nutrients and buildup of metabolic waste products that inhibits cell growth. However, grow could still occurs due to lysing of dying cells to provide more nutrients (McKellar and Lu 2003).

The death phase is characterized by a net loss of culturable cells (Pepper et al. 2011). During this phase some individual cells are metabolizing and dividing, but more viable cells are dying. The death phase is usually omitted in most growth models in foods because most foods become inedible or unsafe long before the death phase begins, and sometimes even before the stationary phase is reached (Peleg and Corradini 2011).

In a batch cultures (an enclosed growing system) under constant environmental conditions such as temperature and nutrient availability, the bacterial growth can generally be characterized by the sigmoid curve (Figure 1.1) where the dependent variable is the the viable cell concentration and the slope of that curve gives the instantaneous specific growth rate

(Baranyi et al 1993). Mathematical description of the entire microbial growth curve including the effect of nutrient concentration in growth rate was pioneered by Monod (1949), who developed a simple equation describing the relationship between the specific growth rate and the substrate concentration:

$$\mu = \frac{\mu_{max} S}{K_s + S} \quad \text{(Eq. 1.4)}$$

In Equation 1.4, μ is the specific growth rate (1/time), μ_{max} is the maximum specific growth rate (1/time) for the culture, S is the substrate concentration (mass/volume), and K_s is the half-saturation constant (mass/volume) or affinity constant. Monod-based models have been widely used in biotechnology, chemical engineering and food applications. Verrips and Zaalberg (1980), for example, developed a mechanistic Monod´s-based model that predicts growth and survival of microorganisms in water-in-oil emulsions, applying the relationship between substrate concentration and biomass formed in the water droplets size in the aqueous phase of the emulsion.

However, food microbiology aims to prevent microbial growth rather than optimize it, thus, Monod´s model lose their significance within the general food microbiology framework because as often ignored in biotechnology applications, the kinetics of the lag phase are of great importance (Baranyi and Roberts 1994).

1.4. Modeling Microbial Growth

A microbial model is a mathematical description of the number of microorganisms or a parameter related to cell level in food product or system, as a function of intrinsic or extrinsic factors (Marks 2008). Most primary models use the equations of each phase in the growth curve

as a baseline to develop equations describing the whole microbial growth curve. Microbial modeling allows the description and prediction of microbial behavior under specific environmental conditions such as pH, nutrient concentration, temperature, salinity, Aw, food matrix composition, oxygen availability, etc. However, only a few (mostly temperature, pH and nutrients availability) have a significant influence in microbial growth, and it is preferred to use as few variables as possible in models' equations (Fakruddin et al. 2011).

Microbial responses are usually tested under controlled conditions in a laboratory media to obtain a growth curve, then a primary model can be fitted to the growth data and the model-specific kinetic parameters (parameters characterizing a growth curve) are calculated (Cliver and Riemann, 2002). By studying the kinetic parameters and response of bacteria, microbial safety or shelf-life in foods can be predicted because the effect of a factor is independent of whether the microorganisms are in a broth or food, as long as other relevant factors are equivalent (Ross and McMeekin, 1994; Whiting, 1995). However, before predictive microbiology can be applied successfully applied to the food industry, primary mathematical models that adequately describe bacterial kinetics need to be stablished (Pla et al. 2015).

1.5. Limitations of Predictive Microbiology

When empirical models are derived, extrapolations cannot be made outside the ranges tested (e.g. temperature, Aw), because the model is derived by fitting the observed data, thus, do not describe microbial behavior. Models can also be over-conservative, because models are usually conducted in laboratory media where growth is much faster than in foods and may not be applicable in the food industry (Fakruddin et al. 2011).

Models derived in static conditions may not be representative of the changing conditions during the life of the product, e.g. fluctuations in temperature during distribution (Mackey and Kerridge 1988). The predictive capabilities of microbial growth models derived from experimental conditions should be treated with skepticism, and its implementation should be limited to the conditions tested and specific microorganisms studied.

During the past several years, there has been substantial advance in both the concepts and methods used in predictive microbiology. Coupled with 'user-friendly' software and the development of databases with extensive repertory such as ComBase, these models are providing powerful new tools for rapidly estimating the effects of formulation and storage factors on the microbiological relations in foods (Perez-Rodriguez and Valero 2013).

1.6. Model Classifications

Models can be microbiologically classified into kinetic and probability models, by the modeling approach into empirical or mechanistic, and by the variables measured into primary, secondary and tertiary.

General classification of models:

1. Kinetic models include those that describes bacterial responses such as the lag time, the specific growth rate, the maximum population density and inactivation/survival over time.
2. Probability models indicates the likelihood of growth/no growth or toxin production; however, they do not indicate the speed of growth (Roberts, 1989).

Classification of models according to their modeling approach:

1. An empirical model is a mathematical expression that describe and quantify experimentally observed phenomena (Peleg and Corradini 2011).
2. Mechanistic models interpret bacterial responses based on theoretical conjectures of known and reproducible processes (Fakruddin et al. 2011).

Classification of models into primary, secondary and tertiary models:

1. Primary models measure the response of the microorganism with a single set of conditions as a function of time. The response can be microbial growth measured by optical density and Colony Forming Units (CFU) or products of microbial metabolism (Membré and Dagnas 2016).
2. Secondary models mathematically describe the dependence of the parameters of primary models to changes in pH, temperature and/or other factors. These can be reincorporated into the equation of the primary model to produce the tertiary model.
3. Tertiary models are an algebraic expression that it can then be used to predict growth curves under a variety of conditions (Peleg and Corradini 2011).

1.7. The Baranyi Model

The Baranyi model is one of the most widely used primary models in predictive microbiology, due to the fact that it has a good fitting capability, it can be applied for dynamic environmental conditions and kinetic parameters obtained allow biologically interpretation (Pin et al., 2002; Lopez et al., 2004; Van Impe et al., 2005, Yilmaz 2011). It is based in part on the concept that the rate of bacterial growth is controlled by the rate of a "bottleneck" biochemical reaction (Marks 2008), but the main assumption of the Baranyi equations is that growth relies upon the physiological state of cells (Baranyi and Roberts 1994).

The model is a system of two equations, the second being independent of the first. The equations of this model captures a lag phase naturally, without the need of artificially introduce a lag parameter within the model (Vadasz and Vadasz 2007).

As explained by (Sinigaglia, et al. 2012), the starting point of the model is the function describing microbial growth (see Eq. 1.1). However, the Baranyi and Roberts equation takes into account the possibility that cells can be transferred from an environment E_1 to an environment E_2; therefore, the equation of microbial growth is revised through an adjustment:

$$\frac{dX}{dt} = \alpha(t) \cdot \mu(x) \cdot (x) \quad \text{(Eq. 1.5)}$$

Where $\alpha(t)$ is the adjustment function and x is the cell count.

This adjustment relies upon the physiological state of cells (q), a dimensionless parameter ranging from 0 to 1. If $q = 1$, cells are ready to duplicate and there is not lag phase, if $q = 0$, no cell division will occur. The correlation between the adjustment function of the microbial growth and the physiological state can be expressed by the following function:

$$\alpha(t) = \frac{q(t)}{1 + q(t)} \quad \text{(Eq. 1.6)}$$

From the physiological state of cells (q_0), the lag phase can be calculated as follow:

$$\lambda = \frac{\text{In}\left(1 + \frac{1}{q_0}\right)}{v} \quad \text{(Eq. 1.7)}$$

Where λ is the lag phase and v is is the growth rate. The system of two equations of the final Baranyi and Roberts (1994) model is as described by equations 1.8 and 1.9:

$$\frac{dN(t)}{dt} = \left(\frac{q(t)}{1+q(t)}\right) \cdot \mu_{max} \cdot \left(1 - \frac{N(t)}{N_{max}}\right) \cdot N(t) \quad \text{(Eq. 1.8)}$$

With $N_{t=0} = N_0$

The first equation (Eq. 1.8) has three descriptive elements:

1. The first element is the adjustment function:$\left(\frac{q(t)}{1+q(t)}\right) \cdot \mu_{max}$ which introduces the physiological state of cells: *q*.

2. The second element introduces the exponential growth rate: $\mu_{max.}$

3. The third element is the inhibition function: $\left(1 - \frac{N(t)}{N_{max}}\right)$ which describes the transition of population to the stationary phase, inferred by N_{max}, the maximal number of cells attained at the end of the exponential phase.

The second differential equation (Eq. 1.9) describes the exponential increase of the physiological state of cells and its correlation with the growth rate:

$$\frac{dq(t)}{dt} = \mu_{max} \cdot q(t) \quad \text{(Eq. 1.9)}$$

With $q_{t=0} = q_0$

The Baranyi model in its current form is as follows (Baranyi et al. 1993, 1995):

$$y_t = y_0 + \mu_{max} A(t) - \ln\left(1 + \frac{e^{\mu_{max} A(t)} - 1}{e^{y_{max} - y_0}}\right) \quad \text{(Eq. 1.10)}$$

Where:

$$A(t) = t + \frac{1}{v} \ln\left(e^{-vt} + e^{-h_0} - e^{(-vt-h_0)}\right) \quad \text{(Eq. 1.11)}$$

In equaitions 1.10 and 1.11, y_t represents the cell concentration in Log CFU/ml at time t; y_0 represents the initial cell concentration in Log CFU/ml; y_{max} represents the maximum cell concentration in Log CFU/ml; μ_{max} is the maximum specific growth rate in Log CFU/h; *v* is the rate of increase in the limiting substrate, assumed to be equal to μ_{max}; λ is the duration of the lag phase in hours; h_0 is equal to $\mu_{max}\lambda$.

