Reaktor

p-ISSN 0852 – 0798
e-ISSN 2407 – 5973

Acredited: SK No.: 60/E/KPT/2016
Website : http://ejournal.undip.ac.id/index.php/reaktor/

Reaktor, Vol. 19 No. 4, December Year 2019, pp. 152-161

Prediction of Microbial Population in Sorghum Fermentation through Mathematical Models

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(Received: August 7, 2019 Accepted: November 28, 2019)

Abstract

The mathematical models can be used as a tool in predicting microbial population in sorghum fermentation, either spontaneous fermentation or fermentation with the addition of lactic acid bacteria (LAB) inoculum. Gompertz model modified by Gibson, Gompertz model modified by Zwietering, Baranyi-Robert model, Fujikawa model, Richards model, Schnute model were used in predicting the growth of lactic acid bacteria (LAB) and coliform bacteria during spontaneous fermentation, and also the growth of LAB during fermentation with the addition of inoculum. Meanwhile, there was death (inactivation) of coliform bacteria during sorghum fermentation with the addition of LAB inoculum. The Geeraerd model and the Gompertz model modified by Gil et al. were used to predict the inactivation. The accuracy and precision of models were evaluated based on the Root Mean of Sum Square Error (RMSE), coefficient of determination (R^2), and curve fitting. Gompertz model modified by Gibson had the highest accuracy and precision, which was followed by the accuracy of the Fujikawa model and Baranyi-Robert model in predicting the growth of LAB and the growth of coliform bacteria during spontaneous fermentation. Meanwhile, in predicting LAB growth during fermentation with the addition of inoculum, high accuracy and precision was obtained from Richards and Schnute models. In predicting the inactivation of coliform bacteria, Geeraerd model provided higher accuracy and precision compared to Gompertz model modified by Gil et al.

Keywords: *fermentation; inoculum; mathematical; model; sorghum; spontaneous*

How to Cite This Article: Laila, U., Nurhayati, R., Utami, T., and Rahayu, E.S. (2019), Prediction of Microbial Population in Sorghum Fermentation through Mathematical Models, Reaktor, 19(4), 152-161, http://dx.doi.org/10.14710/reaktor.19.4.152-161.

INTRODUCTION

Sorghum is a cereal commodity which its productivity ranks fifth in the world after other cereal crops, i.e. wheat, rice, corn and barley. This plant is predominantly cultivated in Mexico and America which both countries occupy first and second position as the largest producer (Wong *et al.*, 2009). Sorghum is increasingly popular in African and Asian countries because of its ability to survive on dry land. In

Indonesia, exactly in the East Nusa Tenggara province, sorghum is used as a staple food.

The dominant nutrients in dried sorghum grain are protein (7.5 -10%) and starch (70-80%), depending on the type of grain and land. The content of these nutrients is almost similar as wheat. However, protein and starch digestibility in sorghum is very low. In fact, sorghum's starch digestibility is the lowest among other cereals (Kulamarva *et al.*, 2010). The phenomenon is affected by structure and components of sorghum. It is known that grain of sorghum consists of three parts: outer protector part called as pericarp, embryo (germ) part, and endosperm part. The distribution of starch granules of these three parts is different. The percentage of the pericarp and embryo on the grain (kernel) of sorghum is small, so that the starch content in both parts is also small. Meanwhile, the biggest starch content is found in the endosperm. Sorghum's endosperm was divided into an outer part called as aleurone layer endosperm and an inside part called as subaleurone layer (peripheral) endosperm. Peripheral endosperm consists of the part called corneous endosperm and also the deepest part called floury endosperm. Starch granules of the corneus endosperm that are resistant to water or enzymatic hydrolysis are surrounded by body protein and attached to a matrix composed of non-starch protein and carbohydrate. They have hard and glassy appearance. Meanwhile, starch granules in floury endosperrm have dense and soft characteristics (like flour) and they are easy to undergo enzymatic hydrolysis.

Protein in sorghum is divided into two groups, namely the kafirin protein and non-kafirin protein (i.e. globulin, albumin, prolamin and glutelin). Kafirin is the main protein constituent of body protein which surround starch granules of corneus endosperm. Until 70-80% of the total protein of sorghum flour is kafirin. Kafirin is very easy to form disulfide bond which is resistant to undergo lysis by protease enzyme. It automatically reduces digestive capacity and starch gelatination. Non-kafirin protein is also a barrier to starch digestibility because this protein is the constituent of the protein matrix in which the body protein is attached, as it has been known that starch granules are surrounded by body protein. In compiling protein matrix, non-kafirin protein is cross-linked with the kafirin protein. Sorghum cooking facilitates this cross-linking to easily undergo conformation which subsequently form a disulfide complex, so that digestion capacity and starch gelatination will decrease.

