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Biofilm Formation, Adhesion with *Staphylococcus aureus* Against Food Borne Pathogen: A Mathematical Modeling on the Effects of *Adiantum phillippense*

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ABSTRACT

Biofilm formation is a process by which microorganisms irreversibly bind to and grow on a surface and create extracellular polymers that promote the formation of attachments and matrixes, resulting in a change in the organisms' phenotype in terms of growth rate and transcription of genes. *A. philippense* is a fern with many curative properties that is medicinally treasured. Predictive mathematical modeling approach was used to study adhesion of *S. aureus* with biofilm. Out of the eight different primary model, modified Gompertz best fit the effect of the plant extract on the biofilm formation and adhesion with *S. aureus* with the least value for RMSE, AICc and the uppermost value for adjusted R^2. The parameters obtained from the modified Gompertz when compared with control and chloramphenicol were y_{max} 0.980 (95% C.I. 0.889 to 1.070) and 0.637 (95% C.I. 0.604 to 0.670), μ_{max} 0.185 (95% C.I. 0.120 to 0.250) and 0.183 (95% C.I. 0.141 to 0.225), lag (h) 0.180 (95% C.I. -0.764 to 1.124) and 3.343 (95% C.I. 2.933 to 3.753) respectively. A strong model to use to fit sigmoidal growth or formation curves tends to be the modified Gompertz equation. The benefit of using this function is that a constant formation rate is not assumed by the Gompertz equation. Instead, it is a model that can be used to model rates of formation (of biofilm) that change over time.

INTRODUCTION

Biofilm formation is a process by which microorganisms irreversibly bind to and grow on a surface and create extracellular polymers that promote the formation of attachments and matrixes, resulting in a change in the organisms' phenotype in terms of growth rate and transcription of genes. Microorganisms mainly occur in nature by binding to and developing on living and inanimate surfaces. These surfaces may take many types, including those in the soil and marine environments, those in the medical device continuum, and those in living tissues, such as tooth enamel, heart or lung valves, and the middle ear. The common characteristic of this attached growth state is that a biofilm is formed by the cells [1]. For public health, biofilms have great significance because biofilm-associated microorganisms show a significantly reduced vulnerability to antimicrobial agents. Such susceptibility may be intrinsic or acquired (due to transfer of extrachromosomal elements to susceptible organisms in the biofilm) [2]. Almost all micro-organisms (99.9%) have the ability to generate biofilm on a wide variety of surfaces, i.e. Biological and inert surfaces. They generate extracellular polymeric material (eps) and form biofilm when micro-organisms bind to a surface. Owing to its resistant nature to antibiotics and diseases associated with domestic medical devices, biofilm presents a major problem for public health. It is observed that *H. infuenza* can form biofilm in the human body and can escape from it [2].

A. philippense is a fern with many curative properties that is medicinally treasured. Plant-derived extracts are highly known these days because of their lack of side effects, and many are currently being used. Traditionally as ethnomedicine for treatment and prevention of the various forms of infections [3]. In India, *A. philippense* is widely used in the treatment of many medical conditions by local and tribal communities, such as epileptic fits, fever, ulcers, diseases of the blood, erysipelas, dysentery, rabies, fever, emaciation or cachexia, atrophy of muscle pain, paralysis, pimples, wounds and elephantiasis[4].

The existence of phenols, terpenoids, flavonoids and carbohydrates, as a result of phytochemical analysis of this plant, has been observed [5].

Mathematical modeling is the art of transforming problems into tractable mathematical formulas from an application field whose theoretical and numerical analysis offers insight, responses, and guidance useful for the originating application [6]. A model is a framework that serves to explain and measure a conceptual or mathematical representation of a system. The distinction between mathematical and philosophical lies only in the manner in which the representation is formulated. A model is often a condensed representation, which the scientist wishes to understand and calculate, of the reference system. In the end, it serves as a way of systematizing the information and understanding available of a given phenomenon and the facts about it [6,7]. For the first time the predictive mathematical modeling of the effect of *A. philippense* on biofilm formation and adhesion with *staphylococcus aureus* against foodborne pathogens was studied using various models (**Table 1**).

Table 1. Growth models used in modelling the growth curve of nile tilapia.

