Chapter 14
Microbial Growth Models

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14.1 Introduction

Foodborne illness, or foodborne disease, is a growing public health issue around the world, primarily resulting from contaminated or toxic food. In the United States alone, it was estimated that 48 million cases of foodborne diseases occurred in 2016 and approximately 128,000 people were hospitalized, and 3000 people died from the ingestion of contaminated food, in the same year (FDA 2018). The most common foodborne pathogens, including *Salmonella serotypes*, *Staphylococcus aureus*, *Campylobacter coli*, *Escherichia coli* O157:H7, *Bacillus cereus*, and *Listeria monocytogenes*, frequently cause illness in the United States and all over the world (FSIS 2018). Therefore, it is of greatest importance to examine food raw or ready-to-eat materials or final products for the existence of pathogenic bacteria and their growth during the storage. In general, conventional detection methods including traditional microculture, molecular biology, immunological, and metabonomic methods, etc. are extensively employed to test food products' safety to ensure the public human health and reduce the risk of infection by pathogens (Cho and Ku 2017).

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A. Demirci et al. (eds.), *Food Safety Engineering*, Food Engineering Series,
https://doi.org/10.1007/978-3-030-42660-6_14

Studying microbial physiologies and predicting their behavior under different circumstances are a constant need for securing microbial food safety (Bazin 2018). The demand for microbial growth modeling began to be realized with the critique that food quality control based on the challenge tests of the final products are expensive, laborious, and time-consuming (Baranyi and Pin 2001; Ross and Mcmeekin 2003). As early as 1980s, microbial growth modeling was reported as an interdisciplinary research area that combined microbiology, statistics, mathematics, and computer science, either in food safety or microbial natural habitats in ecosystems or in bioprocessing applications, where microbial growth is beneficial for productions of the value-added products (Widder et al. 2016; Zwietering et al. 1990; Mitchell et al. 2004).The risks of foodborne pathogens are extremely vast. Even astronauts have not been safe from these risks as the pathogens may find their way into the space stations and threaten the health of astronauts, and thus there is a definite need to experimentally study and mathematically model the growth of these pathogens in such peculiar environments (Van Houdt et al. 2018). Fundamental principles and methods from aforementioned fields are commonly employed to describe and predict microbial growth in specific foods under defined conditions (Baranyi and Pin 2001; Esser et al. 2015). Additionally, these growth models have also been used to predict the shelf life and assess risks in food safety programs such as Hazard Analysis and Critical Control Point (HACCP) and Quantitative Microbial Risk Assessment (QMRA). Furthermore, ComBase Predictor (CDPM 2018) serves as a repository for data to estimate microbial growth in different food environments, and helps to define data gaps, and standardize the work and results of different risk assessors, which plays a significant role in international trade (Baranyi and Tamplin 2004).

Therefore, microbial growth models are widely used as tools for process optimization in food safety control systems (Skinner et al. 1994). In this chapter, it was aimed to summarize and provide an update for existing microbial growth models, including primary predictive models, secondary predictive models, and tertiary models. In addition, some representative models are described in detail covering basic assumptions, limitations, a summary of parameters, possible enhancements, and the needed improvement. The reason for this is that complete framework and knowledge of microbial growth models can assist research or modify existing models; meanwhile, more typical models can be employed in food safety engineering for enhancing public health.

14.2 Compilation of Current Literature

Compared to large animals, microorganisms have a high rate of growth and reproduction. Depicting, understanding, and predicting microbial growth is of great concern for food safety engineering (Esser et al. 2015). To perform assessment studies, different models of microbial growth have been proposed, which can be

classified by a systematic analysis of their final purpose, the types of microorganism, and their impact on food spoilage or food safety (Pérez-Rodríguez and Valero 2013; Whiting 1995).

Standard terminology and classification of models with specific functions make predictive models more precise and simpler to use (Baranyi and Roberts 1992). Several different model classification schemes related to microbial growth models have been used in food safety research, including empirical, mechanistic, and kinetic and probabilistic models. Notably, the classification method proposed by Whiting and Buchanan (1993) is often used that groups most model types into primary, secondary, and tertiary models (Table 14.1):

(i) Primary models: describe the kinetic processes of microbial growth and inactivation phases using only a few parameters and record the increase (or decrease) of population density over time.
(ii) Secondary models: characterize the environmental factors on the parameters of a primary model, such as temperature, moisture, pH, and concentration of preservatives.
(iii) Tertiary models: combine one or more primary and secondary models through computer software and present a model system that establishes a user-friendly interface.

This chapter summarizes several typical sub-models contained in primary, secondary, and tertiary models, and introduces the microorganisms, materials, conditions, verification, validation, advantages and disadvantages of each model.

Table 14.1 Classification of microbial growth model

Primary models	Secondary models	Tertiary models
Gompertz model Jefferies and Brain (1984)	ANNs (artificial neural networks) Gruenreich (1995)	Pathogen Modelling Programme Buchanan (2010)
Logistic model Jason (1983)	Bayesian network models Adcock (2010)	Food MicroModel McClure et al. (1994)
The Rosso model Rosso et al. (1993)	The square root model Ratkowsky et al. (1982)	Growth Predictor Baranyi et al. (1999)
Baranyi and Roberts model Baranyi et al. (1993)	Response surface model (polynomial model) Draper (2006)	Pseudomonas Predictor Neumeyer et al. (1997)
Monod model Monod (1949)	Arrhenius model Labuza and Riboh (1982)	ComBase Baranyi and Tamplin (2004)
Compartmental model Vanier and Bower (1999)		Sym'Previus Leporq et al. (2005)
Weibull model Farewell (1982)		IPMP 2013 Huang (2014)

14.2.1 Primary Models

The kinetic parameters related to primary models have been developed for predicting the growth of microorganisms on food, including environmental factors, food ingredients, and the growth stage of microorganisms. Primary models predominantly estimate the changes in population density versus time during the lag phase, exponential phase, stationary phase, and death phase (Oscar 2005; Ross and Mcmeekin 2003) (Fig. 14.1).

Primary models and their modifications were developed using different theoretical bases and hypotheses (Table 14.1). For instance, the Baranyi and Roberts model assumes that during the lag phase, bacteria need to synthesize substrate(s) for further growth (Baranyi et al., 1993; Bursova et al. 2017; Kowalik and Lobacz 2015), Weibull model (Eq. 14.1) assumes that every microorganism has its own resistance to a lethal agent; as a simple model, bacteria can be divided into two sub-populations: growing or non-growing (Farewell 1982; Mishra and Puri 2013; Ngnitcho et al. 2018).

$$\lg N = \lg N_0 - \left(\frac{t}{\delta}\right)^p \tag{14.1}$$

where N_0 is the initial number of the microbial population; N represents the number of microorganisms that survived after the different treatments have been applied; t is the treatment time; δ is the characteristic time scale parameter, and p is the dimensionless shape parameter. When p is less than 1, the survivor curve displays upward concavity; when p is greater than 1, the survivor curve possessed a downward concavity; and when p equals 1, it represents a linear curve.

Monod model (Fig. 14.2 and Eq. 14.2) can be used under the condition that microbes grow in limited nutrient(s) (Koch et al. 1998; Monod 1949).