Besides being due to endosperm protein factor, low starch digestibility is also caused by the presence of tannin compound in sorghum. Tannin forms a complex with proteins and has the characteristic as inhibitor of the X-Amilose enzyme (Dreher *et al.*, 1984). The protein digestibility of sorghum is also low, which is only 30-70% (Silano, 1977). This value is lower than protein digestibility of corn, which is 78.5%. This fact is due to amino acid bioavailability and inhibition of protein digestibility by tannin (Bach Knudsen *et al.*, 1988). Another factor of sorghum's starch disgestibility is cooking which can reduce prolamin solubility (Hamaker *et al.*, 1986).

One process that can be applied to improve starch and protein digestibility of sorghum is fermentation. Yousif and El Tinay (2001) said that fermentation will increase protein availability through partial degradation of protein complex by enzymatic activity (enzymatic hydrolysis). In the fermentation process, the target of enzymatic hydrolysis (proteolytic activity) is the kaffirin-rich protein matrix. In this process, water soluble protein is produced within kafirin in the form of its originality. In the process of fermented-sorghum cooking, kafirin will form aggregates that are not water-soluble. The process will release starch granules which are previously bound to kafirin (Elkhalifa *et al.*, 2007). The fermentation process will also increase the fraction of albumin and globulin, and also reduce the tannin content, even until 92% of the total tannin that is bound to the complex with protein. It is might due to the activity of microorganisms.

Fermentation of sorghum flour can be done spontaneously or with the addition of lactic acid bacteria (LAB) inoculum. One of studies was conducted by Utami *et al.* (2015). In that study, spontaneous fermentation was carried out through fermentation naturally by original microorganisms of sorghum flour. Meanwhile, in the fermentation with the addition of LAB starter (inoculum), the bacterial inoculum (*Lactobacillus plantarum* inoculum) isolated from sorghum flour was added to the sorghum fermentation system without sterilizing sorghum flour first, so there were still contribution of microorganisms from sorghum flour. The target in that study was to increase starch and protein digestibility through microorganism activity. In that study, it was found that spontaneous (natural) fermentation induced the population of LAB and coliform bacteria respectively from 1.45 log and 2.85 log to 9.14 log and 7.98 log for fermentation time of 24 hours. The final population of coliform bacteria was high because organic acids (lactic acid, etc.) produced by the activity of lactic acid bacteria had not been able to suppress coliform bacteria yet. The fact had been strengthened by the final pH of 4.75. Meanwhile, in fermentation with the addition of the *Lactobacillus plantarum* starter, the population of LAB and coliform bacteria changed respectively from 7.58 log and 3.49 log to 9.28 log and 0 log for fermentation time of 24 hours. From the Utami's data of sorghum fermentation with addition of LAB inoculum, the population of LAB reached maximum value at time fermentation of 6 hours and there was the suppression of the coliform population. It was due to the high population of lactic acid that automatically increase the concentration and amount of organic acid produced. Furthermore, the pH would decrease with the final pH of 3.41 (coliform bacteria can not live at pH below 4).

The decreasing of coliform bacteria by the increasing of the LAB population is the target aspect in the process of sorghum fermentation. Sorghum

fermentation can be interpreted as an effort to achieve the food safety of sorghum product. In the fermentation and controlling microorganisms field, to determine the status of bacterial populations, sampling every time have not to be done. This can provide efficiency of time, effort and cost (Teleken *et al.*, 2018). The mathematical model is one of the tools for predicting bacterial population, both in the scope of fermentation and also the shelf life and distribution of product. Increasing the scale of fermentor (scaling-up) can also be done through mathematical model of bacterial population during fermentation process. Mathematical models can also help in ensuring food security through prediction of pathogen population during product storage and distribution.

There are various mathematical models of bacterial population kinetic. Generally, those are divided into two groups, namely empirical-based models and mechanistic-based models. The empirical model describes population kinetics without involving parameters that related to microorganism growth. One empirical model is Monod model which is very old and familiar in the microbiology scope, but this model has limitation because bacterial population is dependent variable of the substrate. This model is reliable for the growth kinetics of the logarithmic phase. However, by the fact that the population curve of the growth of microorganisms is sigmoid, then other empirical models such as Gompertz model, Fujikawa model, Baranyi-Robert model, Richards model, and Schnute model are more reliable to be used. The empirical model usually describes sigmoidal function between microorganism populations and time. Meanwhile, mechanistic model is a model that describe the mechanism and the causes of changes in microorganism population. This model is developed fundamentally from theoretical and experimental data by involving parameters related to the growth of microorganisms, such as pH, temperature, water activity, substrate content, CO_2 levels, etc.

This study was aimed to predict microorganism population in spontaneous fermentation and also fermentation with inoculum addition of sorghum flour by applying several empirical models, such as the Gompertz model, Fujikawa model, Baranyi-Robert model, Richards model, and Schnute model. The parameters involving with the model were estimated by minimizing the Root Mean of Sum Square Error (RMSE) and coefficient of determination (R^2). The model was selected based on the value of RMSE and R^2. Through the mathematical model application, it is expected to obtain suitable models for describing microbial population in sorghum fermentation. It is expected that the application of mathematical models as tools in predicting microorganism populations will provide benefits, those give efficiency in controlling microorganisms in the fermentation process and also provides growth data for scaling-up of fermentation. Regarding with few national publications in which use mentioned empirical model besides Monod model, this research is expected for introducing other empirical models already mentioned to national academician and microbiologist.