Model	P	Equation
Modified Logistic	3	$y=\frac{A}{\left\{1+\exp\left[\frac{4\mu_m}{A}(\lambda-t)+2\right]\right\}}$
Modified Gompertz	3	$y=A\exp\left\{-\exp\left[\frac{\mu_m e}{A}(\lambda-t)+1\right]\right\}$
Modified Richards	4	$y=A\left\{1+\nu\exp(1+\nu)\exp\left[\frac{\mu_m}{A}(1+\nu)\left(1+\frac{1}{\nu}\right)(\lambda-t)\right]\right\}^{\left(\frac{-1}{\nu}\right)}$
Modified Schnute	4	$y=\left(\mu_m\frac{(1-\beta)}{\alpha}\right)\left[\frac{1-\beta\exp(\alpha\lambda+1-\beta-\alpha t)}{1-\beta}\right]^{\frac{1}{\beta}}$
Baranyi-Roberts	4	$y=A+\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h_0}-e^{-\mu_m x-h_0}\right)-\ln\left(1+\frac{e^{\mu_m x+\frac{1}{\mu_m}\ln\left(e^{-\mu_m x}+e^{-h0}-e^{-\mu_m x-h0}\right)}-1}{e^{(y\max-A)}}\right)$
Von Bertalanffy	3	$y=K\left[1-\left(\frac{A}{K}\right)^3\right]\exp^{-\left(\mu_m x/3K^{\frac{1}{3}}\right)^3}$
Huang	4	$y=A+y\max-\ln\left(e^{-A}+\left(e^{Y\max}-e^{-A}\right)e^{-\mu_m B(x)}\right)$ $B(x)=x+\frac{1}{\alpha}\ln\frac{1+e^{-\alpha(x-\lambda)}}{1+e^{\alpha\lambda}}$
Buchanan Three-phase linear model	3	Y = A, IF X < LAG Y=A + K(X−λ), IF λ ≤ X ≥ X_{MAX} Y = Y_{MAX}, IF X ≥ X_{MAX}

Note:
A= growth lower asymptote;
Y_{max}= growth upper asymptote;
M_{max}= maximum specific growth rate;
V= affects near which asymptote maximum growth occurs.
L=lag time
E = exponent (2.718281828)
T = sampling time
A,b, k = curve fitting parameters
H_0 = a dimensionless parameter quantifying the initial physiological state of the reduction process.
The lag time (h^{-1}) or (d^{-1}) can be calculated as $h_0=m_{max}$

MATERIALS AND METHODS

A previously published data [3] was processed using the software Webplotdigitizer 2.5 (Rohatgi 2018).

Statistical analysis
In the selection for the best models, statistical analysis or error function analysis was carried out using discriminatory factors such as accuracy factor (AF), bias factor (BF), adjusted determination coefficient (R^2), root-mean - square error (RMSE) and one based on information theory which is the AICc (corrected Akaike information criterion) [36].

Fitting of the data
Nonlinear regression analysis was carried out using the curve expert professional software (version 1.6). Several popular growth models were utilized in this study. The μmax of the estimation was performed by the steepest ascent rifle of the curve, whereas the x-axis crossing of this line is an estimate of λ. For the purposes of modeling, the model that demonstrates the highest growth was adopted.

RESULTS AND DISCUSSION

The growth curves were replotted and converted to log units prior to modelling (**Fig. 1**). In the modeling process, the highest signal was used to pick the best model. The adequate fitting of all the models to the growth curve was evident (**Figs 2** to **9**). The best model was found using the modified Gompertz model (**Fig 4**) with the least value for RMSE, AICc and the uppermost value for adjusted R^2. For the model, the AF and BF values were shown to be superb and their values were closest to unity. Biofilm formation was modelled using the modified Gompertz model (**Fig. 10**). Kinetic of biofilm formation and adhesion with *S. aureus* was assumed that the growth rate of the bacteria is greatly affected by the effect of the plant extract (*A. philippense).*

The modified Gompertz equation has been used successfully to explain nonlinear responses. In food microbiology, the modified Gompertz equation has been used mainly to model the asymmetrical sigmoid form of microbial growth curves. The modified Gompertz equation was further used in conjunction with different statistical methods to explain single and multiple effects of factors affecting microbial development [8]. The least performance was the modified logistic model (**Table 2**). The near absence of lag period for growth is likely the reason for the superiority of the modified Gompertz model. The coefficients for the modified Gompertz model are shown in **Table 3**.