1.8. The Huang Model

This model is also considered a mechanistic model since it is based on the biological growth of bacteria. According to Huang (2008), without the lag and stationary phase, the growth of bacteria in food follows a first-order kinetics, i.e., at a high substrate concentration bacterium should grow exponentially, this is described by equation 1.12, where *C* is the bacterial population:

$$\frac{dC}{dt} = \mu_{max} C \quad \text{(Eq. 1.12)}$$

In any environment where bacteria is present, the bacterial growth is limited by the maximum cell density reached at the stationary phase, C_{max}, thus, the transition between the exponential and stationary phase is modeled by equation 1.13:

$$\frac{dC}{dt} = kC(C_{max} - C) \quad \text{(Eq. 1.13)}$$

Where *k* is the rate constant for exponential phase (not the specific growth rate), with units (CFU/ml *x* time)$^{-1}$. And *kC* is equal to μ_{max}. However, equation 1.13 describes a process on which bacteria starts to multiply right after inoculation and therefore is not suitable to describe the entire microbial growth curve. Then, according to Huang (2008), the complete growth process can be described as follow:

$$\frac{dC}{dt} = 0, \text{if } t \leq \lambda \quad (\text{Eq. } 1.14)$$

$$\frac{dC}{dt} = kC(C_{max} - C), \text{if } t > \lambda \quad (\text{Eq. } 1.15)$$

Equation 1.14 is a representation of no growth during the lag phase ($t \leq \lambda$) and therefore no growth can be observed, thus $\frac{dC}{dt} = 0$. After the lag phase ($t > \lambda$) (equation 1.15), growth begins, and exponential phase starts. Although equations 1.14 and 1.15 can be used to describe the entire growth process, it is a discontinuous model that requires 2 separate equations. A single equation was developed using a unit step function to combine the 2 equations (Huang 2008):

$$\frac{dC}{dt} = U(t - \lambda)\, x\, kC(C_{max} - C) \quad (\text{Eq. } 1.16)$$

If $t \leq \lambda$ (within the lag phase) then the unit function, $U(t - \lambda) = 0$ And if $t > \lambda$, then the unit function $U(t - \lambda) = 1$. With $U(t - \lambda) = 0$, there is no growth but with $U(t - \lambda) = 1$, the bacterial growth follows Equation 1.13. The unit step function used in equation 1.16 joins 2 separate expressions, equations 1.14 and 1.15 into a single equation. However, the unit step function in equation 1.16 is still a discrete function (Huang 2008) and to make it into a continuous function, a transitional functions, *f (t),* can be used to allow the smooth transition from the lag phase to the exponential phase in the model according to the following equation proposed by Huang (2008):

$$f(t) = \frac{1}{1 + e[(-\alpha(t - \lambda)]} \quad (\text{Eq. } 1.17)$$

This transitional function *f (t)* has the mathematical property that if $t << \lambda$ then *f (t)* = 0; and if $t >> \lambda$ then *f (t)* = 1. When *t* is close to λ, *f (t)* gradually changes from 0 to 1. The

coefficient α defines the sharpness of the transition in the growth curve. With *f (t)* available, the entire growth process can be described in equation 1.17:

$$\frac{dC}{dt} = \frac{kC(C_{max} - C)}{1 + e[(-\alpha(t - \lambda)]} \quad (\text{Eq. } 1.17)$$

The differential growth equation expressed in equation 1.14 is now a continuous expression that can be solved analytically by separation of variables; thus the general Huang's growth model can be expressed as:

$$y(t) = y_0 + y_{max} - \text{In}[e^{y_0} + (e^{y_{max}} - e^{y_0})e^{\mu_{max} B(t)}] \quad (\text{Eq. } 1.18)$$

Where:

$$B(t) = t + \frac{1}{\alpha} In \frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{\alpha\lambda}} \quad (\text{Eq. } 1.19)$$

Where $y(t)$ represents the cell concentration in log CFU/ml at time *t*, y_0 represents the initial cell concentration in Log CFU/ml; y_{max}represents the maximum cell concentration in Log CFU/ml ; μ_{max} is the maximum specific growth rate in Log CFU/h; λ is the duration of the lag phase in hours.

1.9. Applications of the Baranyi and Huang Models

Predictive microbiology is the integration of traditional microbiology with tools from the disciplines of mathematics, statistics and information systems and technology to describe microbial behavior under different environmental factors, in order to prevent food spoilage and food-borne illnesses (Fakruddin et al. 2011). Predictive microbiology aims to develop models that may assist in food safety evaluation, estimation of the shelf-life of foods, identification of critical points during production and distribution processes, and fundamentally, to describe the

relationship between the environment and the response of pathogenic or spoilage bacteria (Grijspeerdt and Vanrolleghem 1999).

A dynamic predictive model for the growth of *Salmonella spp.* in liquid whole egg under nonisothermal (continuously varying temperature) conditions was developed using the Baranyi model (Singh et al. 2011). Maximum population densities reached approximately 8.5 Log CFU/ml at 25°C, with initial inoculum sizes between 2.5 and 3.0 Log CFU/ml. Root mean square values varied between 0.46 and 1.02 for a temperature profile between 10°C and 43°C.

The effect of tagatose (a low-calorie sweetener) in the growth dynamics of *Listeria monocytogenes* and *Salmonella Typhimurium* in a chemically defined medium fitting the Baranyi model has been explored (Lobete et al. 2017). Behavior of *L. monocytogenes* was not affected by the additions of tagatose; however, *S. Typhimurium* showed a reduced growth with increasing tagatose concentrations. Spore-forming pathogens such as *Bacillus cereus* have also been studied using the Baranyi model (Tango et al. 2014).

Comparisions between the Baranyi and Huang models have been made in structured broth media and in food matrixes. Huang et al. 2013 compared the growth of Lactic Acid Bacteria in vacuum-packaged beef at different tempratures using the Gompertz, Logistic, Baranyi, Huang models. While Juneja et al. 2019 compared the growth of *Bacillus cereus* in rice using the Barnayi, Huang and other primary models. Direct comparisions evaluation the performance of the Barnayi and Huang models with *Clostridium sporogenes* in cooked beed have also been made (Hong et al. 2016).

Despite being a viable alternative to the Baranyi model, the Huang model has been less explored for modeling growth of pathogenic bacteria, thus the need to evaluate its performance under different conditions against the predominant model in the field.

Chapter 2. Modeling the Growth of *Escherichia Coli* O157:H7, *Listeria Monocytogenes* and *Salmonella Typhimurium* Using the Baranyi and Huang Models

2.1. Introduction

Microbial growth can be characterized by parameters such as lag time, growth rate, and maximum population density at any specific point of time. Mathematical models that predict microbial growth of foodborne pathogens are increasingly used in the food industry as a viable alternative to traditional methods of microbial enumeration. Primary models measure the response of the microorganism with a single set of conditions as a function of time (Peleg and Corradini 2011). The Baranyi model (Baranyi and Roberts 1995) and the Huang model (Huang 2008) are examples of mechanistic and semi-theoretical primary models, i.e., mathematical descriptions upon which models are constructed are based in biologic phenomena (Huang et al. 2013).

The Baranyi model has been widely used as the primary model of choice by many authors (Lobete et al. 2016; Kowalik and Lobacz 2014; Tango et al. 2014; Tyrovouzis et al. 2014; Dung et al. 2012; Singh et al. 2011; Alavi et al. 1999) because its performance and accuracy over other primary non-mechanistic models such as the Gompertz and Logistic models (Menezes et al. 2018; Tarlak et al. 2018; Mytilinaios et al. 2012). However, the most recently developed Huang model (Huang 2008) has been less extensively implemented and few comparisons between the Baranyi and Huang models have been made using selected microorganisms; *Bacillus cereus* (Juneja et al. 2019), *Clostridium sporogenes (*Hong et al. 2016*), Staphylococcus aureus* (Li et al. 2015), Non-O157 Shiga Toxin-Producing *Escherichia*

coli (Huang et al. 2012) and Lactic Acid Bacteria (Li et al. 2013). Other foodborne pathogens have not been thoroughly studied using the Huang model.

E.coli O157:H7, *Listeria monocytogenes* and *Salmonella spp.* are the three major foodborne pathogens and are implicated in several outbreaks (Hoelzer et al. 2018; Walsh et al. 2014; Zweifel and Stephan 2012). A report from the World Health Organization (WHO, 2015) listed infections with *Salmonella* and Enteropathogenic *E.coli* as the leading global causes of foodborne deaths. The kinetic parameters estimated from the Baranyi and Huang models can be used to describe the growth of these pathogens and comparison between models may assist in the selection of the primary model with the best fitting capabilities when modeling growth of *E.coli* O157:H7, and *L.monocytogenes*, *S. Typhimurium*.

Chemically defined media are used in food microbiology in order to conduct reproducible experiments and avoid confounding by extraneous, often unknown factors originating from the composition of more rich and complex growth media (Tyrovouzis et al. 2014) and growth under adequate conditions allows for evaluation of undisturbed kinetic behavior. Besides, when comparing model predictions in broth media with observations in foods, one can assume that results will be fail-safe, that is, the predicted growth in liquid media is much faster than that observed in food (Perez-Rodriguez and Valero 2013).

Thus, the objectives of this study were to (1) compare the microbial behavior of three major foodborne pathogens in a liquid system at strict control of nutrients and (2) to evaluate its impact in their kinetic response using the Baranyi and Huang models.

2.2. Materials and Methods

2.2.1. Bacterial Strains and Chemically Defined Minimal Media

Pure cultures of *Escherichia coli* O157:H7 ATCC 43895, *Salmonella Typhimurium* ATCC 14028 and *Listeria monocytogenes* V7 (serotype 1/2a) strains were stored at −80 °C in Tryptic Soy Broth (TSB; Acumedia 7164, Neogen Corporation, Lansing, Michigan, USA) with 20% v/v of glycerol and sub-cultured overnight in Brain-Heart Infusion broth (BHI; Acumedia 7116, Neogen Corporation) at 37 °C for 24 h.