RESEARCH METHOD

The data obtaining

Data was taken according to Utami's research including spontaneous (natural) fermentation and fermentation with addition of lactic acid bacteria (LAB) inoculum (Utami *et al.*, 2015). Briefly, the experiment was conducted according to Sudanese housewives (Mohammad *et al.*, 1991). The experiment was initiated by mixing sorghum flour with sterilized water in ratio of 1:2 (w/v). Furthermore, the mixture was incubated and fermented in sterilized jar at 30ºC for 24 hours. Because the sorghum flour was not sterilized, the original microorganisms performed in spontaneous fermentation. Meanwhile, fermentation with addition of lactic acid bacteria (LAB) inoculum involved with either original microorganism or lactic acid bacteria *Lactobacillus plantarum* S4512 inoculum that was prepared previously by isolation from natural fermentation. The inoculum was inoculated by concentration of 1% v/v (about 10^9 cfu/ml) into mixture medium consisting of unsterilized sorghum flour and sterilized water in ratio of 1:2 (w/v). Furthermore, the fermentation was conducted at 30ºC for 24 hours. Sampling for microbial counts was carried out at 0, 2, 4, 6, 8, 12, 16, 20, and 24 h. The microbial data are shown at Table 1 and Table 2.

Table 1. Population of lactic acid bacteria (LAB) and coliform bacteria during sorghum spontaneous fermentation

Fermentation Time (hour)	Lactic acid bacteria (LAB) (log cfu/ml)	Coliform bacteria (log cfu/ml)
0	1.45	2.85
2	1.60	3.50
4	2.52	3.73
6	4.10	4.01
8	4.94	4.81
12	7.22	6.21
16	8.39	7.73
20	9.10	7.79
24	9.14	7.98

Table 2. Population of lactic acid bacteria (LAB) and coliform bacteria during sorghum fermentation with addition of lactic acid bacteria (LAB) inoculum

Fermentation Time (hour)	Lactic acid bacteria (LAB) (log cfu/ml)	Coliform bacteria (log cfu/ml)
0	7.58	3.49
2	8.49	3.55
4	8.65	3.52
6	8.89	3.35
8	9.04	2.83
12	9.10	1.31
16	9.22	0
20	9.25	0
24	9.28	0

Brief description of empirical models

Generally, the kinetics of microbial population consist of growth kinetics and inactivation kinetics. The difference lies in the rate of change of the population. Microbial growth has a positive rate of population change, while microbial inactivation has a negative rate (Bernaertz *et al.*, 2006).

Microorganism growth kinetic model

The growth kinetics of microorganism (bacteria) include several phases, namely lag phase, logarithmic/exponential phase, and stationary phase. Lag phase is the phase where microorganism adapt and survive in a new environment. After microorganism can adapt to the new environment, the microorganism will grow in a number until the growth rate reach maximum rate. This phase is called as logarithmic/exponential phase. This condition is supported by sufficient substrates. As time goes on, environmental condition and adequacy of the substrate have begun to be diminished, so that the population of microorganisms will be in constant value. This means that the amount of microorganism growth is the same as the amount of microorganism mortality. This phase is called as stationary phase. The several growth kinetic models of microorganism that had been developed are as follow:

Gompertz model

The Gompertz model is a classical model that was originally formulated to describe human mortality data (Okpokwasili and Nweke, 2005). This model illustrates the exponential relationship between population density and specific growth rate, with the following formula:

$$N = C.\exp\left\{\exp\left[-B\left(t-M\right)\right]\right\} \quad (1)$$

Where t = time, N = population density at time t, C = upper asymptotic value, that is the maximum population density, M = time at which the absolute growth rate is maximal, and B = relative growth rate at M time.

Gibson *et al.* (1987) modified the Gompertz model, so that it can describe the relationship between cell population over time, with the following formula:

$$\log(N)=A+D.\exp\left\{-\exp\left[-B\left(t-M\right)\right]\right\} \quad (2)$$

Where N = population density at time t, A = value of the lower asymptote (log N(-∞)), D = difference in value of the upper and lower asymptote [log N(∞) – log N(- ∞)], M = time at which the exponential growth rate is maximum.

Zwietering *et. al* (1990) conducted reparameter to the Gompertz model by including biological parameters such as the Umax and lag time (λ), through the following formula:

$$\ln\left(\frac{N}{N_0}\right)=A.\exp\left\{-\exp\left[\frac{\mu_{max}.e}{A}(\lambda-t)+1\right]\right\} \quad (3)$$

Where N = population density at time t, N_0 = population density at initial time t_0, Umax = maximum specific growth rate, λ = lag time, and A = asymptote ($\ln(N_\infty/N_0)$).