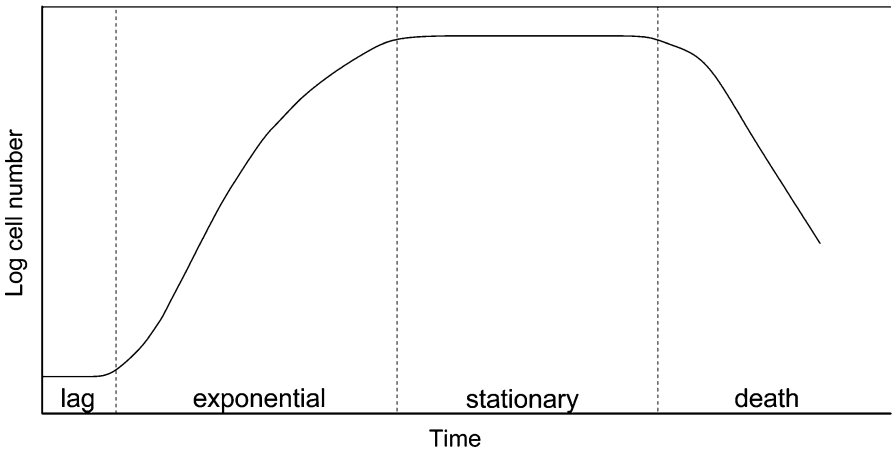

Fig. 14.1 Four-phase kinetics in a microbial growth curve

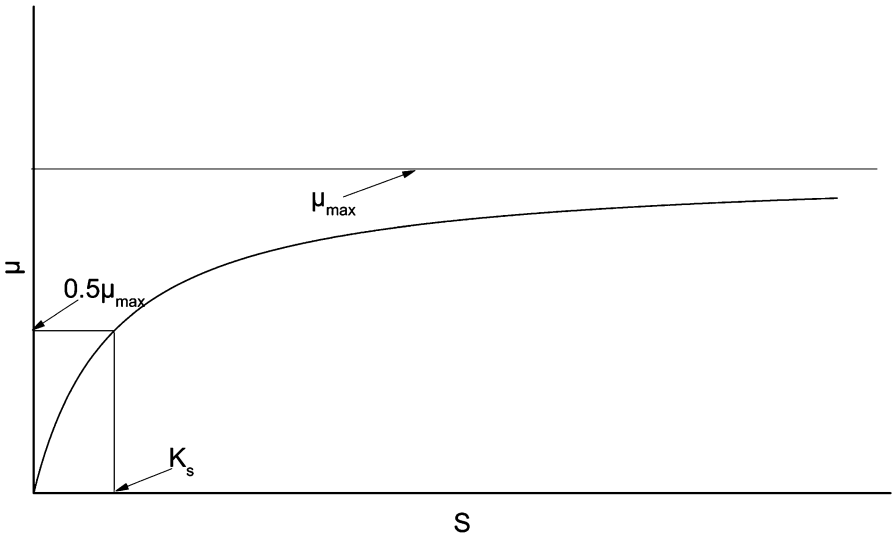

Fig. 14.2 The Monod model illustration

$$\mu = \mu_{\max} \frac{S}{K_s + S} \tag{14.2}$$

where μ is the specific growth rate; μ_{max} is the maximum specific growth rate;S is the concentration of the limiting substrate for growth; and K_s is the half-velocity constant, the value of S when $\mu/\mu_{max} = 0.5$.

Sometimes, researchers start with statistical models to first identify the effective factors on microbial growth and then use these effects and outcomes of the statistical models in primary mathematical models to better picture the effects (Carrascosa et al. 2014, 2016). Furthermore, each primary model has its own specifications and advantages in different applications. For example, the Logistic model is perhaps the simplest primary model and thus is most convenient and therefore preferable to use in most occasions; or the Weibullian model is best to fit in non-linear behaviors (Franco-Vega et al. 2015). The most important and typical primary models are presented in subsequent subsections.

14.2.1.1 Logistic Model

The logistic function model (Fig. 14.3 and Eq. 14.3), a common sigmoid curve first proposed in 1845 (Verhulst 1845), is increasingly used to describe microbial growth as a function of initial microbial density, time, growth rate, and final microbial density (Volterra 1928; Wachenheim et al. 2003). Subsequently, it was applied to food, ecology, demography, biology, medicine applications for predicting the growth of microorganisms, tumors, animals or plants, as well as in economy for

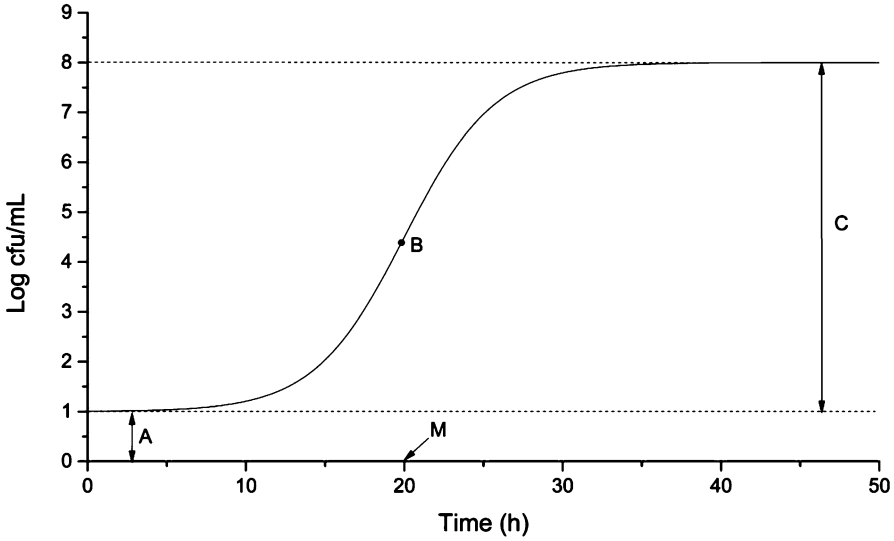

Fig. 14.3 The modified logistic function – A typical illustration

the illustration of how innovation spreads (Giovanis and Skiadas 2007; Román-Román and Torres-Ruiz 2012; Tsoularis and Wallace 2002).

$$y(t) = A + \frac{C}{1 + \exp\left[-B(t - M)\right]} \tag{14.3}$$

where y(t) is the cell concentration at time t; A is the lower asymptotic line of the growth curve as t decreases to zero (initial population level, N_0); C is the difference between the upper asymptotic line of the growth curve (maximum population level, N_{max}) minus the lower asymptotic line; B is the relative maximum growth rate at time M; and M is the time at which the growth rate is maximum.

Numerous modifications of the logistic model have been extensively employed to describe microbial growth in food systems. For example, a log-logistic model was employed to predict the survival of *Y. enterocolitica* and achieved an excellent agreement with the observed survival behavior in mayonnaise and milk (Little et al. 1994; Stern et al. 2010). Similarly, a log-logistic model was proposed for the deli meat industry to select optimum processing conditions of near infrared (NIR) heating through investigation of inactivation kinetics of *E. coli* O157:H7, *S.* Typhimurium, and *L. monocytogenes* in ready-to-eat sliced ham (Ha and Kang 2014). With the improved logistic models, the growths of *E. coli*, *S. aureus*, *V. parahaemolyticus*, and *P. fluorescens* at various temperatures in food have been researched, which is becoming a prototype of an alert system for microbial food safety (Fujikawa 2011; Fujikawa et al. 2004; Fujikawa et al. 2009; Kahraman et al. 2016; Walter et al. 2016). *L. monocytogenes* as a significant food-borne pathogen has a high mortality rate among the high-risk populations (Kuan et al. 2017). Several

models for *L. monocytogenes* growth have been developed with the logistic function as their basis (Fang et al. 2013; Hassan et al. 2001; Pal et al. 2008). For example, a molecular predictive model was developed for rapid detection of *L. monocytogenes* growth in vacuum-packaged chilled pork through appropriate real-time polymerase chain reaction (PCR) detection technology (Ye et al. 2013). Similar to *L. monocytogenes*, many logistic models concentrated on *C. perfringens* which as an anaerobic Gram-positive pathogen has a history of a serious threat to human health (Corradini et al. 2006; Dors et al. 2016; Huang et al. 2017; Juneja et al. 2001). For instance, a probability model was developed to define the threshold of *C. perfringens* growth and was validated using experiment data, suggesting that the combination of sodium tripolyphosphate (STPP), sodium lactate (NaLA),and sodium chloride (NaCl) could prevent microbial growth in meat and poultry and thus food poisoning outbreaks (Huang et al. 2017). In addition, controlling microbial quality of food plays a critical role in proper sensory quality and food safety. A logical background-dependent non-dimensional model was provided to estimate aerobic bacterial growth in pan-fried meat patties at various temperatures and was verified by experimental data (Sojung and Dongsun 2015).

14.2.1.2 Gompertz Model

In recent years, the Gompertz equation (Fig. 14.4) for modelling the asymmetrical sigmoid shape of microbial growth curves has been widely and successfully used to describe and predict nonlinear responses, which was originally employed in humans to record the mortality (Jefferies and Brain 1984). Gibson et al. (1987) first modified

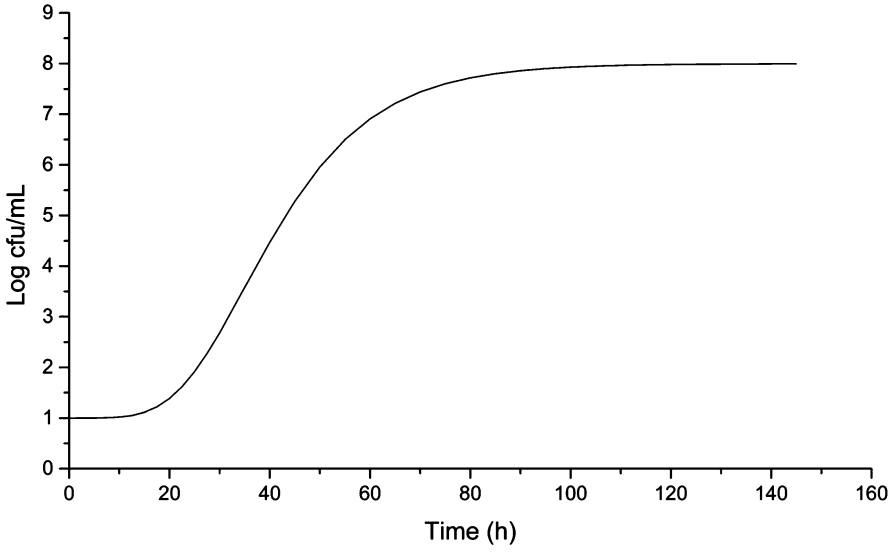

Fig. 14.4 The modified Gompertz model – A typical illustration

the Gompertz model to fit the growth curve of *C. botulinum* in pork in the presence of natural spoilage organisms, and calculated the lag times, growth rates, generation times, and time to maximum growth rates.