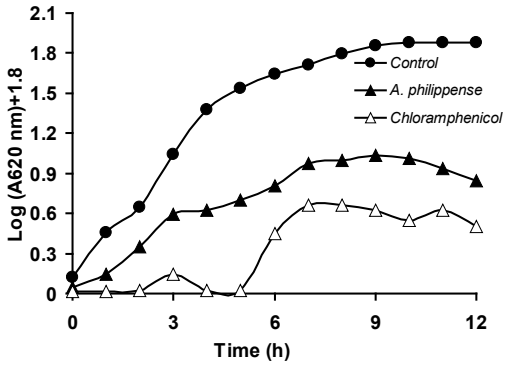

Fig. 1. Growth of *S. aureus* biofilm (control) in the presence of *A. philippense* and a positive control (chloramphenicol).

Table 2. Statistical analysis of the various fitted models.

Model		RMSE	Adr^2	AF	BF	AICc
Huang	4	0.06	0.99	1.03	1.01	-53.08
Baranyi-Roberts	4	0.04	0.99	1.03	1.00	-60.07
Modified Gompertz	3	0.04	1.00	1.08	1.01	-68.04
Buchanan-3-Phase	3	0.08	0.98	1.08	1.02	-48.88
Modified Richards	4	0.04	0.99	1.05	1.01	-60.56
Modified Schnute	3	0.04	0.99	1.05	1.01	-60.56
Modified Logistics	3	0.05	0.99	1.08	1.04	-60.94
von Bertalanffy	4	0.04	0.99	1.04	0.99	-65.05

Note:
p no of parameters
$AdJR^2$ adjusted coefficient of determination
RMSE Root Mean Square Error
BF bias factor
AF accuracy factor

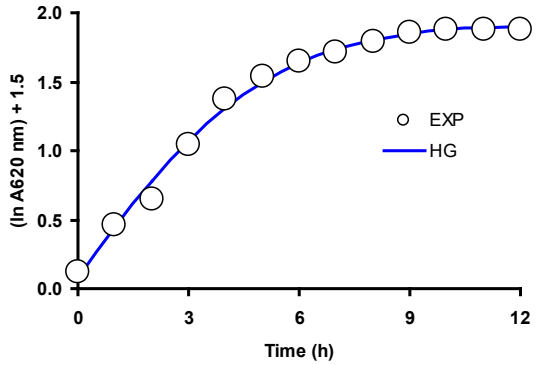

Fig. 2. Growth of *S. aureus* biofilm (control) fitted to the Huang model.

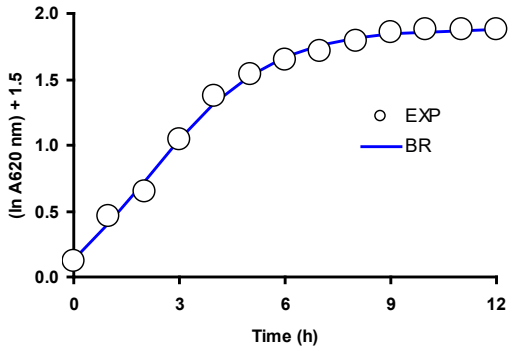

Fig. 3. Growth of *S. aureus* biofilm (control) fitted to the Baranyi-Roberts model.

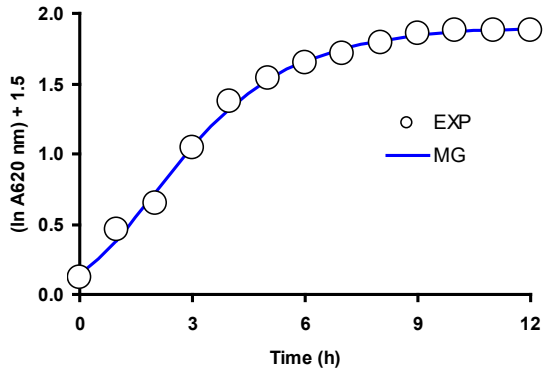

Fig. 4. Growth of *S. aureus* biofilm (control) fitted to the modified Gompertz model.