Richards Model

The original equation of Richards model (Richards, 1959) was as following:

$$y=a\left\{1+\nu.\exp\left[k(\tau-x)\right]\right\}^{-1/\nu} \quad (4)$$

By Zwietering's reparameter (Zwietering *et al.*, 1990), the following formula was produced:

$$\ln\left(\frac{N}{N_0}\right)=A\left\{1+\nu.\exp(1+\nu).\exp\left[\frac{\mu_{max}}{A}.(1+\nu)^{\left(1+\frac{1}{\nu}\right)}.(\lambda-t)\right]\right\}^{(-1/\nu)} \quad (5)$$

Where ν = shape parameter

Schnute Model

The Schnute model equation (Schnute, 1981) was as follow:

$$y=\left\{y_1^b+\left(y_2^b-y_1^b\right).\frac{1-\exp\left[-a(t-\tau_1)\right]}{1-\exp\left[-a(\tau_2-\tau_1)\right]}\right\}^{1/b} \quad (6)$$

With Zwietering's reparameter (Zwietering *et al.*, 1990), the following formula was produced:

$$\ln\left(\frac{N}{N_0}\right)=\left(\mu_{max}\frac{(1-b)}{a}\right)\left[\frac{1-b.\exp(a.\lambda+1-b-at)}{1-b}\right]^{1/b} \quad (7)$$

Where a and b are mathematical parameters in sigmoidal curve.

Fujikawa Logistic Model

The logistic model was first developed by Verhulst, 1838 with the existence of inhibitory functions to describe the stationary phase (Horowitz *et al.*, 2010), with the following formula:

$$\frac{dN}{dt} = \mu.N.\left[1-\frac{N}{N_{asympt}}\right] \quad (8)$$

Where N_{asympt} is the asymptotic population, which correspond to the population size at the "stationary
phase

There were several modifications to the logistics model, one of them was Fujikawa modification (Fujikawa and Morozumi, 2005) with the following formula:

$$dN/dt=\mu_{max}.N.\left[1-\left(\frac{N}{N_{max}}\right)^m\right]\left[1-\left(\frac{N_{min}}{N}\right)^n\right] \quad (9)$$

Where m and n are constant parameters (m and n > 0) which related to the curvature of the deceleration phase and the period of the lag phase, respectively (Fujikawa *et al.*, 2014).

The modified logistic model (Fujikawa model) accommodates the adaptation phase (lag phase) where the growth rate at this phase is very low. To represent

it, a parameter (factor) that control the rate of bacterial growth was developed. In this case the parameter is N_{min}, which is almost the same as the bacterial population initial time, N_0. Fujikawa recommended that Nmin value is 1 ppm smaller than N_0 ($N_{min} = (1\text{-}10^{-6})$ x N_0.

Baranyi and Robert Model

This model is a combination of the Michaelis-Menten Model and the logistic model (Baranyi and Robert, 1995), with the following formula:

$$\frac{dN(t)}{dt} = \left[\frac{Q(t)}{1+Q(t)}\right].\mu_{max}\cdot\left[1-\frac{N(t)}{N_{max}}\right].N(t)$$

$$\frac{dQ(t)}{dt} = \mu_{max}.Q(t) \quad (10)$$

$Q(t)$ is physiological state of cell growth.

The Baranyi and Robert model provides biological interpretations for lag phases through physiological state parameters (Baranyi and Robert, 1995). Because the Baranyi and Robert model can illustrate a few external factors related to the growth of microorganisms through physiological state parameters for the lag phase, this model is often called a semi-mechanistic model, even some scientist classify it into the mechanistic model (Teleken *et al.*, 2018).

Inactivation model (death phase)

The empirical models of microbia growth have mentioned already does not accommodate inactivation/death phase because it usually accommodates until the stationary phase. Generally, bacterial inactivation includes mechanisms, namely shoulder phase that describes a slow microbial inactivation response, a log-linear phase that describes the maximum rate of inactivation, and the tailing phase that describes microbial populations that are resistant to inactivation (Bernaerts *et al*, 2004). Several empirical models of microorganism inactivation had been developed, including:

Geeraerd model

The Geeraerd model which is a sigmoid model accommodates three mechanisms in microbial inactivation processes, namely shoulder, log-linear, and tailing phase (Geeraerd *et al.*, 2000), with the following formula:

$$\frac{dN(t)}{dt} = -\left[\frac{1}{1+C_c(t)}\right].k_{max}\cdot\left[1-\frac{N_{res}}{N(t)}\right].N(t)$$

$$\frac{dC_c(t)}{dt} = -k_{max}.C_c(t) \quad (11)$$

Where $C_c(t)$ is physiological state of cell inactivation, k_{max} is specific inactivation rate, and N_{res} is residual population.