To some extent, microbial growth data are not sufficiently accurately described by the standard Gompertz model due to the fixed values of reliability at the inflection points (Kececioglu et al. 1994). To evaluate the accuracy, the Gompertz model was often used with some modifications such as those by Zwietering et al. (1991), e.g., *S. aureus* growth in Feta cheese, mold growth in long-grain rough rice during storage, *P. fluorescens* in fresh meat in different temperatures and pH, *Salmonella spp.* in processed meat products and microbial inactivation with high-pressure processing (Atungulu et al. 2016; Gonçalves et al. 2017; Jimyeong et al. 2016; Serment-Moreno et al. 2017; Zhu et al. 2011). Specifically, the microbial (aerobic plate counts, total coliforms, and lactic acid bacteria) growth in salted cabbages at different temperatures was investigated, and a modified Gompertz model was developed to determine the shelf-life, which provided proper guidance for food quality control (Kim et al. 2018). In addition, the effect of silver nanoparticles on the growth kinetics of *E. coli* and *S. aureus*, was evaluated by a modified Gompertz model, and it was found that the modified Gompertz model (Fig. 14.4 and Eq. 14.4), incorporating cell death, was useful for microbial growth kinetics research under the influence of antimicrobial agents (Chatterjee et al. 2015).

$$y(t) = y_0 + C * \exp\left\{-\exp\left\{\left[(2.7182 * \mu_{\max}) * \frac{LPD - t}{C}\right] + 1\right\}\right\} \qquad (14.4)$$

where y(t) is the cell concentration at time t; C is the asymptotic increase in population density; μ_{max} is the maximum specific growth rate; LPD is the lag phase duration; and t is the storage time.

14.2.1.3 Baranyi and Roberts Model

Besides the logistic and Gompertz models, a semi-mechanistic biologically-based growth model was developed by Baranyi and Roberts to describe microbial growth under dynamic time-varying temperature conditions. The empirical primary models were developed at isothermal conditions, in which the physiological state of the microorganism is represented by a single variable, and during lag phase bacteria need to synthesize an unknown substrate (Baranyi and Roberts 1994; Baranyi et al., 1993; Gospavic et al. 2008).

Yersinia enterocolitica, as a foodborne pathogen, which can cause acute intestinal tract diseases in humans, is easily observed in foods during production and storage (Stern and Pierson 2010). Accordingly, a large number of Baranyi and Roberts models were developed to model growth of *Y. enterocolitica* (Divya and Varadaraj 2015; Geeraerd et al. 2000; Sarka et al. 2017). For detailed examination, a model was developed to investigate the behavior of *Y. enterocolitica* in Camembert cheese under refrigerated conditions, serving for the consumers who are interested in using

cheese to prepare salads and sandwiches (Kowalik and Lobacz 2015). Since the storage temperature cannot prevent the proliferation of *Y. enterocolitica*, the growth dynamics of *Y. enterocolitica* during storage temperatures (8 °C and 24 °C) were studied with a modified Baranyi and Roberts model for assessing the potential risk to consumers (Bursova et al. 2017). In addition, Baranyi and Roberts model was also employed to estimate *L. monocytogenes* growth in fresh-cut romaine lettuce (Alavi et al. 2001), cantaloupe and sterilized whole milk (Guzel et al. 2017) and *S.* Enteritidis growth in chicken juice (Noviyanti et al. 2018), leading to useful risk assessment methods. Most recently, a novel rearrangement of the Baranyi and Roberts model was used to fit the growth of *E. coli* and *S.* Typhimurium under mild conditions of temperature (25–37 °C), salt concentration (0.086, 0.51 and 1.03 mol·L^{-1}), and pH (4.5–6.85), which showed a great compatibility with standard data and highly accurate growth rates and lag phase duration (Mytilinaios et al. 2015). To explore the effect of oregano essential oil on the shelf-life of vacuum-packed cooked sliced ham, lactic acid bacteria growth at various temperatures was evaluated. It was concluded that the Baranyi and Roberts model accurately fitted to microbial growth curves with R^2 and RMSE values ($R^2 \geq 0.884$, RMSE $\leq$0.270) better than Gompertz model (Menezes et al. 2018). Similarly, the growth of *Pseudomonas spp.* on sliced mushrooms stored between 4 °C and 28 °C were also fitted to Baranyi and Roberts models with the lowest MSE and highest R^2 compared to the modified Gompertz and logistic models (Tarlak et al. 2018). Overall, Baranyi and Roberts model and its modifications have been widely used in food microbiology, and have become a significantly important member of the most popular models applied in daily life (Acai et al. 2016; Kim et al. 2016; Liu and Puri 2007; Mai and Huynh 2017; Vadasz and Vadasz 2007).

14.2.2 Secondary Models

Secondary models are mainly used to predict how environmental factors (e.g., temperature, moisture, pH, concentration of preservatives and initial bacterial count) affect the parameters (e.g., growth rate and lag time) in primary models. With the advancement of mathematics and computer science, various secondary models, including response surface models, Arrhenius models, and square root models, are established and developed (Table 14.1). In many studies, primary models were first utilized by researchers to investigate the effective factors and then the results were used in suitable secondary models to further investigate the individual effects of every factor (Nyhan et al. 2018; de Oliveira Elias et al. 2018). Three of the most commonly applied secondary models, e.g. artificial neural networks, square root models and response surface methodology models, are introduced below.

14.2.2.1 Artificial Neural Networks

Artificial neural networks (ANNs) are computing systems known as analogous mechanisms of the biological neural networks, relying on a batch of nodes called artificial neurons (Fig. 14.5). Generally, microbial growth, inactivation, and probability of growth under complicated environmental conditions can be predicted and described by ANN models (Najjar et al. 1997; Pérez-Rodríguez and Valero 2013). Since decades ago, ANNs models, as an alternative and powerful technique, present high accuracy and generalization ability in modeling, leading to the extensive application in predicting the non-linear relationship between input (e.g. temperature, pH, and initial bacterial) and output in food microbial systems (Kavuncuoglu et al. 2018; Lou and Nakai 2001a, b; Ozturk et al. 2012; Zheng et al. 2017).

For instance, AANs were applied to predict residual pathogenic bacteria such as coliforms and *E. coli* on tomato fruits and lettuce leaves for more realistically assessing the risk of fresh produce consumption (Keeratipibul et al. 2011). *S.* Typhimurium is a harmful pathogenic bacteria contained in intermediate product or final product during processing and storage; a great many of ANNs are used to control it (Ozturk et al. 2012; Raoufy et al. 2011; Siripatrawan et al. 2006). For example, to extend the shelf-life of surimi, citric acid was used to control *S.* Typhimurium growth, combined with the models of back-propagation ANN and particle swarm optimization-based back-propagation artificial neural network (PSO BP-ANN) for ensuring food safety (Qin et al. 2018). Meanwhile, emphasis was also put on *L. monocytogenes* (Ramosnino et al. 2010; Rebuffo et al. 2006). An

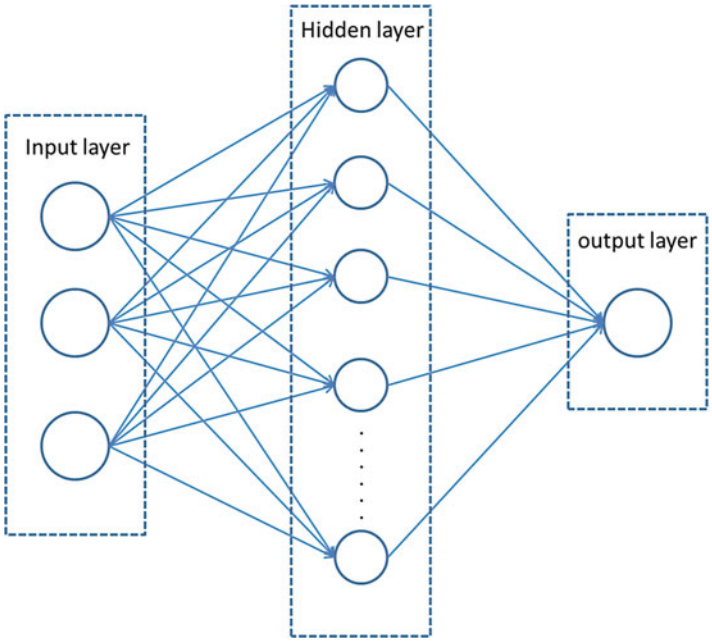

Fig. 14.5 Input, hidden and output layer in ANNs

autoregressive network with an exogenous input (NARX) model was developed to perform real time *E. coli* growth prediction with high accuracy with an emphasis to find hidden neurons and delays selection in the prediction process, which was possible only using ANN (Shamsudin et al. 2017).

Due to the excellent fault tolerance, ANN models are more suitable for modelling complex relationships in uncertainties and variations of conditions in predictive microbiology. However, it is still limited in use because of its complexity and high-cost of learning.

14.2.2.2 The Square Root Model

The square root model was proposed by Ratkowsky et al. (1982), which has been used to describe a linear relationship between the square root of growth rate and temperature. Some commonly used models are also called Ratkowsky models or Huang square root models (Huang et al. 2011).