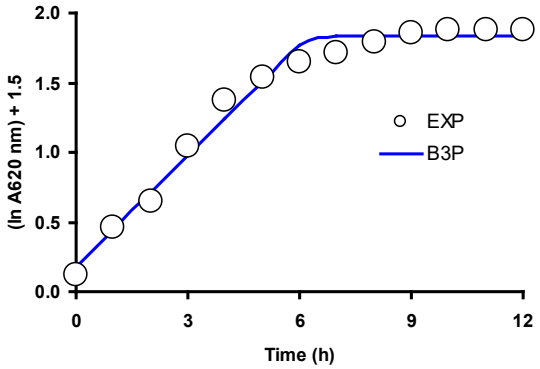

Fig. 5. Growth of *S. aureus* biofilm (control) fitted to the buchanan-3-phase model.

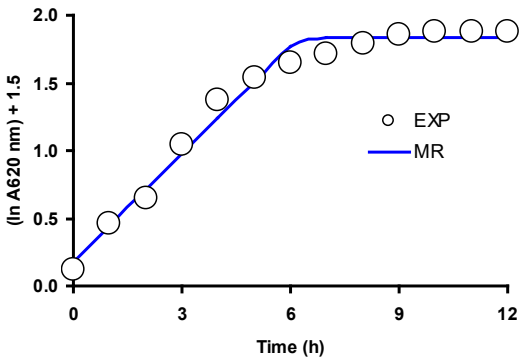

Fig. 6. Growth of *S. aureus* biofilm (control) fitted to the Modified Richards model.

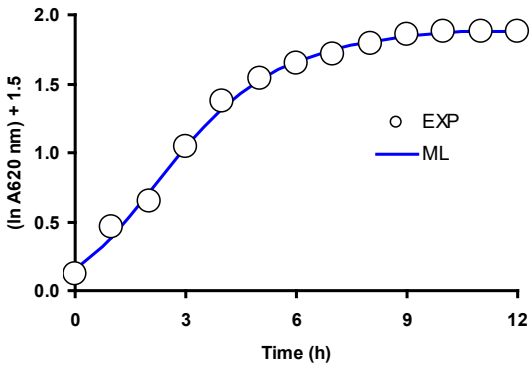

Fig. 7. Growth of *S. aureus* biofilm (control) fitted to the Modified Logistics model.

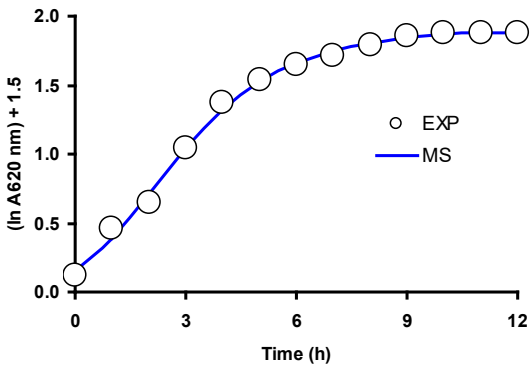

Fig. 8. Growth of *S. aureus* biofilm (control) fitted to the Modified Schnute model.

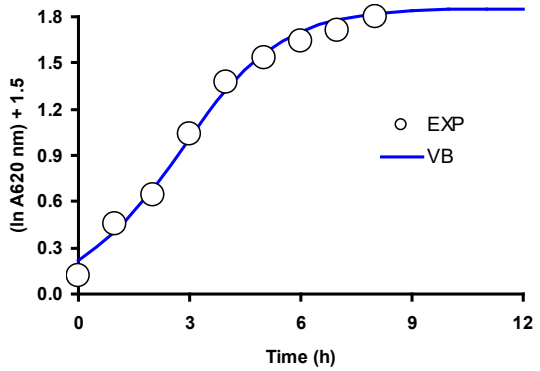

Fig. 9. Growth of *S. aureus* biofilm (control) fitted to the Von Bertalanffy model.

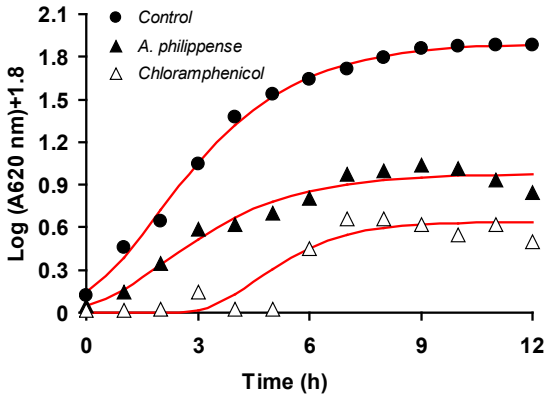

Fig. 10. Growth of *S. aureus* biofilm (control) in the presence of *A. philippense* and a positive control (chloramphenicol) fitted to the modified Gompertz model (red lines).