Gompertz model modified by Gil *et al.*

Gil, Miller, Silva, and Brandao modified the Gompertz model to predict microbial inactivation through a similarity approach to the modification of the Gompertz model by Zwietering (Gil *et al.*, 2011), so that the model was obtained as following:

$$\log\left(\frac{N}{N_0}\right)=\log\left(\frac{N_{res}}{N_0}\right)\exp\left(-\exp\left(\frac{k_{max}e}{\log\left(\frac{N_{res}}{N_0}\right)}(L-t)+1\right)\right) \quad (12)$$

Where N is population at time (t), N_0 is initial population, and L is time parameter or shoulder.

Assumptions

The models mentioned previously are usually applied to single microorganism. However, in spontaneous fermentation there was not only the desired growth of microbes (lactic acid bacteria), there was also the growth of gram-negative bacteria, namely coliform bacteria. In spontaneous fermentation, the two bacteria grew with a positive growth rate. Prediction of microbial populations in spontaneous fermentation applying the growth kinetic models mentioned previously used the assumption that the two bacteria did not compete and interact each other. This was due to abundant substrate and low initial concentration of bacteria in the fermentation medium, so that the two bacteria did not collide and interact each other.

The same assumption was also applied to fermentation with the addition of lactic acid bacteria (LAB) inoculum. However, there was an added assumption that was the use of an inactivation model on the prediction of the population of coliform bacteria because the rate of change in the coliform population was negative. It was marked and initiated by slow response of microbial inactivation (shoulder phase).

Factors evaluated in the assessment of the accuracy of the kinetic model

For comparing the accuracy of among models, several parameters in the model had to be searched by minimizing Root Mean of Sum Square Error (RMSE) The minimizing was conducted by Matlab software. Some of the parameters that had to be observed are listed in Table 3.

Based on Ruhanian and Movagharnejad (2016), the RMSE value is formulated as follows :

$$RMSE=\sqrt{\frac{\sum_{i=1}^{N}(f_i-y_i)^2}{N}} \quad (13)$$

With y_i is experimental data, f_i is model (prediction) data, where N is the number of experimental points.

Table 3. Growth and Inactivation Models with Their Observed Parameter

Model	Observed Parameter
Growth Model	
Gompertz model modified by Gibson	*A,B,D,M*
Gompertz modified by Zwietering	*A*
Richards model	*A,v*
Schnute model	*a,b*
Fujikawa model	*m,n*
Baranyi and Robert model	*Q (0)*
Inactivation Model	
Geeraerd model	C_e (0)
Gompertz model modified by Gil *et al*	-

Evaluation of the accuracy and performance of the model was based on the RMSE value. Small RMSE value indicates better model fitting which mean that the model has a higher level of accuracy. From a number of references, it is found that the RMSE value of 0.7 is included in the low category for data with a range of values 0 to 1000. However, for data with a value range of 1 to 10 (data difference of 10 points), the RMSE value of 0.7 is not included in low category (Aryadoust and Raquel, 2019).

Besides RMSE, the coefficient of determination (R^2) was used to evaluate the performance of the model in predicting microbial populations, which are formulated as the following formula:

$$R^2 = 1 - SS_{res} / SS_{tot}$$

$$SS_{res} = \sum_i (y_i - f_i)^2$$

$$SS_{tot} = \sum_i (y_i - \bar{y})^2 \quad (14)$$

With y_i is experimental data, f_i is model (prediction) data , and $\bar{y}$ is the average of the experimental data.

R^2 value range from 0 to 1. The high R^2 value which is close to 1 shows that the model prediction and fitting for the experiment is good, while a low R^2 value (<0.5) illustrates the lack of accuracy of the model (Di Bucchianico, 2018).

RESULTS AND DISCUSSIONS

Spontaneous Fermentation

In spontaneous fermentation, lactic acid bacteria (LAB) and coliform bacteria underwent population growth and did not undergo inactivation (death), so that the models used to describe the population of the two bacteria were models of microorganism growth with the mentioned assumptions. Figure 1 and 2 show the prediction of the population of LAB and coliform bacteria respectively during spontaneous fermentation of sorghum through several mathematical models.

