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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title	**Description of Kinetic Behavior of Pathogenic *Escherichia coli* in Cooked Pig Trotters Under Dynamic Storage Conditions Using Mathematical Equations**
Running Title (within 10 words)	Growth of *E. coli* in pig trotters
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ABSTRACT

A dynamic model was developed to predict the *Escherichia coli* cell counts in pig trotters at changing temperatures. Five-strain mixture of pathogenic *E. coli* at 4 log CFU/g were inoculated to cooked pig trotter samples. The samples were stored at 10℃, 20℃, and 25℃. The cell count data was analyzed with the Baranyi model to compute the maximum specific growth rate (μ_{max}) (log CFU/g/h) and lag phase duration (*LPD*) (h). The kinetic parameters were analyzed using a polynomial equation, and a dynamic model was developed using the kinetic models. The model performance was evaluated using the accuracy factor (A_f), bias factor (B_f), and root mean square error (*RMSE*). *E. coli* cell counts increased (p<0.05) in pig trotter samples at all storage temperatures (10–25℃). *LPD* decreased (p<0.05) and μ_{max} increased (p<0.05) as storage temperature increased. In addition, the value of h_0 was similar at 10℃ and 20℃, implying that the physiological state is similar between 10ºC and 20ºC. The secondary models used were found to appropriately evaluate the effect of storage temperature on *LPD* and μ_{max}. The developed kinetic models showed good performance with an *RMSE* of 0.618, B_f of 1.02, and A_f of 1.08. In addition, performance of the dynamic model was also appropriate. Thus, the developed dynamic model in this study can be applied to characterize the kinetic behavior of *E. coli* in cooked pig trotters during storage.

Key words: Escherichia coli, pig trotters, dynamic model, mathematical model

INTRODUCTION

Cooked pig trotters, also called *Jokbal*, are a popular food in Korea. However, a survey by the Ministry of Food and Drug Safety found that 80.1% of cooked pig trotters were stored at room temperature, with 50.1% of respondents believing that there is a risk for foodborne illness associated with cooked pig trotters (MFDS, 2012). In fact, in June 2013, a foodborne illness was caused by pig trotters in a high school in Incheon, South Korea, which was identified to be caused by enteroaggregative *Escherichia coli* (EAEC) (Shin et al., 2015). A report by the Korea Consumer Agency (KCA, 2017) showed that 1 in 6 cooked pig trotters were contaminated with *E. coli*. Thus, the infection of cooked pig trotters with *E. coli* needs to be evaluated to allow for implementation of appropriate measures of food hygiene control.

Most foodborne illnesses are caused by bacteria. According to the World Health Organization (WHO), *E. coli* is the leading cause of foodborne illness (Thangavel and Subramaniyam, 2019, WHO, 2018). *E. coli* are facultative anaerobic, gram-negative bacilli that are mainly isolated from human or animal feces (Djaja and Wispriyono, 2018). Thus, animal-derived foods are likely to be contaminated with *E. coli*. Pathogenic *E. coli* are categorized into five major pathotypes: enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and EAEC (Jang et al., 2017, Olsen et al., 2000). In addition, *E. coli* grows well at room temperature. As pig trotters are generally stored at room temperature, their consumption is likely to result in the spread of foodborne illness (Park et al., 2013, Park et al., 2014).

In 2018, the WHO recommended storing foods at safe temperatures as a way to prevent the transmission of *E. coli*. However, changes in temperature during the transport of food products, among other factors, hinders the characterization of the kinetic behavior of *E. coli*. A predictive model is needed to help characterize the kinetic behavior of *E. coli* using

parameters, such as lag phase duration (*LPD*) and maximum specific growth rate (μ_{max}), followed by the development of a dynamic model using kinetic parameters (Ha et al., 2019, Lee et al., 2019).

We developed a dynamic model to predict the pathogenic *E. coli* cell counts in cooked pig trotters at a range of temperatures.