The composition of the chemically defined minimal media (CDMM) per liter was: Na_2HPO_4, 46 mM; KH_2PO_4, 22 mM; NaCl, 8.5 mM; NH_4Cl, 18.70 mM; $MgSO_4$, 2 mM; $CaCl_2$, 0.1 mM; and 0, 5, 10 or 15 mM of glucose as the sole carbon source. Final pH was adjusted to 6.8 ± 0.2 with a 1 M solution of NaOH. 100 µL of overnight cultures (4.0 – 4.5 Log CFU/mL) were inoculated into 500 ml of CDMM supplemented with glucose and incubated at 24 °C with constant agitation (200 rpm).

2.2.2. Microbial Enumerations and Glucose Depletion Analysis

Duplicate samples were aseptically removed at regular time intervals to estimate microbial growth by serially diluting in Phosphate-buffered saline (PBS) and plated onto Nutrient Agar. Plates were incubated at 37°C for 48 h and colony counts in the 25-250 range were expressed as Log CFU mL^{-1}. Three repetitions for the growth experiments were conducted. Supernatants of cultured CDMM after centrifugation at 5,000 rpm for 2 min were enzymatically analyzed for glucose consumption using a Glucose Assay Kit (GAGO-20, Sigma, St. Louis, MO).

2.2.3. Data Analysis and Mathematical Modelling

Two models were chosen to fit the growth curves of pathogens in a CDMM, the Baranyi model (Baranyi and Roberts 1995) and the most recently developed primary model, the Huang model (Huang 2008). Kinetic parameters from growth curves were obtained using the USDA Integrated Pathogen Modeling Program (Huang 2013a), a software designed for the analysis of data in predictive microbiology (Huang 2014).

The Baranyi model in its current form is as follows (Baranyi et al. 1993, 1995):

$$y_t(t) = y_0 + \mu_{max}A(t) - \ln\left(1 + \frac{e^{\mu_{max}A(t)} - 1}{e^{(y_{max} - y_0)}}\right) \quad (\text{Eq. } 2.1)$$

Where:

$$A(t) = t + \frac{1}{\mu_{max}} \ln(e^{-\mu_{max}t} + e^{-h_0} - e^{-\mu_{max}t - h_0}) \quad (\text{Eq. } 2.2)$$

In equaition 2.1 and 2.2, y_t represents the cell concentration in Log CFU/ml at time *t*; y_0 represents the initial cell concentration in Log CFU/ml; y_{max} represents the maximum cell concentration in Log CFU/ml; μ_{max} is the maximum specific growth rate in Log CFU/h; μ_{max} is the specific grwoth rate; h_0 is the physiological state of the microorganism and is equal to $\mu_{max}\lambda$.

Huang growth model can be expressed as:

$$y(t) = y_0 + y_{max} - \ln\left[e^{y_0} + (e^{y_{max}} - e^{y_0})e^{\mu_{max}B(t)}\right] \quad (\text{Eq. } 2.3)$$

Where

$$B(t) = t + \frac{1}{\alpha} In \frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{\alpha\lambda}} \text{ (Eq. 2.4)}$$

In equations 2.3 and 2.4, $y(t)$ represents the cell concentration in log CFU/ml at time *t*, y_0 represents the initial cell concentration in Log CFU/ml; y_{max}represents the maximum cell concentration in Log CFU/ml ; μ_{max} is the maximum specific growth rate in Log CFU/h; λ is the duration of the lag phase in hours.

2.2.4. Statistical Analyses

An analysis of variance (ANOVA) test was performed to determine whether there were any significant differences among means of parameter estimates, at a 95.0% confidence level (α 0.05). If the ANOVA test indicated significant differences between the parameters, a Tukey test was used to identify which means were significantly different. All statistical analyses were performed using the SAS package (Version 9.4, SAS Institute Inc., N.C., U.S.A.).

2.2.5. Validation

The statistic indicators used to externally validate the performance of the models were determined based on the bias factor (BF) and the accuracy factor (AF) (Ross 1996). The BF is an index of the model performance in terms of the average of the ratios between predicted and observed values (equation 2.5). The AF averages the distance between each point and the line of equivalence as a measure of how close, on average, predictions are to observed values (Te-Giffel and Zwietering 1999) (equation 2.6):

$$BF = 10^{\frac{\sum Log\left(\frac{predicted}{observed}\right)}{n}} \text{ (Eq. 2.5)}$$

$$AF = 10^{\frac{\sum|Log\left(\frac{predicted}{observed}\right)|}{n}} \quad \text{(Eq. 2.6)}$$

Predictions made by the Baranyi and Huang models for each treatment were also evaluated using the Acceptable Prediction Zone (APZ) analysis (Oscar, 2005). Predicted values were subtracted from observed values to generate the Prediction Error (PE) for each observation with Log CFU ml^{-1} units. Positive PE values were considered fail-dangerous and PEs with a negative value were considered fail-safe, while a PE of 0 indicated a perfect prediction (Oscar 2005). Acceptable prediction zone limits were set between −1.0 and 0.5 Log CFU ml^{-1} (Mishra et al. 2017, Juneja et al. 2019).

Alternative methods for comparing models are the Akaike's Information Criterion (AIC), based on information theory (Burnham and Anderson, 2002; Motulsky and Christopoulos, 2004); and goodness-of-fit measures such as the Mean Square Error (MSE) and Root Mean Square Error (RMSE) based on the approach of Li et al. (2013) were also evaluated for each model.

The AIC is given by equation 2.7:

$$AIC = nIn\left(\frac{RSS}{n}\right) + 2\,(p+1) + \frac{2(p+1)(p+2)}{n-p-2} \quad \text{(Eq. 2.7)}$$

Where *n* is the number of data points and *p* is the number of parameters of the model. The method considers the change in goodness-of-fit and the difference in number of parameters between two models (Lopez et al. 2004).

The MSE and RMSE are given by equations 2.8 and 2.9, respectively:

$$MSE = \frac{RSS}{df} = \frac{\sum(\mu_{observed} - \mu_{predicted})^2}{df} \quad \text{(Eq. 2.8)}$$

$$RMSE = \sqrt{\frac{\sum(\mu_{observed} - \mu_{predicted})^2}{df}} \quad \text{(Eq. 2.9)}$$

2.3. Results and Discussion

2.3.1. Bacterial Growth in a CDMM with Glucose as the Sole Carbon Source

The generation time was 1.73 h for *E.coli* O157:H7, 2.01 h for *L. monocytogenes* and 1.66 h for *S. Typhimurium* (Data not shown). The generation time in a CDMM did not changed as the glucose concentration increased. Prachaiyo and McLandsborough (2003) while studying *E.coli* O157:H7 in a chemically defined medium of similar composition found slightly shorter generation times, between 1.02 h (at 0.4% of glucose) and 1.11 h (at 1% of glucose).

CDDM supplemented with 5 mM of glucose showed an exponential decrease in glucose concentration close to 8 h. *L. monocytogenes* consumed less glucose over a 24 h period compared to *E.coli* O157:H7 and *S. Typhimurium*, conversely, at higher concentrations of glucose *L. monocytogenes* depleted higher amounts of glucose over a 24 h period.

In all experiments, results indicate that the capability of cells to respond faster to surrounding glucose as the sole-carbon source in a CDMM was dependent on the concentration; at 5 mM, changes in glucose concentrations were observed until 7 hr; at 10 mM a more rapid glucose depletion was observed from the beginning but little changes were detected between 12 h and 24 h either at 10 or 15 mM of glucose, with the exception of *L. monocytogenes* since depletion was near to 50% in both concentrations at 24 h. Increasing concentration from 10 to 15 mM had little effect in the amount of glucose consumed after 24 h (Figure 2.1).

In most cases, bacteria reached stationary phase at 24 ± 2 h, however *E.coli* O157:H7 and *L. monocytogenes* at 5 mM reached stationary phase approximately at 30 ± 2 h. No growth was observed at 0 mM of glucose.

2.3.2. Fitting Curves of Primary Models

Figures 2.2 to 2.4 show the fitting curves of the Baranyi and Huang models to experimental data of *E.coli* O157:H7, Figures 2.5 to 2.7 and 2.8 to 2.10 show the fitting curves of *L. monocytogenes* and *S. Typhimurium*, respectively. Graphically, both models fit the data well, which means that pathogenic growth as function of time was well described by both models, as reported by other authors who have challenged the models under a variety of conditions (Juneja et al. 2019, Lobete et al. 2016, Huang et al. 2013).

Nevertheless, graphic representation varied depending on the model, the Huang model in some cases tend to have a sharp angle between the lag and exponential phases, which is intended to clearly differentiate between these two phases (Huang et al. 2011).