The combination of Baranyi model with the Ratkowsky square root model has been used to quantify the influence of temperature on the growth of bacteria, such as *B. cereus* and *E. cloacae* in liquid whole egg products (Grijspeerdt and De Reu 2005). *V. vulnificus*is is a Gram-negative bacterium responsible for food-borne illnesses related to the consumption of oysters (Hald et al. 2016). By using a square root model, a predictive model for *V. vulnificus* in postharvest oysters as a function of temperature to minimize the risk against consumers was developed (DaSilva et al. 2012). The *L. monocytogenes* growth in sterilized whole milk for a range of temperature values (4–35 °C) was calculated by the Zwietering square root model with maximum relative error of 10.42% and the RMSE of 0.28 log CFU/ml (Alavi et al. 1999). In addition, the growth parameters of *L. monocytogenes* on vacuum packed sliced Mortadella and the growth and survival models for *S. enterica* and *L. monocytogenes* in leafy greens were modeled by the square root model, as well as the effect of storage temperature on growth rate of *L. monocytogenes* (RMSE = 0.014–0.099) (Bolivar et al. 2018; Daminelli et al. 2014; Mishra et al. 2017). Furthermore, Ratkowsky square root and Huang square root are models widely used to study the effect of temperature on *Salmonella* growth (Fujikawa et al. 2015; Sabike et al. 2015; Sakha and Fujikawa 2012). Fang et al. (2015) showed that the Huang square root model was more applicable to predict the effect of temperature on *Salmonella* growth, while the model of Ratkowsky square root was usually more suitable for the background microorganisms with a wider temperature range.

14.2.2.3 Response Surface Model (Polynomial Model)

Response surface model (RSM) is a mathematical-statistical method established by Box and Wilson (1951) that can be used to research the relationships between one or more response variables and factors (e.g., pH, temperature, pressure, etc.). RSM is a

powerful practical tool widely used in food science and technology, not only in food microbial predictions but in other fields of study (Huang et al. 2016; Mohammadi et al. 2016; Xu et al. 2016). In response surface analysis, a regression equation should be obtained first, and then the optimal value can be obtained by reasonable value of the independent variable (Baş and Boyacı 2007; Bezerra et al. 2008). The regression may be a curve or surface relationship; hence this model is referred to as the response surface model.

Basically, RSM utilizes statistically precision to design experiments in most efficient way in order to minimize the number of experiments required to ensure the desired efficiency. RSM designs are mostly used to optimize procedures where the experiments are costly or time-consuming and thus not easily replicable (Box and Wilson 1951). RSM models use various experimental designs, each with certain advantages, to create such efficiency in the experiments. For instance, the most commonly used design is a Central Composite Design (CCD) where a second order (Full quadratic) model is used based on orthogonality without needing to use a complete three-level factorial experiment, where the number of experiments can be significantly more (Mahdinia et al. 2018a). Furthermore, a Box-Behnken design can be used instead of a CCD and the modeling precision can be preserved (if cross-effects permit) with even fewer number of experiments (Mahdinia et al. 2018b). For example, for three continuous variables and each variable with three levels, a complete three-level variable design requires 27 experiments to cover all combinations whereas, a CCD design comes with 20, and a Box-Behnken design with only 15 (Mahdinia et al. 2018c). Most of the times, prior to an RSM design, there are a number of candidate factors that are hypothesized to affect response(s) and researchers need to screen through them to determine effective ones from ineffective ones. In these situations, researchers use screening methods such as the Plackett-Burman design to reduce the number of experiments and therefore save time and money (Izmirlioglu and Demirci 2015).

The inactivation effect of high-pressure processing in combination with mild heat on *L. monocytogenes*, the influence of UV-C light and trans-cinnamaldehyde on mesophiles and yeasts in grapefruit juice and the effect of electrolyzed oxidizing water based clean-in-place technique for cleaning milking system inoculated by four common microbial in milk were modeled by RSM (Ates et al. 2016; Dev et al. 2014; Ochoa-Velasco et al. 2018).

The RSM makes it possible to understand the interactions between experimental variables, and helps to determine and adjust operating conditions in the parametric amplification process in food microbiology (Buchanan and Bagi 1994; Han et al. 2001; Jha et al. 2017; Krishnamurthy et al. 2008; Yoon et al. 2014); however, optimization is often a compromise among variables since one response variable impacts the other variables (Pinzi et al. 2010). Obviously, the application of RSM is not just limited to food technologies or food safety. These days, RSM designs are also applied in fermentation technologies and synthetic biology, even in human psychology (Berenjian et al. 2011; Izmirlioglu and Demirci 2016; Coban and Demirci 2014; Ercan and Demirci 2014; Mahdinia et al. 2017a, 2019c).

14.2.3 Tertiary Models

The tertiary models are powerful, user-friendly microbiological prediction tools that include one or more primary and secondary models. Based on the informative database, the effects of different conditions on microorganisms can be expediently calculated and compared, and the inaccuracy of predictions in microbiology can be reduced. Scientific research institutions can not only input their own data, but also exchange data with other institutions.

14.2.3.1 ComBase (The Combined Database for Predictive Microbiology)

A large amount of data on the effects of various factors on microorganism lays the foundation of microbial predictive model packages, such as the Pathogen Modeling Program (PMP) and the former Food MicroModel (FMM), which was gradually replaced by the ComBase (Baranyi and Tamplin 2004; Koseki 2009; Mcmeekin et al. 2006). ComBase was established by the University of Tasmania and the United States Department of Agriculture, Agricultural Research Service (USDA-ARS), encompassing ComBase database and ComBase models. The goal is to help companies reduce the investment of time and money in testing, and contains over 60,000 growing records, for describing how environmental factors (e.g. temperature, pH, a_w, preservatives, and atmosphere) affect the food microbial growth (CDPM 2018).

A large number of researchers use ComBase individually or combined with other tertiary models (Aaslyng et al. 2014; Doona et al. 2005; Kapetanakou et al. 2017; Madden et al. 2017; Marc et al. 2005; Garre et al. 2017). For instance, a multiple food predictive model systems was developed to study the effect of food (micro) structure on microbial dynamics via ComBase, and further compared the results with the maximum specific growth rate values of *S. Typhimurium* and *S. aureus* estimated by the Baranyi and Roberts model at different temperature (4, 8 and 12 °C) in fish products (Baka et al. 2017). Meanwhile, the growth rate and lag-phase of *S. aureus* in fresh Minas cheese from Brazil at different pH (5.0, 5.5 and 6.5), salt concentrations (1.1, 2.1, and 4.5%) and temperatures (7.5, 10, 12.5, 15 and 17 °C) were evaluated by PMP and ComBase for improving the risk assessment in food security (Nunes and Caldas 2017). Additionally, Lobacz et al. (2013) modified the Gompertz and Ratkowsky square root models used to predict and validate the *L. monocytogenes* growth during the ripening and cold storage in mold-ripened cheeses. The results were compared with PMP and ComBase, which offered a typical example for the extensive use and applications of microbial predictive models in the food processing industry.

14.2.3.2 Integrated Pathogen Modelling Program

The Integrated Pathogen Modeling Model (IPMP 2013) was developed by USDA-ARS (USDA 2018). Though most researchers prefer to use MATLAB, SPSS, or R to analyze data, it is rather difficult to master them for common users without programming knowledge. Fortunately, IPMP 2013 is a free program convenient for researchers to analyze microbial data and develop the knowledge of predictive microbiology, where the logistic model, Baranyi model, re-parameterized Gompertz model, Weibull model, Ratkowsky square root model, Huang square root model, and Arrhenius model are included (Huang 2014).

The growth of *S. aureus* under various storage temperatures (10, 15, and 25 °C) in raw pork was predicted via IPMP. Based on a comparative study, the re-parameterized Gompertz model was assessed as the most accurate model at 10 and 15 °C, and the Baranyi model at 25 °C, in which the critical control points for storage temperature in the HACCP can be set up to improve product safety for meat (Lee et al. 2015). In addition, the growth of *C. botulinum* in ground beef under different temperature conditions under anaerobic conditions were also analyzed by IPMP with the Huang model and cardinal parameters model, validated by Laplace distribution showing a high accuracy (60% of the residual errors are ±0.5 log CFU/g) (Huang 2018). Similarly, the specific growth rates, lag times, and minimum temperature for growth of nonpathogenic *E. coli* at different incubation temperatures (10, 15, 22, and 30 °C) in ground chicken meat was analyzed by IPMP with the Huang primary and secondary square root models. Approximately 83.9% of the residual errors of ±0.5 log CFU/g suggested the accuracy in predicting the growth of uropathogenic *E. coli*. (Sommers et al. 2018).

14.2.4 Summary of Predictive Models

Predictive models in food safety engineering describe not only the growth but the survival or inactivation of microorganisms in foods under various conditions, giving the opportunity to minimize the risk of pathogenic outbreaks (Ross and Mcmeekin 2003). While microbial growth modeling is not limited to food safety applications, perhaps the vast number of applications are in the food industry (Mitchell et al. 2004). On the basis of their structure, the models and their modifications are introduced in three categories; i.e. primary, secondary, and tertiary models. Logistic and Gompertz models tend to fit isothermal growth curves with 3–4 intuitive parameters, such as the maximum growth rate and the asymptotic population size (Esser et al. 2015). Different from logistic and Gompertz models, the Baranyi and Roberts model is a mechanistic model. Due to the advantages (i.e., accurate, simple and practical), the Baranyi and Roberts model has become the most used primary model in food microbial prediction. Furthermore, the parameters in the model have physiological significance (Baranyi and Roberts 1994; Mytilinaios et al. 2012).