Table 3. Coefficients of bacterium biofilm (control) in the presence of *A. philippense* and a positive control (chloramphenicol) fitted to the best model.

	Control		*S. aureus*		Chloramphenicol	
	Value	(95% C.I.)	Value	(95% C.I.)	Value	(95% C.I.)
Y_{max}	1.900	1.850 to 1.950	0.980	0.889 to 1.070	0.637	0.604 to 0.670
M_{max} (h^{-1})	0.344	0.310 to 0.378	0.185	0.120 to 0.250	0.183	0.141 to 0.225
Lag (h)	-0.097	-0.372 to 0.177	0.180	-0.764 to 1.124	3.343	2.933 to 3.753

Note: 95% C.I. denotes 95% confidence interval.

The study carried out was to study the effect of plant extract A. *Phillippense* on biofilm formation and adhesion with *S. aureus* on foodborne pathogens using mathematical models' approach. Some of the tested predictive models include Baranyi-Roberts [7,9] and logistic, modified Gompertz [10–16], Richards, Schnute [17,18], Von Bertalanffy [19,20], Buchanan three-phase [13,21–25] and more recently the Huang model [26]. The Modified Gompertz model is the most popular model as it is the simplest (having three parameters).

The asymmetrical sigmoidal form of the modified Gompertz model provides greater flexibility compared to the Logistic model. Sigmoidal models such as Logistics and Gompertz differ primarily at the point of inflection between the lower and upper asymptotes. There is a distance of 1/2 and 1/e, respectively, between the lower and upper asymptotes of the logistic and Gompertz models [27]. Most growth models, in general, have a flexible slope and variable inflection point feature between the lower and upper asymptotes. Such functions are either individual or simplified instances of a parent model. As an example, the modified logistics, modified Gompertz and the von Bertalanffy growth models originates from the parent Richard's model [18,27,28]. The model has its limitations and with some primary problems. Firstly, in the static version, $y_{(t=0)}$ is not equal to y_o. Secondly, the inherent property of the sigmoidal curve is an inflection point, allowing the model to have a systematic difficulty representing the exponential phase. Ultimately, the model appears to overestimate the importance of its parameters [29–31]. Notwithstanding this, the modified Gompertz model has been commonly used to model the development of the processing of bacteria and secondary bacterial products including biohydrogen, methane, lactic acid, biofuel and bacterioricin to name a few [32–36] including callus growth [37–39].

For more secondary modeling, parameters derived from the fitting exercise may later be used. These mechanistic models aim to gain a better understanding of the processes of chemistry, physics and biology. Mechanistic models, such as the modified Gompertz, are more effective compared to purely empirical model, as mechanistic models tell you about the underlying mechanism or mechanisms that drive the changes in the observed growth rates [40]. Modified Gompertz support the exemplified this plant's strong inhibitory activity against some bacteria (*S. aureus*). Consequently, this research offers evidence of the ethanomedicinal application of *A. philippensis* in the treatment of a number of diseases caused by pathogenic microorganisms and infections [3]. To maximize the effect of *S. aureus*, the merging of A. philippense crude extract and chloramphenicol was imperative. Futuristic experiments are also important for the testing of antibacterial resistance to other drugs.

CONCLUSION

In conclusion, the Modified Gompertz model was the best model in modelling the biofilm formation curve of the bacterium *S.Aeureus* based on statistical tests such as root-mean-square error (RMSE), adjusted coefficient of determination (R^2), bias factor (BF), and accuracy factor (AF) and corrected AICC (akaike information criterion).
A strong model to use to fit sigmoidal growth or formation curves tends to be the Gompertz equation. The benefit of using this function is that a constant formation rate is not assumed by the Gompertz equation. Instead, it is a model that can be used to model rates of formation that change over time. However, [41,42] justified the use of the Gompertz equation from a mathematical point of view to model microbial growth. He stated that the Gompertz equation better estimates early lag phase, end of lag phase, and maximum growth for sigmoidal microbial growth curves than the logistic equation