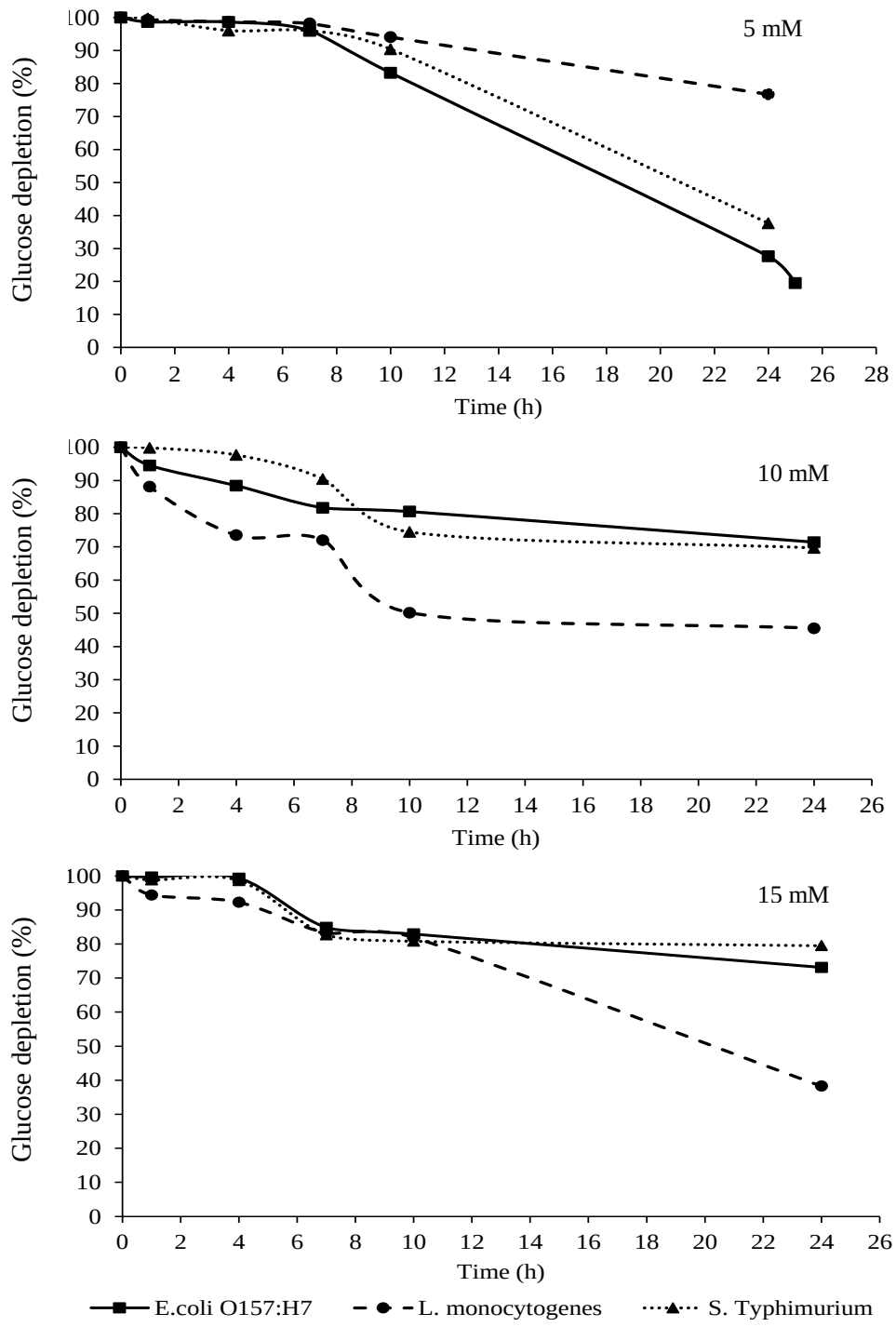

Figure 2.1. Glucose depletion (%) of *E.coli* O157:H7, *L. monocytogenes* and *S.Typhimurium* at 5, 10, and 15 mM as a function of time.

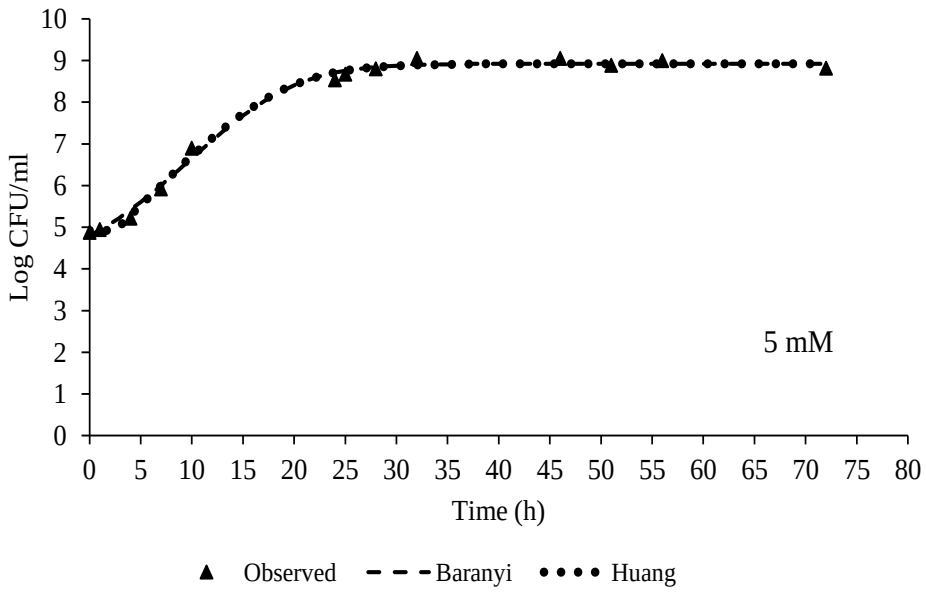

Figure 2.2. Observed growth of *E.coli* O157:H7 at 5 mM and fitted Baranyi and Huang growth models.

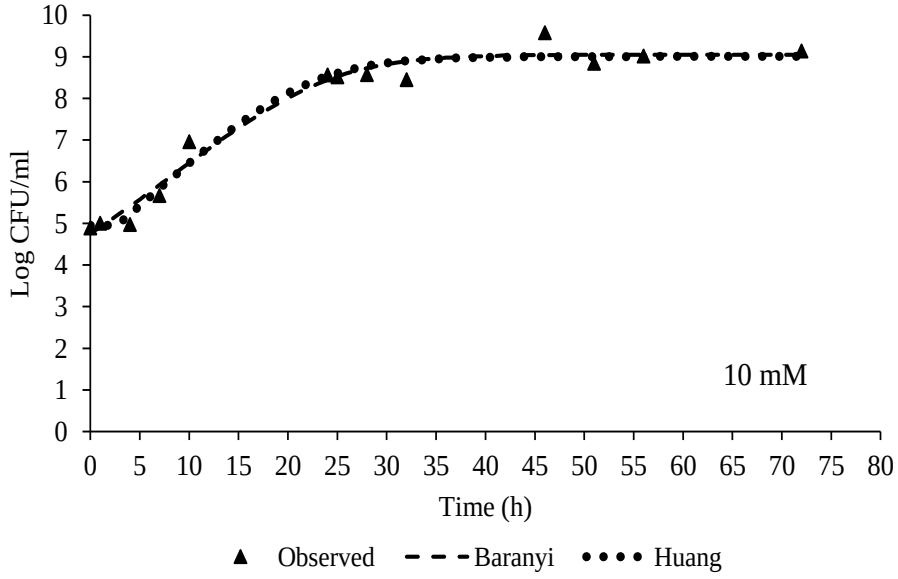

Figure 2.3. Observed growth of *E.coli* O157:H7 at 10 mM and fitted Baranyi and Huang growth models.

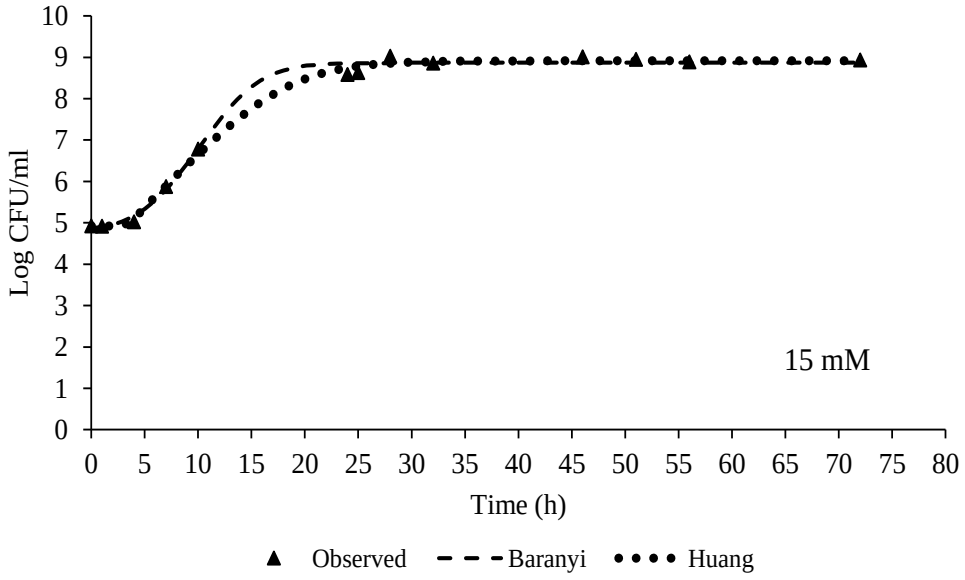

Figure 2.4. Observed growth of *E.coli* O157:H7 at 15 mM and fitted Baranyi and Huang growth models.

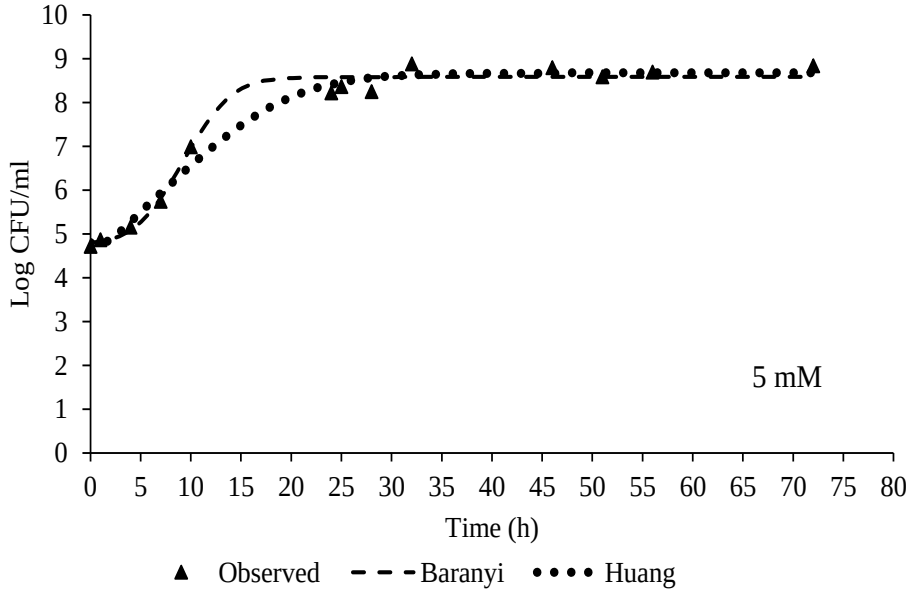

Figure 2.5. Observed growth of *L. monocytogenes* at 5 mM and fitted Baranyi and Huang growth models.