Secondary models aim to describe the microbial growth and the effect of external factors such as temperature and pH; serving as prediction tools for risk assessment in foods. Typically, the square root model describes a linear relationship between the square root of growth rate and temperature (Ratkowsky et al. 1982). Because of the fewer parameters, user-friendly processing and accurate prediction, many researchers tend to use this model in food research. Even so, the efforts of improvement are always continuing. Huang et al. (2011) reported a new secondary square root model, which can accurately estimate the minimum and maximum growth temperatures of bacteria. With the significant progress made in the past few decades in ANNs, our understanding of interacting parameters has been considerably enhanced. Compared with traditional models, ANNs often show better characteristics in regard to food microbial prediction and parameter optimization; moreover, the accuracy of prediction by ANNs can be further improved by algorithm optimization.

Tertiary mathematical models such as ComeBase and IPMP 2013 are derived from the primary or secondary models or their combinations. The IPMP 2013 is one of latest predictive microbiology tools (Huang 2014). It offers a user-friendly interface with high-accuracy in microbial prediction. Like other tertiary models, the IPMP 2013 has been used for predictive microbial data and to develop predictive models.

In summary, all of the models mentioned are very practical and significant in food safety engineering. To make them more user-friendly for novice modelers, the typical Baranyi and Roberts model, square root model, RSM, and ANNs, and the software IPMP 2013 are selected for further detailed description and comparison in the next section.

14.3 Examples of Specific Growth Models

14.3.1 Baranyi and Roberts Model

14.3.1.1 Basic Assumptions

Baranyi and Roberts model (Baranyi and Roberts 1994; Baranyi et al. 1993a, b) is a typical semi-mechanistic growth model for the microorganism's growth. The lag time is determined by the initial variable value at inoculation and post-inoculation. With the standardized cultivation methods, the growth state of microorganism including the lag parameter and maximum specific growth rate of microorganisms are relatively constant and independent on the subsequent growth conditions. Srivastava and Volesky (1990) proposed that the microorganisms do not grow under the conditions when the bottleneck-substance titer is lower than the minimum level, and the accumulation rate changes with temperature. Combining previous studies (Baranyi et al. 1993a, b, 1995) with the aforementioned theory, the model successfully predicted the growth of *Brochothrix thermosphacta* at temperatures ranging from 5 °C to 25 °C.

Below are the equations of the Baranyi and Roberts model, and the entire derivation of the model is available in Baranyi and Roberts (1994) and Baranyi et al. (1995).

$$\begin{aligned}\frac{d}{dt}q &= vq \\ \frac{d}{dt}x &= \mu_{\max}\frac{q}{1+q}\left(1-\frac{x}{x_{\max}}\right)x\end{aligned} \tag{14.5}$$

The variable y(t) denotes the natural logarithm of the cell concentration x(t). The solution of the above differential equations is:

$$y(t) = y_0 + \mu_{\max}A(t) - \ln\left(1+\frac{e^{\mu_{\max}A(t)}-1}{e^{y_{\max}-y_0}}\right) \tag{14.6}$$

where $y_0 = \text{In } x_0$, $y_{\max} = \text{In } x_{\max}$

$$\begin{aligned}A(t) &= t + \frac{1}{v}\ln\left(e^{-vt}+e^{-h_0}-e^{-vt-h_0}\right) \\ h_0 &= -\ln\left(\frac{q_0}{1+q_0}\right) = -\ln(\alpha_0) = \mu_{\max}\lambda\end{aligned} \tag{14.7}$$

where q is a measure of the initial state of cells, μ_{max} is maximum specific growth rate, v is the rate of increase of the limiting substrate, assumed to be equal to μ_{max}, y (t) is the cell concentration at time t, y_{max} is maximum cell concentration, λ is lag-phase duration.

14.3.1.2 Limitations and Possible Enhancements

There are some shortcomings and limitations in the Baranyi and Roberts model, even though it has been extensively used in various microorganisms and environments of the food safety engineering (Alavi et al. 2001; Bursova et al. 2017; Liu and Puri 2007; Lobete et al. 2017; Longhi et al. 2016; Tarlak et al. 2018).

Michaelis-Menten constant (K_p), assumed to be independent of actual environment (E_2), is one of the most important assumptions of the Baranyi and Roberts model. Based on that, the equation of $q(t) = P(t)/ K_p$ was applied to predict the physiological state of the microorganism by only one variable, which appears to be an oversimplification (Baranyi and Roberts 1994; Li et al. 2007). Additionally, the assumption of q_0 is a constant is only suitable for the positive temperature changes, and q_0 actually decreases with the reduction of incubation temperatures (Alavi et al. 1999; Swinnen et al. 2004; Yilmaz 2011).

For nearly two decades, great efforts had been made to enhance the Baranyi and Roberts model (Mytilinaios et al. 2015). Under some circumstances, the

non-autonomous form of the Baranyi and Roberts model may impede drawing accurate conclusions. Vadasz and Vadasz (2007) developed a more biologically meaningful autonomous version of Baranyi and Roberts model to keep the accuracy, leading to a meaningful interpretation for the physiological state of the cells after inoculation.

The modification of the model has been of urgent concern since the traditional models cannot fit the real conditions especially in some extreme environments (Julio et al. 2016; Mellefont and Ross 2003; Robinson et al. 1998). For example, Zhou et al. (2011) explored the growth of *Salmonella* Enterica under a range of osmotic stress conditions, critical to the growth or no-growth regions, to propose that microorganism may build a protection against harsh environments. Once the protection reached the minimum level, the microorganisms start growing rather than dying. This suggested that the classical definition of the lag via inoculum level is not suitable, resulting in an extension of the Baranyi and Roberts model. At a constant temperature, the Baranyi and Roberts model can be formulated as a series of coupled equations with analytical solution. However, under dynamic temperature conditions, the equations do not have an analytical solution and were usually solved using the Runge–Kutta method (Gumudavelli et al. 2007; Koseki and Isobe 2005; Singh et al. 2011; Velugoti et al. 2011; Zhu and Chen 2015). With the aim to simplify the Baranyi and Roberts model, Zhu and Chen (2015) derived a numerical equation to estimate model parameters through combining numerical solution with simulated microbial growth data. In addition, these equations can be easily used in computer programs or commercial software.

14.3.1.3 Comparison with Other Models

The Baranyi and Roberts model is well-known and extensively applied in many aspects of biology, and inevitably led to comparisons with Gompertz and Logistic models. Numerous studies suggested that the mechanistic growth models like Baranyi and Roberts model, were more precise than empirical models, such as Gompertz model and Logistic model (Baty et al. 2002; Huang 2008; Li et al. 2014; Longhi et al. 2014; Menezes et al. 2018). But it is not always the case since the Baranyi and Roberts model appears to be more time-consuming than other models in some specific conditions. For example, the growth data of *Staphylococcus aureus* in sandwich fillings at different temperatures was determined by the Gompertz model, Logistic model, and Baranyi and Roberts model. The Gompertz model showed the best performance in coefficient of determination (R^2), the standard deviation (Sy.x), and the Akaike's information criterion (AIC) (Ding et al. 2010). Additionally, the Baranyi and Roberts model, modified Gompertz model, Logistic model and Huang model were used to evaluate the effect of essential oils on the growth of *Salmonella* Typhimurium in rainbow trout stored under aerobic, vacuum and modified atmosphere conditions. Based on comparisons, the empirical models (modified Gompertz model, the Logistic model) were better than the other two mechanistic models (Baranyi and Roberts model and Huang model) (Yilmaz 2011).

14.3.2 Square Root Model

14.3.2.1 Basic Assumptions

Even though Arrhenius equation was modified and applied to describe bacterial growth, the modified law relationship was not suitable for the complex microbial growth processes related to numerous substrates and enzymes, and thus Ratkowsky et al. (1982) proposed a linear relationship between the square root of the growth rate constant (r) and the temperature (T) (Eq. 14.4). Initially, Ota and Hirahara (1977) discovered empirically that a plot of the square root of the rate of nucleotide breakdown in cool-stored carp muscle versus temperature was nearly linear. Unfortunately, there is no theoretical foundation, but it has an excellent fit to the data.

$$\sqrt{r} = b(T - T_0) \tag{14.8}$$

where b is a regression coefficient and T_o is a conceptual temperature-independence of metabolic rate, which is an intrinsic property of the organism. However, at a higher temperature, the previous equation does not work because of the inactivation or denaturation of proteins and other factors . Therefore, Ratkowsky et al. (1983) modified the equation and named the optimized equation as "Ratkowsky Square Root model", suitable for the description of bacterial growth throughout the entire temperature range (Eq. 14.5). Moreover, it fits data well and has meaningful statistical properties, for instance, the least-squares estimators of the parameters were almost unbiased and normally distributed.

$$\sqrt{r} = b(T - T_{\min})\{1 - \exp[c(T - T_{\max})]\} \tag{14.9}$$

where T_{min} and T_{max} are the minimum and maximum temperatures, respectively, at which the growth rate is zero, b is the regression coefficient of the square root of growth rate constant below the optimal temperature and c is an additional parameter to enable the model to fit the data for temperatures above the optimal temperature.