MATERIALS AND METHODS

Sample and *E. coli* inoculum preparation. Pig trotter samples were cut aseptically into 25-g portions and transferred into filter bags. Single colonies of *E. coli* strains NCCP11142 (EHEC; isolation sources have not been identified and serotype is O157), NCCP14037 (ETEC; isolated from ascites and the serotype is O6), NCCP14038 (atypical EPEC negative for the *bfpA* gene; isolated from stool and the serotype is O15), NCCP14039 (EAEC; isolated from stool), and NCCP15661 (typical EPEC positive for the *bfpA* gene; isolation sources have not been identified) were each inoculated into 10 mL tryptic soy broth (TSB; Becton, Dickinson and Company, Franklin Lakes, NJ, USA), and incubated at 37℃ for 24 h. Thereafter, 0.1 mL aliquots of the cultures were each inoculated into 10 mL fresh TSB, followed by incubation at 37℃ for 24 h. The subcultures were harvested by centrifugation at 1,912 × *g* and 4℃ for 15 min. The resulting pellets were washed twice with phosphate-buffered saline (PBS) (KH_2PO_4 (0.2 g), $Na_2HPO_4 \cdot 7H_2O$ (1.5 g), NaCl (8.0 g), and KCl (0.2 g) in distilled water (1 L) [pH 7.4]). The suspension of each strain was mixed and serially diluted using PBS to adjust the *E. coli* count to 5-6 log CFU/mL for use as the inoculum.

Inoculation and growth analysis. The 0.1-mL of *E. coli* inoculum was inoculated onto the sample surface in filter bags. The samples were then rubbed vigorously to spread and attach

the bacterial cells onto their surface. Pig trotter was usually stored at room temperature after cooking, and thus, it was stored at 10℃ to 25℃. *E. coli*-inoculated samples were stored aerobically at 10℃, 20℃, and 25℃ for up to 192 h, and analyzed at appropriate intervals during storage to determine the *E. coli* cell counts. The samples were homogenized with 50 mL 0.1% BPW using a pummeler for 1 min and the resulting homogenates were plated on Petrifilm™ *E. coli*/Coliform Count plates. The plates were incubated at 37℃ for 24 h, and the resulting colonies were then counted manually.

Calculation of the kinetic parameters. To calculate the kinetic parameters, such as *LPD* (h) and μ_{max} (log CFU/g/h), the *E. coli* cell counts determined for each storage temperature were fitted with Baranyi model using DMfit curve-fitting software (Institute of Food Research, Norwich, United Kingdom) for primary modeling (Baranyi and Roberts, 1994). To analyze the effect of temperature on *LPD* and μ_{max} were fitted with a polynomial equation in SigmaPlot 10.0 (Systat Software, San Jose, CA) for secondary modeling.

Validation of model performance. The performance of the developed models was validated with the root mean square error (*RMSE*), bias factor (B_f), and accuracy factor (A_f). The *RMSE*, B_f, and A_f were calculated by comparing the predicted and observed values obtained from different sets of experiments at 15℃ and 23℃. The following equations were used:

$$RMSE = \sqrt{1/n \times \sum(\text{observed data} - \text{predicticed data})^2} \quad \text{(Eq. 1)}$$

$$B \text{ factor} = 10^{[\sum \log((predictive\ values/observed\ values)/n]} \quad \text{(Eq. 2)}$$

$$A \text{ factor} = 10^{[\Sigma|\log(predictive\ values/observed\ values)|/n]} \quad \text{(Eq. 3)}$$

where *n* represents the number of data points.

Kinetic behavior of *E. coli* under changing temperature. To elucidate the kinetic behavior of *E. coli* during the transportation and storage of pig trotters, a dynamic model was developed using the equation presented in a study by Baranyi and Roberts (1994). To compare the cell counts simulated by the dynamic model with recovered *E. coli* cell counts at the same temperature profile used for model simulation, the samples inoculated with *E. coli* inoculum were exposed to changing temperatures from 10℃ to 25℃. During storage, the *E. coli* cell counts were determined as described previously.

Statistical analysis. The kinetic parameters (*LPD*, μ_{max}, and h_0) of *E. coli* in pig trotters were analyzed with the general linear model procedure in SAS® (version 9.4 SAS Institute, Cary, NC). The LS mean comparison in the data was analyzed with a pairwise *t*-test at α=0.05.