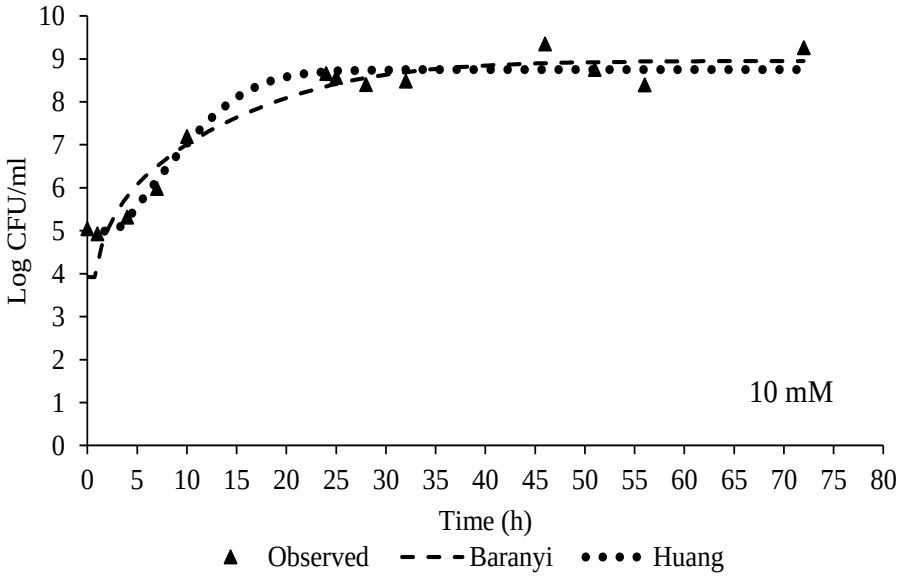

Figure 2.6. Observed growth of *L. monocytogenes* at 10 mM and fitted Baranyi and Huang growth models.

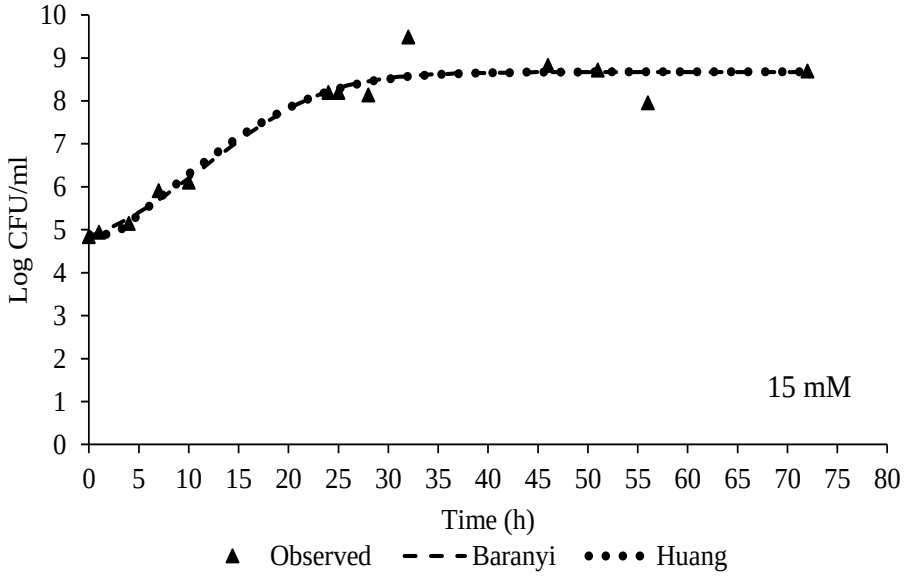

Figure 2.7. Observed growth of *L. monocytogenes* at 15 mM and fitted Baranyi and Huang growth models.

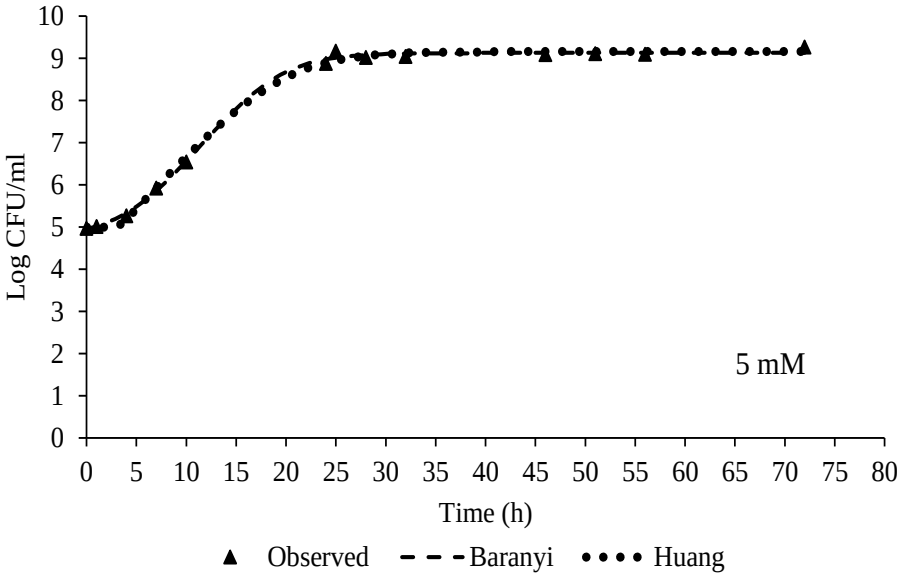

Figure 2.8. Observed growth of *S. Typhimurium* at 5 mM and fitted Baranyi and Huang growth models.

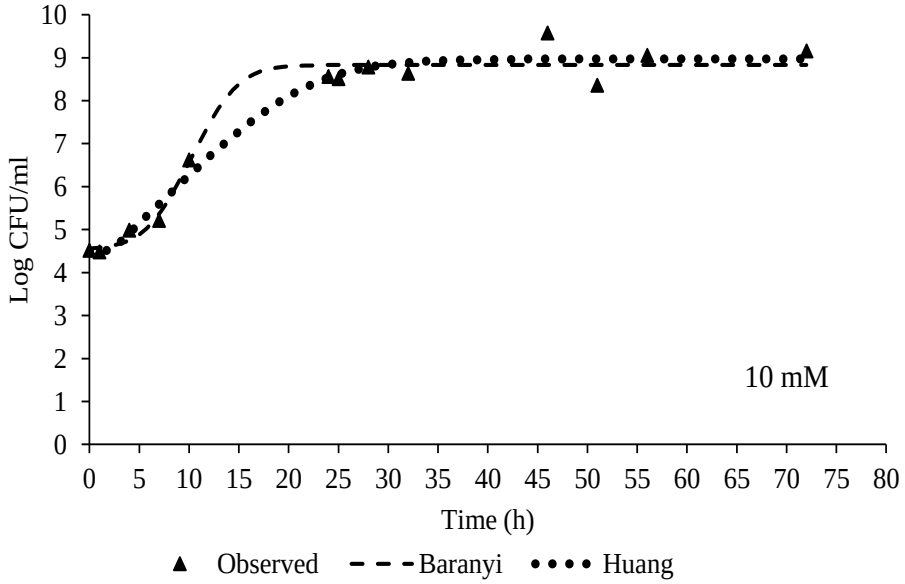

Figure 2.9. Observed growth of *S. Typhimurium* at 10 mM and fitted Baranyi and Huang growth models.

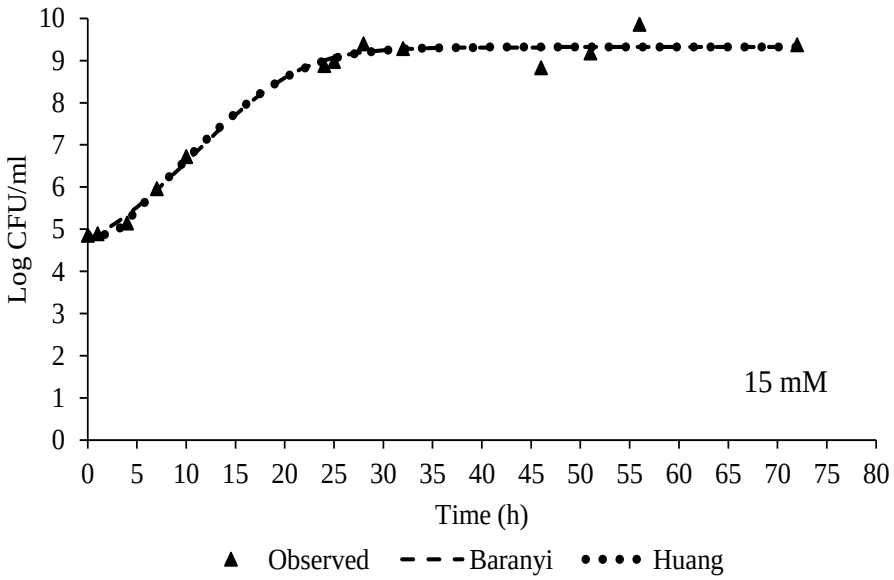

Figure 2.10. Observed growth of *S. Typhimurium* at 15 mM and fitted Baranyi and Haung growth models.