REFERENCE

1. Donlan RM. Biofilm Formation: A Clinically Relevant Microbiological Process. Clin Infect Dis. 2001;33(8):1387–92.
2. Mohammad Reza S. Bacterial Biofilm and its Clinical Implications. Ann Microbiol Res. 2018:[19];2(1).
3. Adnan M, Patel M, Deshpande S, Alreshidi M, Siddiqui AJ, Reddy MN, et al. Effect of *Adiantum philippense* Extract on Biofilm Formation, Adhesion With Its Antibacterial Activities Against Foodborne Pathogens, and Characterization of Bioactive Metabolites: An in vitro-in silico Approach. Front Microbiol. 2020;11:823.
4. Kuo L-Y, Hsu T-C, Chang Y-H, Huang Y-M, Chiou W-L. Systematics of the Adiantum philippense complex (Pteridaceae, Polypodiales) in Taiwan. Phytotaxa. 2016;263(2):113. 5.

5. Mengane SK. Phytochemical Analysis of *Adiantum lunulatum*. Int J Curr Microbiol Appl Sci. 2016;5(11):351–6.
6. Torres NV, Santos G. The (Mathematical) Modeling Process in Biosciences. Front Genet . 2015;6.
7. Halmi MIE, Shukor MS, Johari WLW, Shukor MY. Evaluation of several mathematical models for fitting the growth of the algae *Dunaliella tertiolecta*. Asian J Plant Biol. 2014;2(1):1–6.
8. Gil MM, Brandão TRS, Silva CLM. A modified Gompertz model to predict microbial inactivation under time-varying temperature conditions. J Food Eng. 2006;76(1):89–94.
9. Baranyi J, Roberts TA. A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol. 1994;23(3–4):277–94.
10. Wang J, Wan W. Kinetic models for fermentative hydrogen production: A review. Int J Hydrog Energy. 2009;34(8):3313–23.
11. Wu D, Yang Z, Tian G. Inhibitory effects of Cu (II) on fermentative methane production using bamboo wastewater as substrate. J Hazard Mater. 2011;195:170–4.
12. Nikhil GN, Mohan SV, Swamy YV. Systematic approach to assess biohydrogen potential of anaerobic sludge and soil rhizobia as biocatalysts: Influence of crucial factors affecting acidogenic fermentation. Bioresour Technol. 2014;165(C):323–31.
13. Tornuk F, Ozturk I, Sagdic O, Yilmaz A, Erkmen O. Application of predictive inactivation models to evaluate survival of *Staphylococcus aureus* in fresh-cut apples treated with different plant hydrosols. Int J Food Prop. 2014;17(3):587–98.
14. Germec M, Turhan I. Ethanol production from acid-pretreated and detoxified tea processing waste and its modeling. Fuel. 2018 Nov 1;231:101–9.
15. Zhu H, Yang J, Xiaowei C. Application of Modified Gompertz Model to Study on Biogas production from middle temperature co-digestion of pig manure and dead pigs. In 2019.E3S Web of Conferences. Vol. 118. EDP Sciences, 2019.
16. Uba G, Yakasai HM, Abubakar A, Shukor MYA. Predictive Mathematical Modelling of the Total Number of COVID-19 Cases for Brazil. J Environ Microbiol Toxicol. 2020 Jul 31;8(1):16–20.
17. Gibson AM, Bratchell N, Roberts TA. Predicting microbial growth: growth responses of salmonellae in a laboratory medium as affected by pH, sodium chloride and storage temperature. Int J Food Microbiol. 1988;6(2):155–78.
18. Zwietering MH, Jongenburger I, Rombouts FM, Van't Riet K. Modeling of the bacterial growth curve. Appl Environ Microbiol. 1990;56(6):1875–81.
19. Cloern JE, Nichols FH. A von Bertalanffy growth model with a seasonally varying coefficient. J Fish Res Board Can. 1978;35(11):1479–82.
20. Darmani Kuhi H, Kebreab E, Lopez S, France J. A derivation and evaluation of the von Bertalanffy equation for describing growth in broilers over time. J Anim Feed Sci. 2002;11(1):109–25.
21. McClure PJ, Beaumont AL, Sutherland JP, Roberts TA. Predictive modelling of growth of Listeria monocytogenes. The effects on growth of NaCl, pH, storage temperature and NaNO2. Int J Food Microbiol. 1997;34(3):221–32.