14.3.2.2 Limitations and Possible Enhancements

The Ratkowsky Square Root model is not suitable for predicting positive values of bacterial growth rate if the temperature is above T_{max}. Zwietering et al. (1991) modified the traditional model so that above the maximum growth temperature, T_{max} predicts no positive values of the growth rate (Eq. 14.6).

$$r = [b(T - T_{\min})]^2\{1 - \exp[c(T - T_{\max})]\} \tag{14.10}$$

Considering the temperature and water activity, a_w, and that temperature and pH independently affect microbial growth rate, McMeekin et al. (1992) proposed a

modified Square Root model (Eq. 14.7) to describe the rate in response to a combination of temperature, water activity, and pH values. However, the interactions of a factor are inevitable, e.g., in the case of acid potentiated ions such as nitrite.

$$\sqrt{r} = c\sqrt{(a_w - a_{w,\min})(pH - pH_{\min})}(T - T_{\min}) \tag{14.11}$$

Later, Zwietering et al. (1996) proposed an enhanced version of the Square Root model, which was also named as the Gamma model:

$$r = c(a_w - a_{w,\min})(pH - pH_{\min})(pH_{\max} - pH_{\min})(T - T_{\min})^2 \tag{14.12}$$

where T_{min} is defined as a hypothetical temperature, which is the point at which the line of the square root of growth rates intercepts the temperature axis (Heitzer et al. 1991; Huang et al. 2011; Ratkowsky et al. 2005). Many researches showed that T_{min} estimated by the Square Root model is lower than the true minimum growth temperature (Baranyi et al. 1995; Huang 2011; Juneja et al. 2009; Stannard et al. 1985). Therefore, it is necessary to close the gap between the model-calculated T_{min} and measured T_{min}. Huang (2010) used a Bělehrádek-type model (Eq. 14.9) to develop a nonlinear regression equation to describe the relationship between growth rate of *Escherichia coli* O157:H7 in beef and growth temperature and the results demonstrated that the T_{min} estimated by the new model was better than Ratkowsky Square Root model. However, Ross et al. (2011) disagreed that this new model is more suitable than the traditional Square Root model, so more research is needed in the future to make this model more encompassing of and truer to the real world conditions.

$$r = b(T - T_{\min})^{1.5} \tag{14.13}$$

Furthermore, Huang et al. (2011) developed an updated model covering a wider range of temperatures to describe the growth of *L. monocytogenes* in beef frankfurter and the T_{min} was also closer.

$$r = b(T - T_{\min})^{1.5}\{1 - \exp[c(T - T_{\max})]\} \tag{14.14}$$

However, the Square Root model is relatively weak in predicting the conditions beyond the extreme values of the environmental parameters, which has been viewed as the bottleneck of the square root function.

14.3.2.3 Comparison of the Models

Various square root models and their modifications have been used to predict the microbial growth and were compared with other models. Non-linear Arrhenius model (Schoolfield model) (Eq. 14.11) and Square Root model are available to

describe the effects of temperature and other environmental factors on lag phase duration and growth rate (Ratkowsky et al. 1983; Schoolfield et al. 1981). Additionally, the dependent variables expressed as ln *rate* and sqrt *rate* are involved in Schoolfield model and the Square Root model. However, there are incompatibilities between the aforementioned typical models. Through predicting the effect of temperature on the growth of bacteria in foods, Adair et al. (1989) evaluated the ability of the two models with the mean squared error (MSE) among the observed generation, lag time and the predicted data. Based on their study, they proposed the Schoolfield model was a more reliable description of the experimental data than the Square Root model for the two more parameters involved in the Schoolfield model. While Ratkowsky et al. (1991) held the view that the two models performed almost equally via the MSE criterion with the theoretical foundation and published data. The increased variability of data affects the Schoolfield model much more than the Square Root model because sqrt *rate* is constant, but ln *rate* increases progressively with response time. Therefore, the parameter values can vary widely at low temperatures and long times.

$$r = \frac{\rho\left(25^{\circ}\mathrm{C}\right)\frac{T}{298}\exp\left[\frac{\Delta H_A^{\neq}}{R}\left(\frac{1}{298}-\frac{1}{T}\right)\right]}{1+\exp\left[\frac{\Delta H_L}{R}\left(\frac{1}{T_{1/2L}}-\frac{1}{T}\right)\right]+\exp\left[\frac{\Delta H_H}{R}\left(\frac{1}{T_{1/2H}}-\frac{1}{T}\right)\right]} \tag{14.15}$$

where T is the temperature in Kelvin; R is the gas constant; ρ (25 °C) is a constant; $\Delta H \neq A$ is the heat (enthalpy) of activation of the growth rate-controlling reaction; ΔH_L is enthalpy of low temperature denaturation of the rate-controlling enzyme; ΔH_H is enthalpy of high temperature denaturation of the rate-controlling enzyme; $T_{l/2L} = \Delta H_L/\Delta S_L$ and is the temperature at which half of the population of the rate-controlling enzyme is active and the other half has been inactivated by low temperature; ΔS_L is entropy of low temperature denaturation of the rate-controlling enzyme; $T_{l/2H} = \Delta H_H/\Delta S_H$ and is the temperature at which half of the population of the rate-controlling enzyme is active and the other half has been inactivated by high temperature; and ΔS_H is entropy of high temperature denaturation of the rate-controlling enzyme.

Numerous researchers have considered the Square Root model as more accurate than other models (Fernandez-Piquer et al. 2011; Koutsoumanis and Nychas 2000; Martins et al. 2015). Several predictive models such as Square root, Polynomial, and Arrhenius models (Eq. 14.12) have been used for the description of *L. monocytogenes* growth states in different food materials (e.g. meat, fish, egg, milk, dairy products, cheese, vegetables), and the Gamma model (Eq. 14.8) is evaluated as good as other models via MSE, R^2, bias factor and accuracy factor (Ross 1996; te Giffel and Zwietering 1999). Similarly, the impacts of temperature on *L. monocytogenes* growth in salmon roe were modeled by the Ratkowsky Square Root model, Huang Square Root model, and an Arrhenius model, respectively. The Ratkowsky Square Root model was more suitable to describe the effect of temperature on the specific growth rates in unsalted salmon roe because the nominal

minimum temperature was close to the real minimum growth temperature, and Huang Square Root model was more suitable in salted salmon (Cornu et al. 2006; Li et al. 2016).

$$ln\,(r) = ln\,(b) - \left(\frac{E_a}{RT}\right) \tag{14.16}$$

where b is pre-exponential factor; Ea is activation energy for bacterial growth; R is the gas constant; T is the temperature in Kelvin.

However, some researchers held the opposite conclusions (Cayré et al. 2003; Fernandez-Piquer et al. 2011; Giannuzzi et al. 1998). For instance, Mataragas et al. (2006) evaluated the spoilage of cooked cured meat products by an Arrhenius model and Square Root model and proposed that both of them fit well, but the Arrhenius model was more adaptable than the Square Root model. Analogously, Kreyenschmidt et al. (2010) assessed the shelf life of sliced cooked ham based on the growth of lactic acid bacteria and showed that the Arrhenius equation gave a better result.

14.3.3 Response Surface Methodology (RSM)

14.3.3.1 Basic Assumptions

The variables in an RSM model can be continuous (e.g. temperature or length) or categorical (number of participants) in nature. The model is essentially a second-degree polynomial approximation. The effectiveness of each variable is also tested in the model and the model is usually reiterated to only include the most effective variables to ensure parsimoniousness.

Since RSM utilizes statistical estimation to explain the effect of the variables, it is easy to apply the method to any set of variables in the process without the need to profoundly study these variables or the process beforehand. In other words, the flexibility and the fact that the model can be reiterated to great extents make RSM approaches ideal to oversophisticated processes where mathematical models are unable to operate (Myers and Montgomery 1995). The RSM designs employ several features including orthogonality (the property that allows RSM to estimate individual effects without confounding with other effects), rotatability (the property of rotating points of the design about the center of the factor space) or uniformity (used to control the number of center points in the design) (Box and Wilson 1951).

14.3.3.2 Limitations and Possible Enhancements

RSM experiment designs come on different levels of applicability and therefore complexities. The simplest design is a 2- level factorial experiment or a fractional

Table 14.2 An example for a 2 level 3-factor factorial design

	Factors		
Treatment	A	B	C
(1)	−1	−1	−1
a	1	−1	−1
b	−1	1	−1
ab	1	1	−1
c	−1	−1	1
ac	1	−1	1
bc	−1	1	1
abc	1	1	1

factorial design. Fractional designs take on all possible combinations of the factor levels. Therefore, a factorial design is a fully crossed design and thus does not leave out any combinations. But sometimes scientists cannot afford to investigate all the possible combinations. The experiments may be too expensive or too much time-consuming that may take weeks to complete each of them which may be the case in many microbial studies (Mahdinia et al. 2017a, b, 2018d, 2019a, b). In those cases, a fractional factorial design may be used that dismisses a large number of the combinations (possibly more than half). Table 14.2 shows a simple s-level factorial design with 3 factors.