2.3.3. Kinetic Parameters According to the Baranyi Model

According to the Baranyi model (Table 2.1), there were no significant differences between the three bacteria in the initial population (N_0), an expected result since inoculums were prepared under the same condition. Maximal cell density (N_{max}) achieved after reaching the stationary phase in the CDMM was also not significantly different.

On average, maximum growth rate (μ_{max}) was shown to be lower at 5 mM and higher at 10 mM and 15 mM. The longest lag phase (λ) was observed for S. *Typhimurium* at a glucose concentration of 10 mM, with 7.17 h, *S.Typhimurium* also shown the lowest glucose consumption close to 8 h (see figure 2.1), this may indicate a longer period of adaptation is needed for *S.Typhimurium* at this level of nutrient availability according to the Baranyi model.

Table 2.1. Kinetic parameters of *E.coli* O157:H7, *Listeria monocytogenes* and *Salmonella Typhimurium* derived from the Baranyi model[1,2].

Pathogen	mM of Glucose	Kinetic parameters[3]			
		N_0 (Log CFU/ml)	N_{max} (Log CFU/ml)	μ_{max} (1/h)	λ (h)
E.coli O157:H7	0	4.90 ± 0.09	N.G[4]	N.G	N.G
L.monocytogenes	0	4.77 ± 0.02	N.G	N.G	N.G
S.Typhimuirum	0	4.33 ± 0.80	N.G	N.G	N.G
E.coli O157:H7	5	4.81 ± 0.03	8.80 ± 0.07	0.36 ± 0.11^{B}	3.93 ± 0.40^{Bb}
L.monocytogenes	5	4.78 ± 0.11	8.42 ± 0.87	0.30 ± 0.23^{B}	5.20 ± 0.04^{Bab}
S.Typhimuirum	5	4.97 ± 0.05	9.13 ± 0.08	0.36 ± 0.11^{B}	5.66 ± 0.13^{Bab}
E.coli O157:H7	10	4.88 ± 0.15	8.87 ± 0.32	0.47 ± 0.26^{A}	6.33 ± 0.01^{Aab}
L.monocytogenes	10	4.89 ± 0.18	8.63 ± 0.11	0.58 ± 0.04^{A}	6.08 ± 0.30^{Aab}
S.Typhimuirum	10	4.43 ± 0.69	8.80 ± 0.76	0.77 ± 0.01^{A}	7.17 ± 0.06^{Aa}
E.coli O157:H7	15	4.88 ± 0.06	8.80 ± 0.03	0.45 ± 0.08^{AB}	6.15 ± 0.57^{Bab}
L.monocytogenes	15	4.85 ± 0.10	8.66 ± 0.03	0.37 ± 0.13^{AB}	3.62 ± 1.51^{Bb}
S.Typhimuirum	15	4.81 ± 0.07	9.20 ± 0.14	0.37 ± 0.11^{AB}	4.76 ± 1.51^{Bab}

[1]Within each column, different uppercase letters indicate significant differences ($p<0.05$) between glucose concentrations (collapsed across bacteria).
[2] Within each column, different lowercase letters indicate significant differences ($p<0.05$) from one-way ANOVA, comparing means across each bacteria and glucose combination.
[3] Mean ± Standard Deviation.
[4] No Growth.

2.2.4. Kinetic Parameters According to the Huang Model

The initial population size using the Huang model was no different between bacteria (Table 2.2), however, the average predicted maximal cell density achieved between *S. Typhimurium* and *L. monocytogenes* was significantly different (Table 2.2). Nevertheless, on average *L. monocytogenes* at 10 mM grew at a significantly higher rate (0.72 h^{-1}) than the other two bacteria. *L.monocytogenes* at 10 mM also showed the longest lag phase with a duration of 5.70 h.

Table 2.2. Kinetic parameters of *E.coli* O157:H7, *Listeria monocytogenes* and *Salmonella Typhimurium* derived from the Huang model[1,2].

Pathogen	mM of Glucose	Kinetic parameters[3]			
		N_0 (Log CFU/ml)	N_{max} (Log CFU/ml)	μ_{max} (1/h)	λ (h)
E.coli O157:H7	0	4.90 ± 0.09	N.G	N.G	N.G
L.monocytogenes	0	4.77 ± 0.02	N.G	N.G	N.G
S.Typhimuirum	0	4.33 ± 0.80	N.G	N.G	N.G
E.coli O157:H7	5	4.92 ± 0.03	8.90 ± 0.16^{AB}	0.28 ± 0.07^{Bb}	2.89 ± 0.04^{Bb}
L.monocytogenes	5	4.80 ± 0.03	8.56 ± 0.65^{B}	0.24 ± 0.10^{Ab}	2.58 ± 0.40^{Ab}
S.Typhimuirum	5	5.00 ± 0.04	9.17 ± 0.07^{A}	0.26 ± 0.04^{Bb}	3.34 ± 0.06^{Bb}
E.coli O157:H7	10	4.89 ± 0.13	9.15 ± 0.29^{AB}	0.20 ± 0.02^{Bb}	2.11 ± 0.33^{Bb}
L.monocytogenes	10	4.99 ± 0.16	8.63 ± 0.11^{B}	0.72 ± 0.26^{Aa}	5.70 ± 0.62^{Aa}
S.Typhimuirum	10	4.50 ± 0.59	8.99 ± 0.57^{A}	0.23 ± 0.02^{Bb}	2.26 ± 0.13^{Bb}
E.coli O157:H7	15	4.92 ± 0.06	8.83 ± 0.02^{AB}	0.31 ± 0.03^{Bb}	3.58 ± 0.86^{Bb}
L.monocytogenes	15	4.93 ± 0.07	8.57 ± 0.14^{B}	0.28 ± 0.11^{Ab}	3.04 ± 0.11^{Ab}
S.Typhimuirum	15	4.87 ± 0.04	9.24 ± 0.12^{A}	0.27 ± 0.01^{Bb}	2.97 ± 0.70^{Bb}

[1]Within each column, different uppercase letters indicate significant differences ($p<0.05$) between bacteria (collapsed across glucose concentrations).
[2] Within each column, different lowercase letters indicate significant differences ($p<0.05$) from one-way ANOVA, comparing means across each bacteria and glucose combination.
[3] Mean ± Standard Deviation.
[4] No Growth.

2.2.5. Comparison of the Baranyi and Huang Models

When comparing these models, the assumptions upon each model is based on should be considered; the assumptions for the Baranyi model are based on the prior conditions of the inoculum and the accumulations of critical substances for bacterial growth on a new environment. The Huang model is based on the transition from the lag phase to exponential phase after exposed to a new environment (Huang 2013b). The initial population size using the Huang model was no different between bacteria (Table 2.3), however, the average predicted maximal cell density achieved by *S. Typhimurium* was significantly different compared to the maximal cell density achieved by of *L. monocytogenes* (Table 2.3). The Baranyi model precited

significantly higher μ_{max} values for *Salmonella* at 10 mM compared to the Huang model at the same conditions. The Baranyi model predicted longer lag phases than the Huang model in most cases. Lag predictions for both models were the same for *E.coli* O157: H7 at 5 mM, *L. monocytogenes* at 10 mM and 15 mM, and for *S. Typhimurium* at 15 mM. One of the few studies comparing these models found a closer agreement between the Huang and Baranyi model in the determination of maximum specific growth rate, however, longer lag phases were also determined when using the Baranyi model (Huang 2013b). Bovill et al. (2000) suggested that the lag predictions of the Baranyi model are less accurate than growth rate predictions, because the lag phase depends on the physiological sate (a non-autonomous feature), while the maximum specific growth characterize the bacteria and the actual environment.

Juneja et al. (2009) compared the Baranyi and Huang models using *Salmonella* and suggested that any of them could be used to describe bacterial growth under isothermal conditions. However, the Huang model provides a slight advantage since the lag phase is directly calculated from each growth curve.

Table 2.3. Kinetic parameters of *E.coli* O157:H7, *Listeria monocytogenes* and *Salmonella Typhimurium* derived from the Baranyi and Huang models[1]

Model	Bacteria	Kinetic parameters[2]			
		N_0	N_{max}	μ_{max}	λ
Baranyi	*E.coli* O157: H7	4.85 ± 0.09	8.82 ± 0.18^{AB}	0.42 ± 0.17^{AB}	5.25 ± 1.28^{A}
	L.monocytogenes	4.84 ± 0.11	8.57 ± 0.41^{B}	0.42 ± 0.18^{AB}	4.97 ± 1.31^{A}
	S. Typhimurium	4.77 ± 0.35	9.07 ± 0.35^{AB}	0.46 ± 0.20^{A}	5.70 ± 1.28^{A}
Huang	*E.coli* O157: H7	4.91 ± 0.06	8.95 ± 0.20^{AB}	0.26 ± 0.06^{AB}	2.98 ± 0.84^{B}
	L.monocytogenes	4.89 ± 0.11	8.58 ± 0.36^{B}	0.38 ± 0.25^{B}	3.60 ± 1.48^{B}
	S. Typhimurium	4.79 ± 0.37	9.13 ± 0.31^{A}	0.26 ± 0.03^{B}	2.86 ± 0.59^{B}

[1] For each model and pathogen combination, parameter values bearing different lowercase superscripts are significantly different ($p \leq 0.05$).
[2] Mean ± Standard Deviation.