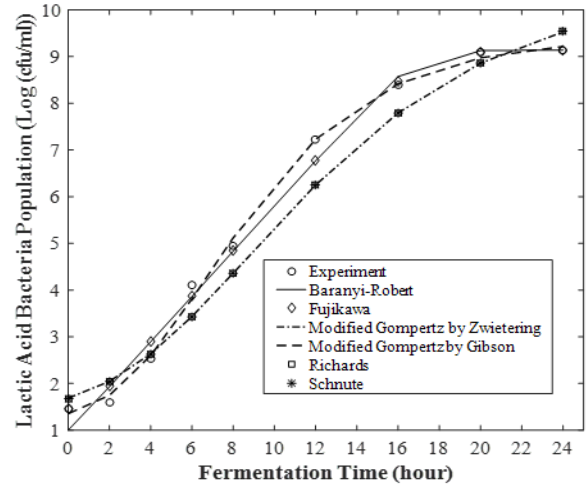

Figure 1. Population Profile of Lactic Acid Bacteria in Spontaneous Fermentation

From Figure 1, it can be seen that the model data that was closest to the experimental data was resulted by the Gompertz model modified by Gibson, which was followed successively by Fujikawa and Baranyi-Robert model. This was supported by the values of RMSE and R^2 in Table 4. The Gompertz model modified by Gibson gave the smallest RMSE value with the R^2 value that was close to 1. As be shown at Figure 1, Gompertz model modified by Gibson provided the best and most appropriate curve fitting, with all parts coincided with the experimental data, including the population at t = 0 (N_0). The Fujikawa model was slightly lower in accuracy than Gompertz model modified by Gibson because there were several population points that shifted toward the experimental points, when t = 2 hours, 4 hours and 6 hours. Meanwhile, the declining in the accuracy of the Baranyi-Robert model was caused by shifting of population at t = 0 (N_0) which was rather far from the experimental point. It made the transition coefficient (α) sharply shaped within inflection point that was not very visible. It seems that there was no lag phase in the Baranyi-Robert model.

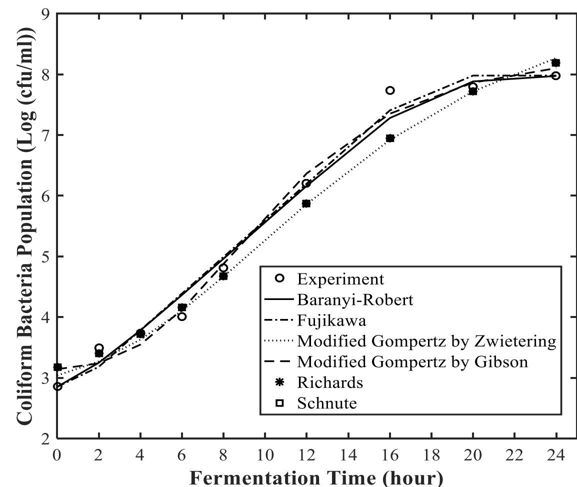

Figure 2. Population Profile of Coliform Bacteria in Spontaneous Fermentation

Table 4. Values of Observed Paramater, RMSE, R^2 Obtained from Various Growth Model Approach to LAB Population in Spontaneous Fermentation

Growth Model	Value of Observed Parameter	RMSE	R^2
Gompertz model modified by Gibson	A = 1.2471; B = 8.1454; C = 0.2204; D = 6.6603	0.1395	0.9978
Gompertz modified by Zwietering	A = 20.7284	0.5319	0.9682
Richards model	A = 20.7911; B = -0.0181	0.5319	0.9682
Schnute model	a = 0.1447; b = 0.0181	0.5319	0.9682
Fujikawa model	m = 0.7018; n = 13.4424	0.2430	0.9934
Baranyi and Robert model	q_0= 9.2256	0.2900	0.9906

From Figure 1, the models that were far from the experiment were Gompertz model modified by Zwietering, Richards model, and Schnute model. The shifting can be seen in each phase including lag phase, logarithmic phase, and stationary phase. From Table 4, RMSE and R^2 values were also similar for the three models, that were RMSE of 0.5319 and R^2 of 0.9682. However, regarding with bacterial population data in the range 0 - 10 (data range difference was 10 points), the RMSE value of 0.5319 was not concluded as small RMSE value. It can be said that the three models had low accuracy compared to other models, moreover they had lower R^2 values.

Meanwhile, the growth profile of coliform bacteria in spontaneous fermentation can be seen at Figure 2. The result of model prediction on the growth of coliform bacteria had similar tendency as the growth of lactic acid bacteria. Data from Gompertz model modified by Gibson provided curve which coincided with experimental data. Moreover, it can also be seen in Table 5 that the model had the lowest RMSE value of 0.2087 and the highest R^2 value of 0.9883. The accuracy of the Gompertz model modified by Gibson was followed by Fujikawa model with the RMSE value of 0.2164 and the Baranyi-Robert model with RMSE of 0.2182. The Gompertz model modified by Zwietering, Richards model, and Schnute model had lower accuracy compared to the three models already mentioned with the value of RMSE 0.3323; 0.3324; 0.3324 respectively. Regarding with the experimental population in range of 0 - 8 (data difference was 8 points), the all of three RMSE value could be concluded as moderate value.