RESULTS AND DISCUSSION

To predict *E. coli* cell counts in pig trotters, *E. coli* cell counts were fitted with the Baranyi model (Baranyi and Roberts, 1994). The primary model was able to appropriately describe the kinetic behavior, with an R^2 of 0.894–0.973 (Table 1). The model showed that the *E. coli* cell counts in pig trotters increased during storage at 10℃, 20℃, and 25℃ (Fig. 1). At 10℃, denoted as the threshold temperature of refrigeration *(8)*, *E. coli* populations were maintained at 4 log CFU/g until approximately 24 h, but gradually increased as storage

time increased (Fig. 1). In Korea, the maximum temperature for refrigeration is 10℃ in the regulation (MFDS, 2020). As expected, the μ_{max} values were higher (p<0.05) at high temperatures than at low temperatures (Table 1). The *LPD* values decreased (p<0.05) as the temperature increased, and the values were 26.90 h for 10℃, 4.58 h for 20℃ , and 3.77 h for 25℃ (Table 1). Even at 10℃, *E. coli* initiated growth after 26.90 h, as indicated by *LPD*, at 0.01 log CFU/g every hour, as indicated by μ_{max} (Table 1). This result can be used to predict *E. coli* growth in pig trotters. h_0 is used to indicate the initial physiological state of *E. coli* in new environments (Baranyi and Roberts, 1994), and the values (0.34 and 0.44) were similar among the storage temperatures, especially at 10℃ and 20℃ (Table 1). This indicates that the physiological conditions of *E. coli* at 10℃ were not different from those at 20℃. This indicates that despite being stored to 10℃ for a long time, if the temperature is raised to 20℃, *E. coli* is able to grow as if exposed only to 20℃. To evaluate the effects of storage temperature on the kinetic parameters, secondary modeling was conducted. The *LPD* and μ_{max} values were fitted to a polynomial model, with fitting completed at an R^2 of 0.897–0.942 (Fig. 2). This means that the secondary model was appropriate for evaluating the effects of temperature on the kinetic parameters of *E. coli* in pig trotters. To validate the accuracy of the developed models, the models were validated with the results from the different sets of studies and conducted at 15℃ and 23℃. The validation resulted in a B_f and A_f of 1.02 and 1.08, respectively. According to Ross (1999), a model is considered to have a “good” performance when the B_f value is between 0.9 and 1.05, and the A_f value is below 1.15. Also, *RMSE* was calculated as 0.618, indicating that the performance of the model developed in this study was determined to be good. The majority of the data points were found to regress to the line, as shown in Fig. 3, denoting the observed data that were the same as the predicted values. These results indicate that the primary and secondary models can be used to characterize the growth of *E. coli* in pig trotters (Fig. 3). However, because pig trotters are

often stored at varying temperatures, we also developed a dynamic model using the equation suggested by Baranyi and Roberts (1994) and the results obtained by the primary and secondary models. When the dynamic model was used to simulate *E. coli* growth in pig trotters stored at temperatures ranging from 10℃ to 25℃, the predicted data were also found to be close to the observed values. Our findings indicate that the dynamic model can be used to describe the fate of *E. coli* in pig trotters. Our simulated results showed that *E. coli* cell counts increased in pig trotters when temperatures varied between 10℃ and 25℃ (Fig. 4).

In summary, the models developed in this study provide an accurate description of the kinetic behavior of *E. coli* in pig trotters. *E. coli* was found to grow in pig trotters even at 10℃, after approximately 26 h, with an increase of 0.01 log CFU/g every hour. In addition, the physiological state of *E. coli* at 10℃ was not very different from that at other high temperatures. Thus, *E. coli* subjected to 10℃ for long may grow at higher temperatures in a manner similar to when exposed to only the higher temperature. Thus, as such, the models can be used to predict of *E. coli* cell counts in pig trotters under changing temperature.

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Author Contributions

Ha J and Yoon Y participated in the design of the study, analyzed the data, and wrote the manuscript. Lee J, Oh H, and Kim HJ helped draft the manuscript. Choi Y, and Lee Y performed the experiments. Kim Y, Lee H, and Kim S performed the experiments and coordination of the study. All authors read and approved the final manuscript.

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FIGURE LEGENDS

Fig. 1. Population of *Escherichia coli* in pig trotters during storage at 10℃ (A), 20℃ (B), and 25℃ (C) for up to 192 h.

Fig. 2. Secondary models for kinetic parameters (*LPD*, A; μ_{max}, B) for *Escherichia coli* in pig trotters. Data are presented as the mean and standard error.

Fig. 3. Comparison between the observed cell counts and the predicted cell counts of *Escherichia coli* at 15℃ and 23℃.

Fig. 4. Dynamic model for *Escherichia coli* in pig trotters (symbol: observed values; solid line: predicted values; short dash line: 95% interval; dotted line: dynamic temperature from 10℃ to 25℃).