The precision may be preserved, yet the potency of the model to address complex cross-effects is definitely reduced. In this fashion, other more complex and more capable designs were fabricated (Montgomery 2017).

14.3.3.2.1 Central Composite Design (CCD)

A CCD uses a second-order quadratic model. Similar to a 3-level factorial design, the CCD uses three levels for the factors and replicates over the middle levels of treatment to probe for the sensitivity and replicability of the experiments (Yolmeh and Jafari 2017). Table 14.3 shows a circumscribed design (CCC) for a three-factor model.

As seen in Table 14.3, the CCC uses factor settings outside the range of the factors in the factorial part and thus provides high quality predictions over the entire design space. A central face-centered design (CCF) uses the same number of runs but do not require settings outside the range. As a result, a CCF design is usually unable to provide precision for estimating pure quadratic coefficients. Nevertheless, both of CCDs require 20 runs for a 3-factor experiment while a full factorial design would require 27, of course.

Table 14.3 A coded CCC for a 3-factor experiment

	Factors		
Experiment	A	B	C
1	−1	−1	−1
2	+1	−1	−1
3	−1	+1	−1
4	+1	+1	−1
5	−1	−1	+1
6	+1	−1	+1
7	-1	+1	+1
8	+1	+1	+1
9	−1.682	0	0
10	+1.682	0	0
11	0	−1.682	0
12	0	+1.682	0
13	0	0	−1.682
14	0	0	+1.682
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

14.3.3.2.2 Box-Behnken Design

When scientists would like to use as few number of runs as possible and are not really expecting to run into convoluted cross-effects between factors; they turn to Box-Behnken designs. Table 14.4 shows the coded design for it.

As seen in Table 14.4, the Box-Behnken design does not require over or under-range settings and only requires 15 runs. As a result, it does not provide as high resolutions over a vide space of prediction like a CCC does. However, its missing corners may be useful when we should avoid combined factor extremes; which prevents a potential loss of data in those cases (Montgomery 2017).

Usually, the CCD models end up giving in a full quadratic model to explain the effects of all variables (A, B and C) on the response (Y) (Yolmeh and Jafari 2017):

$$Y = \beta_0 + \beta_A A + \beta_B B + \beta_C C + \beta_{AB} AB + \beta_{AC} AC + \beta_{BC} BC + \beta_{AA} A^2 + \beta_{BB} B^2 + \beta_{CC} C^2 \quad (14.17)$$

Table 14.4 A coded Box-Behnken design for a 3-factor experiment

	Factors		
Experiment	A	B	C
1	−1	−1	0
2	+1	−1	0
3	−1	+1	0
4	+1	+1	0
5	−1	0	−1
6	+1	0	−1
7	−1	0	+1
8	+1	0	+1
9	0	−1	−1
10	0	+1	−1
11	0	−1	+1
12	0	+1	+1
13	0	0	0
14	0	0	0
15	0	0	0

14.3.3.3 Comparison of Models

Above, we talked about how in theory RSM designs vary for different applications and how they may help us model sophisticated effects without usual difficulties of mathematical models. But what does that mean when it comes to microbial growth modeling? Cole et al. (1990) were one of the pioneers using RSM factorial design to investigate the simultaneous effects of pH, salt and temperature on *L. monocytogenes* survival and growth. They found out that survival at low pH and high salt concentrations is strongly temperature dependent and hence *L. monocytogenes* is the only species that poses great consumer health threats over refrigeration periods. Also, their polynomial model helped understand and develop better preservation conditions to minimize *L. monocytogenes* survival. In another study, García-Gimeno et al. (2002) used RSM to investigate the effects of NaCl concentration, pH and storage temperature on the growth curve of *Lactobacillus plantarum* in comparison with an ANN model. Their findings indicated that the RSM was a more precise model despite the fact that ANN models were more vastly used. As another example, effects of temperature, pH, and sodium chloride on growth of *Staphylococcus attreus* was predicted using a quadratic model in comparison with the modified Gompertz model with high precision by Sutherland et al. 1994.

As the RSM can be used for studying and modeling the effects of factors on microbial growth and survival, further post-modeling optimization techniques can be used to maximize a deactivation method efficiency. In this fashion, Han et al. (2002) used a Box-Behnken design to optimize *E. coli* O157:H7 deactivation using ozone treatment on green peppers. The variables were ozone gas concentration, humidity

and treatment time. The finding of the optimum conditions not only helped reduce the risk of the foodborne microorganisms, but ozone itself as a hazardous gas. Similarly, Skandamis and Nychas (2000) used RSM to obtain a quadratic model to predict the effects of temperatures, pH and oregano essential oil concentrations on the survival of an *E. coli* O157:H7 strain in eggplant salad. In conjunction with a Baranyi and Robert model, they accurately predicted the survival kinetics of the *E. coli* strain, which led to coherent predictions with viable-count measurements.

14.3.4 Artificial Neural Networks (ANNs)

14.3.4.1 Basic Assumptions

Artificial neural networks (ANNs) are an empirical non-linear method based on a set of mathematical equations to imitate the function of the human brain (Zupan and Gasteiger 1991). Basic ANNs contain three layers, the input layer, hidden layer, and output layer. The input layer is made up of the environmental influencing factors. The hidden layer is composed of numerous neurons and links between the input layer and the output layer, which may have one or multiple layers. The number of nodes (neurons) in the hidden layer is variable, and the nonlinearity of the neural network increases over the number of nodes, resulting in a more robust neural network (Gevrey et al. 2003). The output layer consists of the dependent variables (e.g., maximum specific growth rate and lag phase duration). Notably, ANNs do not assume the previous hypothesis of normality and independency between independent factors which are inevitable constraints for other methods. Instead, ANNs derives nonlinear functions directly from experimental data. Meanwhile, from one neural network model, different output can be obtained by various multi-equation models resulting in a smaller estimation error. (Pérez-Rodríguez and Valero 2013).

14.3.4.2 Limitations and Possible Enhancements

ANNs as a black box model stress their flexible behavior and prefer to describe the unknown relationship between microbial growth parameters and environmental influencing factors (Geeraerd et al. 2004; Khayet et al. 2011). However, this advantage brings about a side-effect, though ANNs hold high accuracy and great ability when multiple variables are described, a lack of interpretability limits the application in practical settings (Nelofer et al. 2012).

Back-propagation (BP) technique is the most common training algorithm for fee-forward neural network. It has the advantages of ANNs such as good prediction performances and easy to master (Jiang et al. 2016; Sadrzadeh et al. 2008; Wang et al. 2017a); however, it also has some issues including the local minima, overfitting, and slow convergence rate (Chen et al. 2014). The accuracy and efficiency of traditional ANNs can be improved by modifications. Several evolutionary techniques, such us genetic algorithm (GA) and particle swarm optimization (PSO)

algorithm are regularly used to solve the shortcomings of BP-ANN (He and Zhang 2018; Sun and Zhang 2018; Wang et al. 2017b; Zhang et al. 2016). The particles in PSO follow the trend that bird flocking and fish schooling share information for better living. Based on that, the nonlinear problem involving multiple variables can be solved more effectively (Kennedy and Eberhart 2011). A genetic algorithm is a randomized search method derived from Darwin's evolutionary theory (survival of the fittest) with an efficient and parallel global searching ability (Goldberg 1989). In addition, to enhance BP-ANN, Pruning algorithms and two hidden layers are frequently used to remove the unnecessary node and increase accuracy, respectively (Huang 2003; Reed 1993). Moreover, radial basis function neural networks (RBF-ANN) are another kind of models different from BP-ANN. For any BP-ANN, there is always an RBF-ANN that can replace it, and vice versa; however, the RBF-ANN is superior to BP-ANN in terms of approximation ability, classification ability, and learning speed (Marini 2009). A great many types of ANNs are not mentioned in this part. With the aim to apply ANNs in food microbial growth prediction, much more professional books and papers are strongly recommended to be investigated (Bishop 2006; Haykin 1994; Huang et al. 2007; Mitchell 1997).

14.3.4.3 Comparison of Models

As an unconventional microbial growth model, ANNs are always compared to the traditional microbial growth models such us Response Surface Methodology model (RSM) (Baş and Boyacı 2007; Garcíaa-Gimeno et al. 2003; Huang et al. 2007). RSM requires the order of the model to be stated, while ANNs implicitly match the growth conditions to the kinetic parameters. A further advantage of ANNs is that they allow the inclusion of non-growth data (García-Gimeno et al. 2005). For instance, ANNs and RSM were both used for predicting bacterial growth in a simulated medium of modified-atmosphere-packed cooked meat products. The results showed that the accuracy of ANNs was higher than RSM (Lou and Nakai 2001a, and b). Similarly, ANNs provided better predictions for the maximum specific growth rate of the fungus *Monascus ruber* than RSM (Panagou et al. 2010).