Table 5. Values of Observed Paramater, RMSE, R^2 Obtained from Various Growth Model Approach to Coliform Bacteria Population in Spontaneous Fermentation

Growth Model	Value of Observed Parameter	RMSE	R^2
Gompertz model modified by Gibson	A = 3.1256; B = 5.1658; C = 0.2105; D = 8.3700	0.2087	0.9883
Gompertz modified by Zwietering	A = 14.6097	0.3323	0.9705
Richards model	A = 13.5525; B = 0.4631	0.3324	0.9722
Schnute model	a = 0.1708; b = -0.4631	0.3324	0.9722
Fujikawa model	m = 5.6949; n = 23.8844	0.2164	0.9875
Baranyi and Robert model	q_0= 0.9884	0.2182	0.9873

Fermentation with Addition of LAB Inoculum

In fermentation with the addition of LAB inoculum, according to Figure 3 and Figure 4, it can be seen that the lactic acid bacteria (LAB) grew over fermentation time, while coliform bacteria underwent inactivation (death). By comparing Figure 1 and Figure 3, it can be seen that original LAB in spontaneous fermentation required time to conduct cell division., so that there was lag phase in growth profile. Meanwhile, the addition of LAB inoculum made no lag phase in LAB growth profile. It indicated that inoculum administrated to the medium was exponentially growing culture. Moreover, the addition of LAB inoculum could accelerate LAB growth rate. It was needed faster time (that was 6 hours) to achieve maximum growth (stationary phase) in fermentation with inoculum addition than that of spontaneous fermentation, which was 14 hours. It was in agreement with research of milk fermentation by *Lactobacillus plantarum* Dad 13 which reported that addition of inoculum could reduce lag time and also increase growth rate (Wardani *et al.*, 2017). The same tendency was also found at bacterial cellulose research which obtained that inoculum increased bacterial cellulose production (Yanti *et al.*, 2018). The higher growth rate of LAB would induce an increase in the concentration of its metabolite (acid), so that the pH drop of fermentation with addition of LAB inoculum was greater than that of spontaneous fermentation. It was proven by data of Utami *et al.* (2015) which stated that pH of fermentation with the addition LAB inoculum dropped to 3.41, while pH of spontaneous fermentation altered to 4.75. It had been mentioned that coliform bacteria are difficult to grow at pH below 4.0 (Ray,

1996), so that coliform underwent an inactivation (death) process in fermentation with the addition of LAB inoculum. Meanwhile, coliform bacteria in spontaneous fermentation still had chance to grow.
Regarding with the prediction of model on the growth kinetics of LAB population in fermentation with LAB inoculum addition found at Figure 3, it appears that the Richards model and Schnute model coincided closely with the experimental data. This is agreed with the lowest RMSE values and the highest R^2 values of the two models with the same values, i.e, 0.0759 and 0.9781 respectively according to Table 6. The high accuracy was followed respectively by Gompertz model modified by Gibson, Fujikawa model, Gompertz model modified by Zwietering, Baranyi-Robert model with RMSE and R^2 values which can be seen in Table 6. Based on the RMSE values of Gompertz model modified by Zwietering and Baranyi-Robert model and also the range of data which was from 7.4 to 9.4 (the difference of data range was only 2 points), the RMSE values of two model were not included in the low category. This indicates that the accuracy of the two models were classified to low accuracy. This can also be seen from the shifts which were rather far away during the stationary phase for the two models.

There was a difference between the accuracy of the growth model in predicting the growth of LAB population during inoculum fermentation and the accuracy of the prediction of LAB and coliform bacteria population growth during spontaneous fermentation. The Gompertz model modified by Gibson, Fujikawa model, and Baranyi-Robert model that provided high accuracy and precision and also the best fitting curve for the prediction of LAB and coliform bacteria population growth during spontaneous fermentation did not show similar results in the prediction of BAL population growth during fermentation with addition of inoculum. High accuracy and precision in predicting the growth of LAB population during inoculum fermentation was obtained through Richards model and Schnute model. This difference was due to difference in system characteristics between the two fermentation.

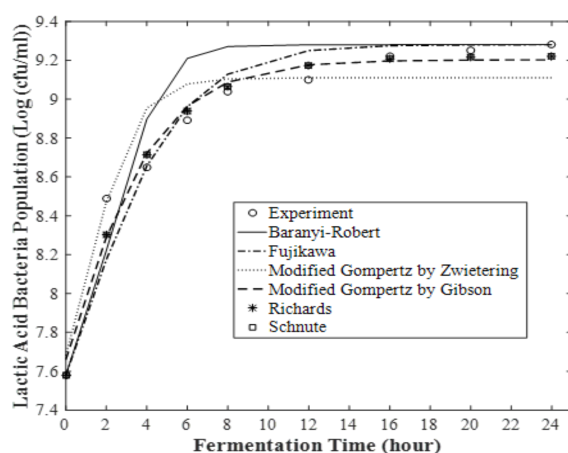

Figure 3. Population Profile of Lactic Acid Bacteria (LAB) in Fermentation with LAB Inoculum Addition

Table 6. Values of Observed Paramater, RMSE, R^2 Obtained from Various Growth Model Approach to LAB Population in Fermentation with LAB Inoculum Addition

Growth Model	Value of Observed Parameter	RMSE	R^2
Gompertz model modified by Gibson	A = 6.7622; B = 2.4401; C = 0.3793; D = 0.0000	0.0908	0.9686
Gompertz modified by Zwietering	A = 3.5229	0.1503	0.9139
Richards model	A = 3.7791; B = -0.9849	0.0759	0.9781
Schnute model	a = 0.2957; b = 0.9849	0.0759	0.9781
Fujikawa model	m = 0.3906; n = 115.8623	0.1243	0.9411
Baranyi and Robert model	q_0= 1.0802	0.1902	0.8621

As already mentioned, the addition of LAB inoculum caused faster adaptation and growth of LAB.