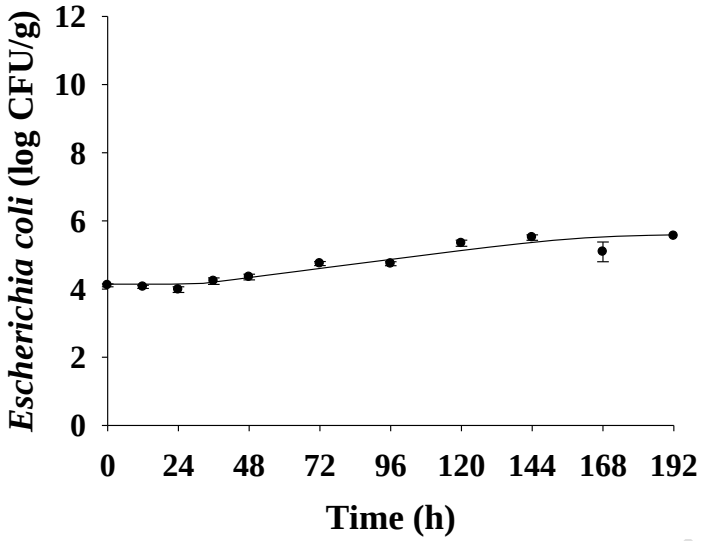
Table 1. Kinetic parameters of primary model fitted by the Baranyi model for *Escherichia coli* in pig trotters during storage at 10°C, 20°C, and 25°C for up to 192 h

Storage temperature (°C)	Lag phase duration (h)	μ_{max} (log CFU/g/h)	h_0	R^2
10	26.90 ± 5.01^{A}	0.01 ± 0.00^{B}	0.34±0.00	0.894
20	4.58 ± 3.35^{B}	0.11 ± 0.05^{AB}	0.44±0.16	0.972
25	3.77 ± 2.51^{B}	0.27 ± 0.07^{A}	1.10±0.95	0.973

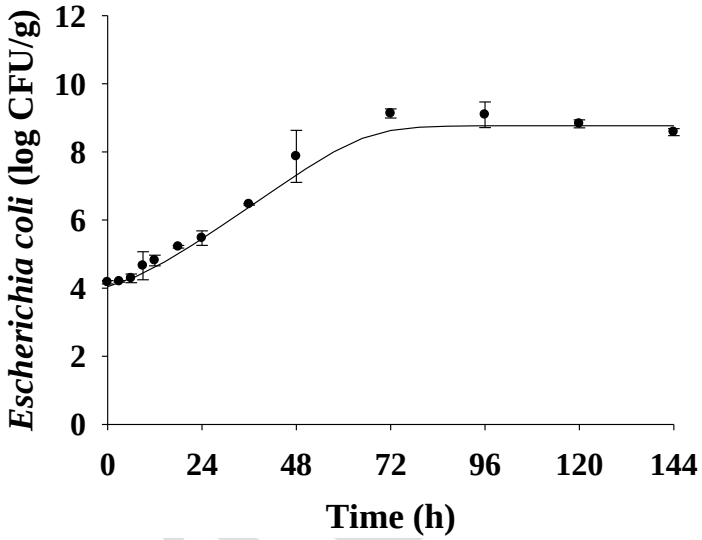
[A-B] Different letters mean significantly different at $p<0.05$.

Figure 1.