Compared to the Arrhenius model, ANNs offered several advantages in its non-linearity, parallelism, noise tolerance, learning, and capability for generalization (Gosukonda et al. 2015). For example, the Arrhenius model, BP-ANN, and RBF-ANN were used to predict the freshness of brined bream fillets stored at different temperatures, respectively. The RBF-ANN exhibited a great ability in function approximation, learning speed (compared to BP-ANN), and multi-output ability and self-learning (compared to the Arrhenius model) (Wang et al. 2015). Meanwhile, the ANNs were more effective than the Arrhenius model in predicting the quality of rainbow trout fillets during storage at different temperatures (Liu et al. 2015). Furthermore, Panagou et al. (2011) compared the partial least squares modelling (PLS) with ANNs for the rapid detection of the microbial spoilage of beef fillets on the basis of Fourier transform infrared spectral fingerprints via bias factor, accuracy factor and root mean square error. They concluded that PLS models

presented better correlation of total viable counts on meat surface with FTIR spectral data (Panagou et al. 2011).

14.3.5 Integrated Pathogen Modeling Program (IPMP2013)

14.3.5.1 Basic Assumptions

As mentioned in the last section, IPMP2013 is a microbial data analysis tool developed by USDA-ARS containing both primary and secondary models (USDA 2018). Twenty-one models are available to describe incomplete growth curves, complete growth curves, microbial survival, and inactivation, as well as the effect of temperature on microbial specific growth rates. The software includes the data window, model window, plot window, and report window. In particular, IPMP 2013 provides an interface allowing the users to adjust the initial guess values of each parameter (Fig. 14.5). The estimated parameters, the associated standard errors, t-values and p-values, and lower and upper 95% confidence intervals are shown in the analysis results. The sum of squared errors (SSE), mean and root mean of squared errors (MSE and RMSE), residual standard deviation, and Akaike information criterion (AIC) are all belong to error analyses (Huang 2014). It is an easy-to-use microbial data analysis tool which can be directly used without any programming knowledge (Fig. 14.6).

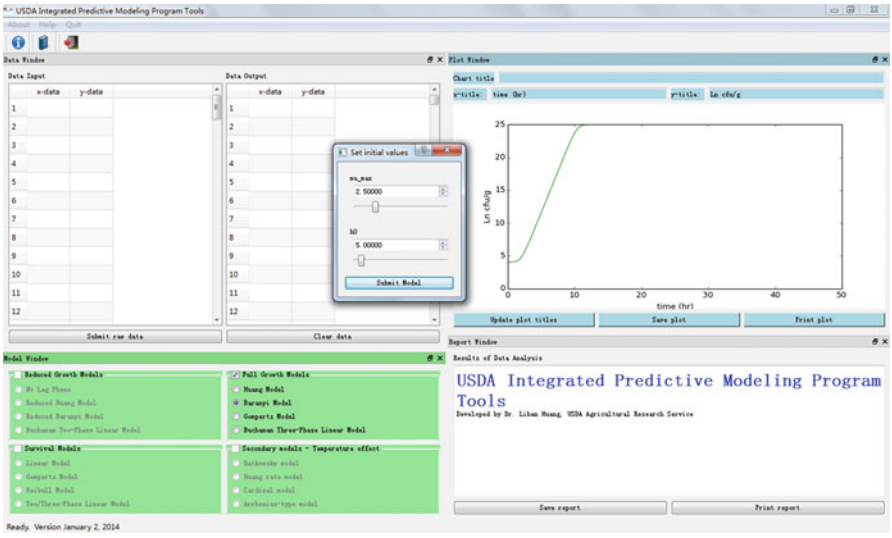

Fig. 14.6 Interface of IPMP2013 (USDA 2018)

14.3.5.2 Comparison of the Models

It is important to note that IPMP2013 is a fitting software. Whereas, Food MicroModel (FMM), Pathogen Modelling and Programme (PMP, and ComBase model are databases (Fig. 14.7).

There are other fitting software programs for microbial prediction. Many researchers use commercially available mathematical tools, such as MATLAB, SAS, and SPSS, while others use open-source (free) statistical analysis tools, such as R, for data analysis. However, these software packages are unfriendly to those individuals who lack in programming knowledge. For instance, R packages can offer considerable flexibility only for the users equipped with R's command line interface and script writing (Kahm et al. 2010). Later, some user-friendly tools have been developed such as two typical free Excel add-in packages (DMFit and GInaFit). The DMFit includes both primary models (reparameterized Gompertz model and the Baranyi model) and secondary models (Gamma model, Ratkowsky model, Cardinal model and polynomial model) (Combase, 2018). Specifically, the DMFit not only fits a primary curve to log CFU counts versus time data, but estimates the kinetic parameters such as growth/death rate, lag time, and maximum population density, the GInaFit includes nine different types (log-linear model, Weibull model, Biphasic model and their modifications) of microbial survival (inactivation) models, if the user does not have a clear idea of the general shape of their survival curves yet, different model types available can be tested and compared (Geeraerd et al. 2005).

Compared with DMFit and GInaFit, IPMP 2013 provides sufficient models, and the analysis results obtained from IPMP 2013 are identical to those from either R or SAS (Huang 2014). Even so, few shortcomings in IPMP2013 were also presented by researchers. For example, it lacks a local regression option and supports a limited number of data points. Additionally, it can only analyze one growth curve at a time

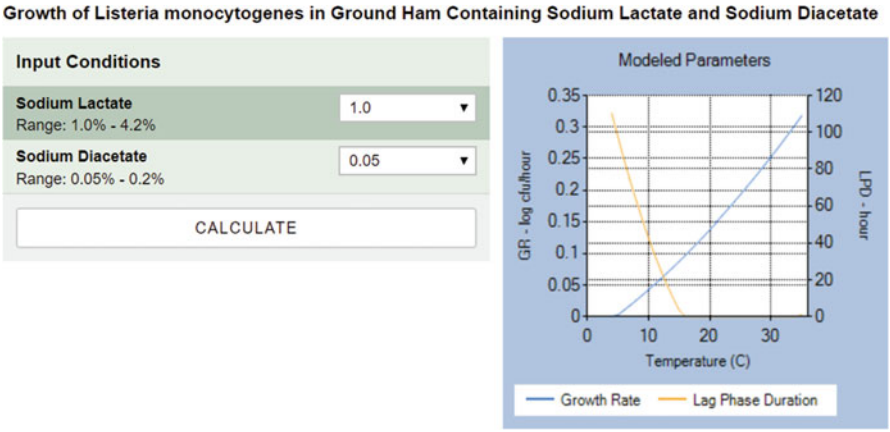

Fig. 14.7 Interface of PMP online (Hwang and Tamplin 2010; USDA 2018)

which limits its use in analyzing high-throughput microtiter plate-based data (Bukhman et al. 2015). Nevertheless, IPMP 2013 remains to be a good microbial growth fitting software.

14.4 Concluding Remarks and Future Trends

In this chapter, the most widely used traditional and novel food microbial predictive models were summarized. Most of these food microbial predictive models have been widely applied in the food industry for estimating risk, identifying critical control points, evaluating reformulations, and education (Whiting 1995). It is helpful for the researchers in the field of food safety engineering to understand and master the fundamentals of microbial growth modeling to enable the development of more reasonable and accurate models.

Microbial growth modeling has progressively become an indispensable part of food engineering. Recognizing its importance and significance, researchers and practitioners are working towards addressing the grand challenge of developing a first-principle-based universal growth model, which is applicable for all microorganisms and foods under all environmental conditions. To that end, significant time and effort have been invested in expanding the scope of and generalizing the current and new models to achieve a universal model. For example, ComBase integrates numerous microbial growth data from many different models, which can be used to assist researchers and food companies in developing new food products, reformulating foods, produce food safety plans, reducing food waste, and helping public health organizations in developing science-based food policies (ComBase, 2018). Currently, the available data is not sufficient for the varieties of food materials and microorganisms. To fill the data gap, a team-effort involving food engineers, food scientists, and microbiologists is urgently needed.

With the increasingly robust and ever-improving microbial models, especially with the constant updating of the database, the food microbial predictive models have been widely used with confidence in HACCP and QMRA programs. However, some limitations restrict their application in food safety and engineering (Amézquita et al. 2005; Halder et al. 2010; Plaza-Rodríguez et al. 2015). For instance, different models and their modifications fit different circumstances; meanwhile, even small discrepancy in the environment (e.g., nutritional ingredient, processing method, etc.) may lead to different results. Moreover, some pathogenic microorganisms such as *C. botulinum* are not allowed in food, suggesting that preventing pathogenic bacterium is just as, if not more, important than predicting its growth (Collins 2010). Additionally, since microbial prediction is mostly used in the safety field, the model should overestimate the microbial growth rate for those special situations requiring a greater margin for error. Accordingly, three guiding principles are recommended in applications:

(i) The real conditions should be in the usable range of the model;
(ii) The model should be used for conservative estimations;

(iii) The widely used and proven models should be slightly modified, if necessary, to fit the actual situation.

In summary, although models are convenient to use in both scientific research and practical applications, they should be combined with traditional or novel and emerging microbial testing in concert with the indispensable experience of experts or practitioners rather than lieu of them.

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