2.2.6. Evaluation of Performance of Primary Models

Mean Square Errors (MSE) and Roots Mean Square Errors (RMSE) values from both models were low, indicating a good fit. For the Baranyi model, values ranged from 0.03 to 0.25 and for the Huang model from 0.03 to 0.26. Significantly higher MSE values ($p<0.05$) in both models were obtained for modeling *S. Typhimurium* at 10 mM. RMSE values were lower than reported by Singh et al. 2011, who observed calculated values between 0.47 and 1.02 when modeling the Salmonellae using the Baranyi model at various temperatures.

Models yielding smaller AIC values are more likely to be correct (Motulsky and Christopoulos 2004). AIC values for the Baranyi model ranged from -29.15 to -2.98 and the Huang model from -21.16 to -2.56. Li et al. 2013 found acceptable AIC values for both models while studying behavior of Lactic Acid Bacteria. Juneja et al. (2009) when comparing the Baranyi and Huang models on the means of AIC, found no difference on the model's performance.

An acceptable range of BF of 0.7–1.15 and AF range of 0.9–1.05 is considered good (Ross 1996). Under all the experiments both models had a good BF and AF, except for the modeling of *L. monocytogenes* at 10 mM using the Baranyi model, which showed only a marginally acceptable AF. However, BF and AF indices should not be used as a statistical comparison between predictive models, since these indicators may not present statistical comparison between the performances of different models for the same set of observed data but are based instead on the deviation between observed and mean response (Pal et al. 2008; te Giffel and Zwietering 1999).

The APZ analysis showed that all predictions for *E.coli* O157:H7 from both models fell within the APZ limits set of −1.0 and 0.5 Log CFU ml-1 (Figure 2.11). Whereas 10% of the Baranyi model predictions for *L. monocytogenes* fell outside the APZ and only 2.6% of predictions for *L. monocytogenes* from the Huang model fell outside the APZ (Figure 2.12). All predictions from for *S. Typhimurium* from the Baranyi model fell within the APZ and 2.6% from the Huang model fell outside (Figure 2.13).

2.4. Conclusion

The growth of three foodborne pathogens were fitted using the Baranyi and Huang models in minimal media under room temperature. Overall, nutrient availability influenced the growth rate and lag phase duration; at 10 mM of glucose growth rate was higher and lag phase longer compared to 5 mM and 15 mM of glucose. Both models performed well as indicating by the MSE, RMSE and AIC. The Baranyi model consistently estimated longer lag phases and higher growth rates than the Huang model, however predictions from the Huang model appeared to be more accurate. The Baranyi model was better at determining differences in kinetic parameters between nutrient concentrations, while the Huang model determined differences in parameters between bacteria. These results provide an insight into modeling growth of pathogens as a function of time and nutrient concentration and may help to choose between the Baranyi or Huang models when determining the best-fitting model. Further studies may use the kinetic parameters estimated here to develop secondary and tertiary models in other food systems.

Table 2.4. Mean Square Error (MSE), Root Mean Square Error (RMSE) and Akaike information criterion (AIC) values and the analysis of statistics.

Statistic	Bacteria	TRT	Model		Mean[1]
			Baranyi-Roberts	Huang	
MSE	*E.coli* O157:H7	5 mM	0.03	0.03	0.03 ± 0.01b
		10 mM	0.13	0.15	0.14 ± 0.10ab
		15 mM	0.07	0.06	0.07 ± 0.02ab
	Listeria monocytogenes	5 mM	0.04	0.05	0.04 ± 0.03b
		10 mM	0.04	0.05	0.04 ± 0.02ab
		15 mM	0.09	0.09	0.09 ± 0.06ab
	Salmonella Typhimurium	5 mM	0.03	0.03	0.03 ± 0.02b
		10 mM	0.25	0.26	0.26 ± 0.24a
		15 mM	0.06	0.05	0.06 ± 0.06ab
RMSE	*E.coli* O157:H7	5 mM	0.16	0.16	0.16 ± 0.02
		10 mM	0.32	0.38	0.35 ± 0.16
		15 mM	0.28	0.24	0.26 ± 0.03
	Listeria monocytogenes	5 mM	0.20	0.20	0.20 ± 0.08
		10 mM	0.22	0.21	0.21 ± 0.05
		15 mM	0.27	0.29	0.28 ± 0.11
	Salmonella Typhimurium	5 mM	0.14	0.18	0.16 ± 0.08
		10 mM	0.43	0.44	0.44 ± 0.28
		15 mM	0.21	0.20	0.21 ± 0.12
AIC	*E.coli* O157:H7	5 mM	-23.44	-21.16	-22.30 ± 6.23b
		10 mM	-5.90	-11.04	-8.47 ± 7.80ab
		15 mM	-12.86	-14.07	-13.46 ± 6.12ab
	Listeria monocytogenes	5 mM	-14.68	-15.06	-14.87 ± 4.95ab
		10 mM	-15.98	-11.92	-14.36 ± 13.30ab
		15 mM	-15.44	-8.09	-12.50 ± 9.54ab
	Salmonella Typhimurium	5 mM	-29.15	-20.10	-24.62 ± 12.72b
		10 mM	-2.98	-2.56	-2.77 ± 8.45a
		15 mM	-16.56	-18.08	-17.32 ± 7.18ab

[1]Different letters indicate means are significantly different ($p<0.05$) within each statistic.

Table 2.5. Bias Factor and Accuracy Factor for the Baranyi and Huang models

Bacteria	Glucose (Mm)	Bias Factor		Accuracy Factor	
		Baranyi-Roberts	Huang	Baranyi-Roberts	Huang
E.coli O157:H7	0	N.D.[1]	N.D.	N.D.	N.D.
	5	0.999	0.999	1.012	1.009
	10	1.004	1.003	1.030	1.026
	15	1.002	1.001	1.013	1.011
Listeria monocytogenes	0	N.D.	N.D.	N.D.	N.D.
	5	1.003	1.003	1.020	1.025
	10	0.986	1.000	1.059	1.029
	15	1.000	1.000	1.026	1.028
Salmonella typhimurium	0	N.D.	N.D.	N.D.	N.D.
	5	1.002	1.002	1.008	1.008
	10	1.002	1.003	1.032	1.027
	15	1.000	1.000	1.019	1.015

[1] N.D. = No Data

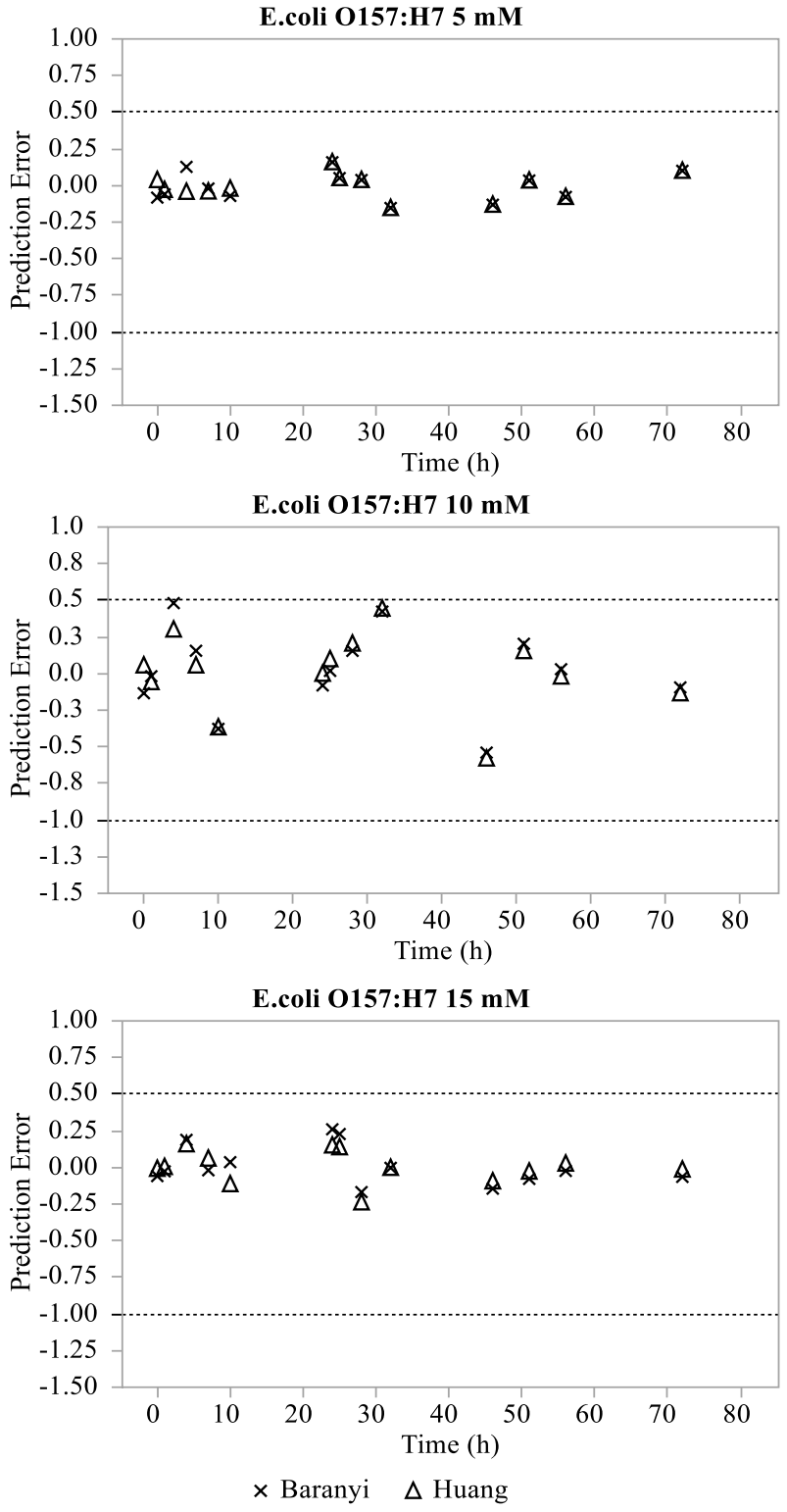

Figure 3.11. Acceptable prediction zone analysis (APZ) of the goodness of fit for the Baranyi (x) and Huang (Δ) models for *E.coli* O157:H7. APZ is indicated by dot lines.