Meanwhile, the kinetic of inactivation (death) of coliform bacteria can be seen in Figure 4. Geeraerd model gave more accurate and more coincidental to experimental data than the Gompertz model modified by Gil *et al*. The Geeraerd model and experimental data coincided each other in almost every part (the shoulder phase, logarithmic phase, and tailing phase), although the model data in the tailing phase were shifted slightly from experimental data.

The accuracy of the Geeraerd model can also be seen from its smaller RMSE value which was 0.1078 and high R^2 value which was 0.9952 at Table 7. RMSE value of the Gompertz model modified by Gil *et al.* provided low accuracy. As can be seen that there was a

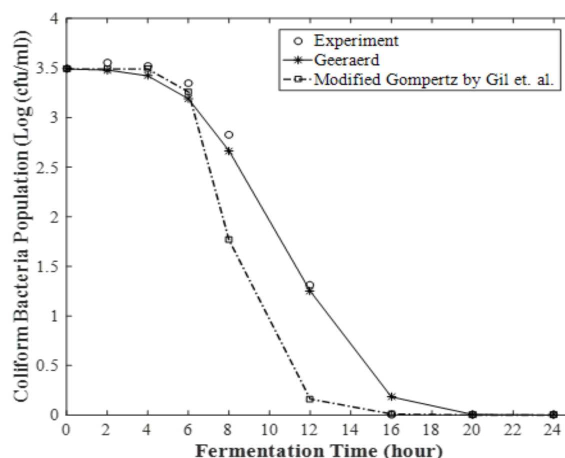

Figure 4. Population Profile of Coliform Bacteria on Fermentation by Addition of LAB Inoculum

Table 7. Values of Observed Paramater, RMSE, R^2 Obtained from Various Inactivation Model Approach to Coliform Bacteria Population in Fermentation with LAB Inoculum Addition

Growth Model	Value of Observed Parameter	RMSE	R^2
Geeraerd model	$C_c(0)$ = 179.3018	0.1078	0.9952
Gompertz model modified by Gil *et al.*	-	0.5229	0.8877

large shifting between the model data at the logarithmic phase, as well as a slight shifiting at the tailing phase. RMSE values of 0.5229 and R^2 of 0.8877 emphasized that the model had a low accuracy by paying attention to the range of bacterial population data from 0 to 3.5 (the difference of data range was 3.5 point).

Some primary (empirical) models were also used by several studies to predict bacterial population. Gompertz model modified by Zwietering and the Baranyi-Robert model had higher accuracy and precision than other models in predicting the growth of *Lactobacillus plantarum* (Longhi *et al*., 2013). Li *et al.* (2013) predicted population of lactic acid bacteria in raw beef product that was vacuum-packed. In that study, mathematical models were used, i.e. Gompertz model modified by Gibson, Baranyi model, Logical model, and Huang model. All four models had high accuracy in predicting bacterial growth. As for bacterial inactivation, the Geeraerd model also provided high precision in the inactivation of *Bacillus pumilus* (Albert and Mafart, 2005). Longhi *et al.* (2005) said that a certain model may be more feasible and accurate in describing the kinetics of bacteria population depending on the specific and characteristic of bacteria that differ one another, as well as the characteristic of fermentation (for example was environmental factor).

CONCLUSIONS

Several mathematical models had been applied to the prediction of the growth of lactic acid bacteria (LAB) and coliform bacteria during spontaneous fermentation of sorghum and prediction of LAB growth during sorghum fermentation with the addition of LAB inoculum. The models including the Gompertz model modified by Gibson, Gompertz model modified by Zwietering, Baranyi-Robert model, Fujikawa model, Richards model, and Schnute model had different accuracy depending on the bacterial characteristic and specification and also characteristic of the fermentation system. In predicting the growth of LAB and coliform bacteria during spontaneous fermentation, high accuracy and prediction were obtained from Gompertz model modified by Gibson, which was then followed by the Fujikawa model and Baranyi-Robert model. While the Richards model and the Schnute model had high accuracy in the prediction of LAB growth during fermentation with LAB inoculum addition. During sorghum fermentation with of LAB inoculum addition, coliform bacteria underwent inactivation (death). By applying the Geeraerd model and Gompertz model modified by Gil *et al.* to the inactivation prediction, it was found that Geeraerd model provided higher accuracy and precision. Finally, those accurate mathematical models can be applied in controlling microorganisms in the sorghum fermentation process or used as kinetic data for scaling-up of sorghum fermentation.

ACKNOWLEDGEMENT

The authors thank to Mrs. Anggita Sari Praharasti for discussing about the experiences in using mathematical models in research.

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