A. 10°C



B. 20°C



C. 25°C

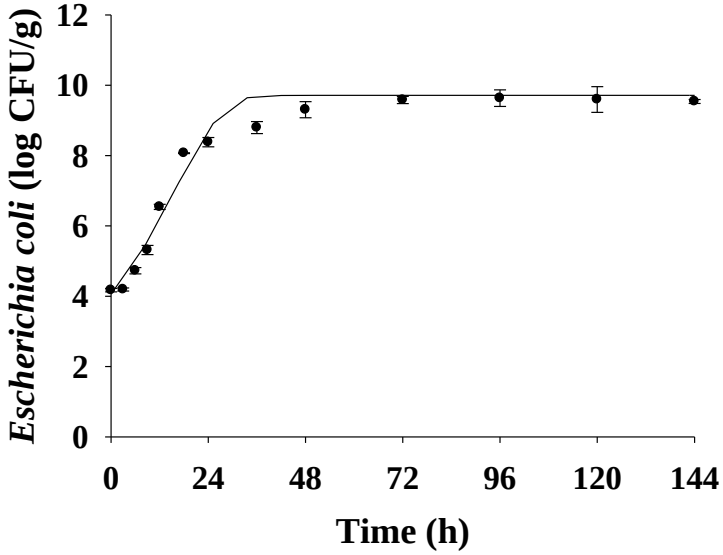
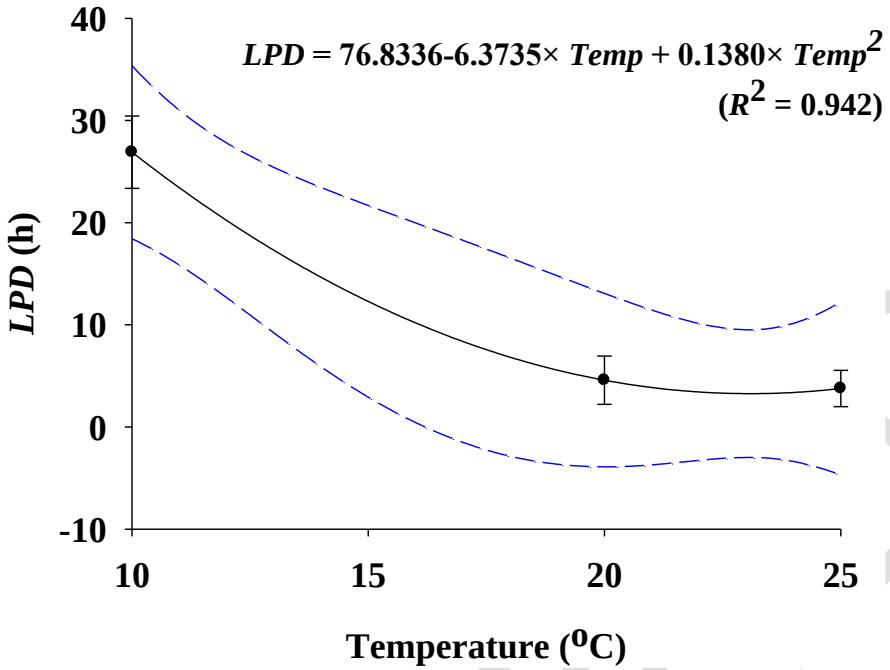
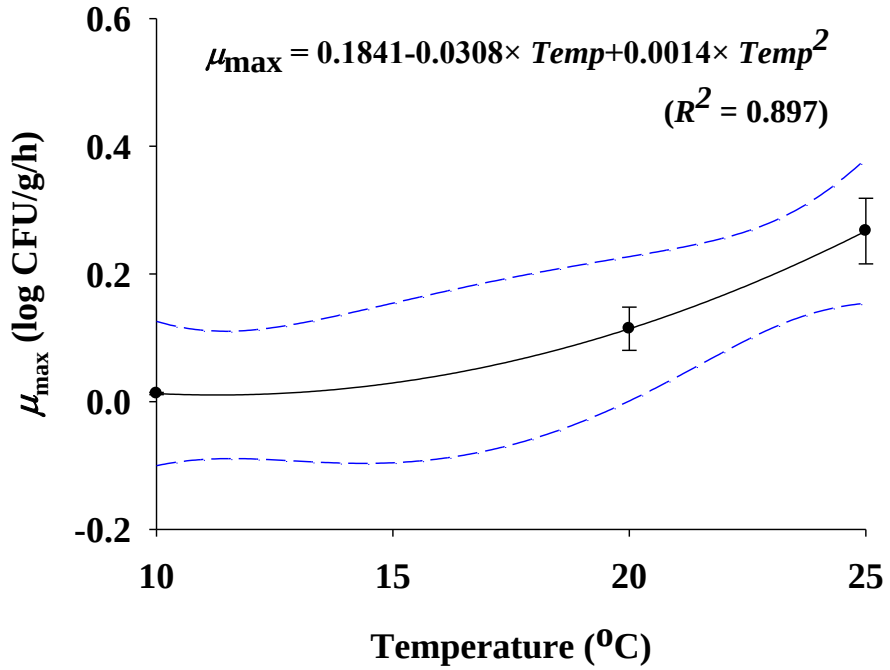


Figure 2.