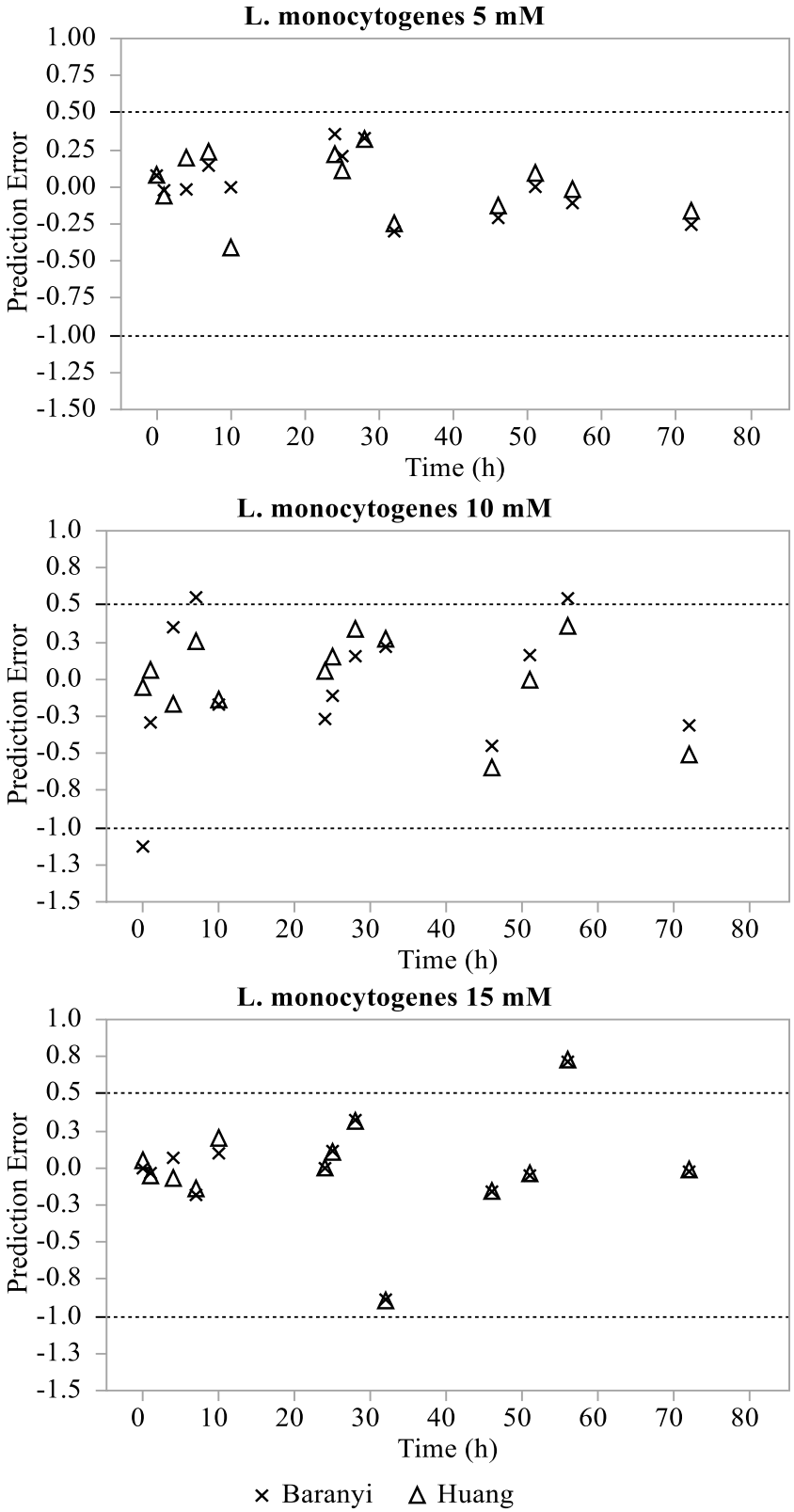

Figure 3.12. Acceptable prediction zone analysis (APZ) of the goodness of fit of the Baranyi (x) and Huang (Δ) models for *L. monocytogenes*. APZ is indicated by dot lines.

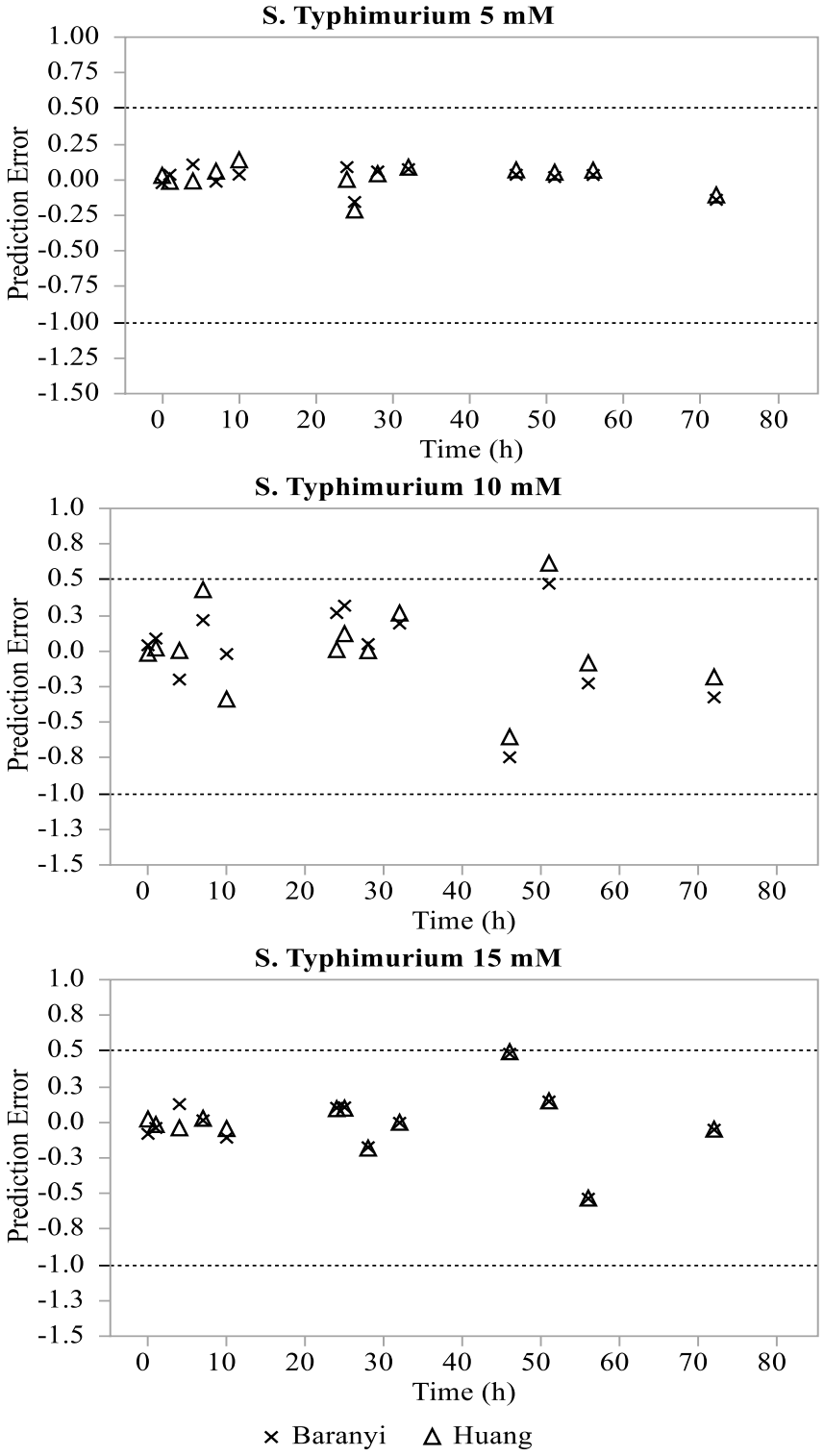

Figure 3.13. Acceptable prediction zone analysis (APZ) of the goodness of fit for the Baranyi (x) and Huang (Δ) models for *S. Typhimurium*. APZ is indicated by dot lines.

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Vita

Jose Isidro Fuentes Rosales was born on October 10 of 1993 in Tela, Honduras. He is the middle child of Jose Isidro Fuentes Rivera and Olga Marina Rosales. In December 2015 he obtained his B.Sc. in Food Science and Technology from Zamorano University in Tegucigalpa, Honduras. His graduation project in Zamorano University focused on identifying nutritional deficiencies in the elderly living in rural communities with limited income. In 2015, he worked as a Visiting Scholar at Texas Tech University in Lubbock, Texas where he investigated the prevalence of pathogens in lamb meat to assess risks of cross-contamination. In 2016, he worked for the Department of Social Development in Honduras where he helped households living in poverty by training them in basic financial management in order to gain access to special financial aid. In 2016 he moved to the U.S. to work in the Food Safety and Microbiology lab at Louisiana State University (LSU) under Dr. Marlene Jane's mentorship. His research focused on predictive microbiology and food safety. At LSU he also worked helping small and middle-size food companies to obtain federal and state approval for their food products. During the summer of 2019, he worked as a Product Design and Food Safety Intern at Eurofins Sensory, Consumer and Product Research in Ithaca, New York. He obtained his M.Sc. in Food Science and Technology from LSU in December 2019.