22. Xiong R, Xie G, Edmondson AE, Sheard MA. A mathematical model for bacterial inactivation. Int J Food Microbiol. 1999;46(1):45–55.
23. Koseki S, Isobe S. Growth of Listeria monocytogenes on iceberg lettuce and solid media. Int J Food Microbiol. 2005;101(1–2):217–25.
24. Okubara PA, Bonsall RF. Accumulation of Pseudomonas-derived 2,4-diacetylphloroglucinol on wheat seedling roots is influenced by host cultivar. Biol Control. 2008;46(3):322–31.
25. Kwak Y-S, Bonsall RF, Okubara PA, Paulitz TC, Thomashow LS, Weller DM. Factors impacting the activity of 2,4-diacetylphloroglucinol-producing *Pseudomonas fluorescens* against take-all of wheat. Soil Biol Biochem. 2012;54:48–56.
26. Huang L. Growth kinetics of Escherichia coli O157:H7 in mechanically-tenderized beef. Int J Food Microbiol. 2010;140(1):40–8.
27. Gibson AM, Bratchell N, Roberts TA. The effect of sodium chloride and temperature on the rate and extent of growth of *Clostridium botulinum* type A in pasteurized pork slurry. J Appl Bacteriol. 1987;62(6):479–90.
28. López S, Prieto M, Dijkstra J, Dhanoa MS, France J. Statistical evaluation of mathematical models for microbial growth. Int J Food Microbiol. 2004;96(3):289–300.
29. McKellar RC, Knight K. A combined discrete-continuous model describing the lag phase of *Listeria monocytogenes*. Int J Food Microbiol. 2000;54(3):171–80.
30. Membré J-M, Ross T, McMeekin T. Behaviour of *Listeria monocytogenes* under combined chilling processes. Lett Appl Microbiol. 1999;28(3):216–20.
31. Whiting RC. Modeling bacterial survival in unfavorable environments. J Ind Microbiol. 1993;12(3–5):240–6.
32. Espeche MC, Tomás MSJ, Wiese B, Bru E, Nader-Macías MEF. Physicochemical factors differentially affect the biomass and bacteriocin production by bovine *Enterococcus mundtii* CRL1656. J Dairy Sci. 2014;97(2):789–97.
33. Kargi F, Eren NS, Ozmihci S. Effect of initial bacteria concentration on hydrogen gas production from cheese whey powder solution by thermophilic dark fermentation. Biotechnol Prog. 2012;28(4):931–6.
34. Karthic P, Joseph S, Arun N, Varghese LA, Santhiagu A. Biohydrogen production using anaerobic mixed bacteria: Process parameters optimization studies. J Renew Sustain Energy. 2013;5(6).
35. Mathias SP, Rosenthal A, Gaspar A, Aragão GMF, Slongo-Marcusi A. Prediction of acid lactic-bacteria growth in Turkey ham processed by high hydrostatic pressure. Braz J Microbiol. 2013;44(1):23–8.
36. Mohammadi M, Mohamed AR, Najafpour GD, Younesi H, Uzir MH. Kinetic studies on fermentative production of biofuel from synthesis gas using *Clostridium ljungdahlii*. Sci World J. 2014;2014.
37. Omar R, Abdullah MA, Hasan MA, Rosfarizan M, Marziah M. Kinetics and modelling of cell growth and substrate uptake in *Centella asiatica* cell culture. Biotechnol Bioprocess Eng. 2006;11(3):223–9.
38. Shukor MS, Masdor NA, Halmi MIE, Ahmad SA, Shukor MY. Test of randomness of residuals for modified Gompertz model used for modelling the growth of callus cultures from *Glycine wightii* (Wight & Arn.) Verdc. Asian J Plant Biol. 2015;3(1):11–3.
39. Shukor MS, Masdor NA., Halmi MIE, Ahmad SA, Shukor MY. Modelling the growth of callus cultures from *Glycine wightii* (Wight & Arn.) Verdc. Asian J Plant Biol. 2015;3(1):20–5.
40. Bolker BM. Ecological Models and Data in R. Princeton, N.J: Princeton University Press; 2008. 408 p.
41. Baranyi J, McClure PJ, Sutherland JP, Roberts TA. Modeling bacterial growth responses. J Ind Microbiol. 1993;12(3–5):190–4.
42. Garthright WE. Refinements in the prediction of microbial growth curves. Food Microbiol. 1991;8(3):239–48.