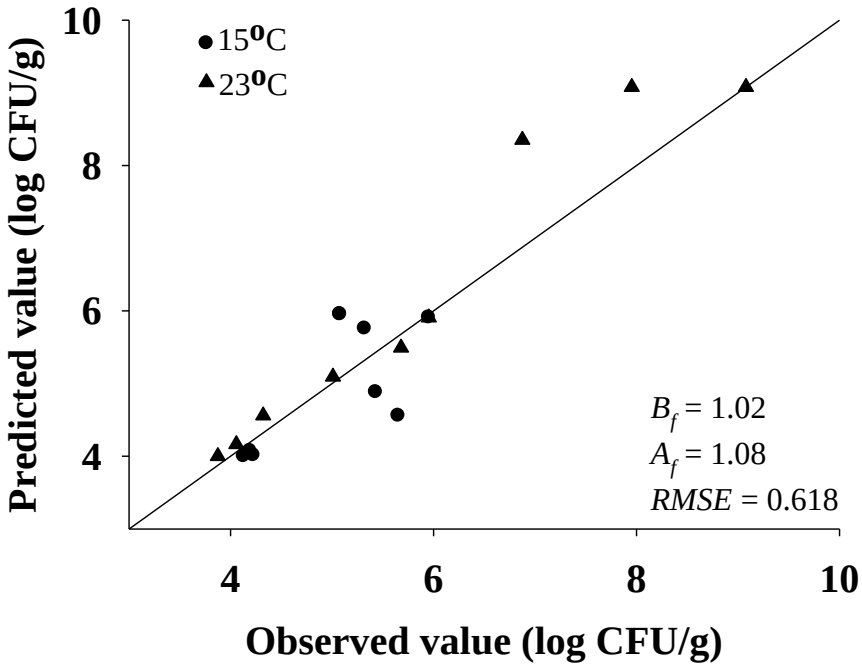
A.


LPD = 76.8336-6.3735× Temp + 0.1380× Temp2
(R2 = 0.942)
LPD (h)
40
30
20
10
0
-10
10
15
20
25
Temperature (oC)

B.


μmax = 0.1841-0.0308× Temp+0.0014× Temp2
(R2 = 0.897)
μmax (log CFU/g/h)
0.6
0.4
0.2
0.0
-0.2
10
15
20
25
Temperature (oC)

269 **Figure 3.**



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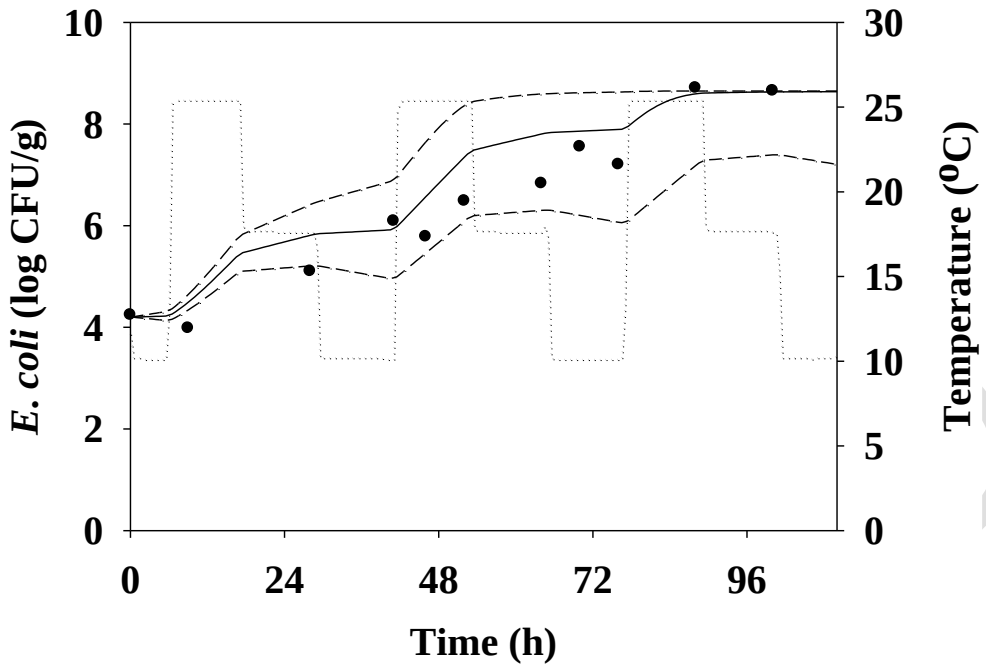
272 **Figure 4.**



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