Simulation of microbial growth based on Euler's method

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ABSTRACT

Microorganisms such as bacteria, fungi and yeast produce valuable metabolites when they are grown in suitable culture conditions. The cultivation condition affects the cell growth, metabolism, and product production in a sophisticated and nonlinear way. Therefore, in this research, the growth of *Lactococcus lactis* NZ9000 in response to the growth conditions was simulated using different growth models. The objective was to simulate the effect of temperature, agitation speed, carbon and nitrogen sources, on the cell growth using the exponential model, logistic, and Monod equations. All equations were solved according to the Euler's method using MATLAB R2021a for simulation. The experimental data used for the simulation were from literature. The accuracy of the model was expressed as percentage relative error between the maximum value of experimental and simulated data. Simulation results show that the optimum conditions for cell growth was achieved at temperature 27 °C, agitation speed of 100 rpm with glucose and peptone as the carbon and nitrogen sources, respectively. The logistic equation's maximum cell concentration yields the lowest percentage error of 0.33% for the effects of agitation and 6.40% for temperature. While Monod equation give the closest accuracy of 1.84% and 7.11% for carbon and nitrogen sources, respectively. Thus, it was shown that the complexity of the microorganism growth was able to be simulated using suitable model such as logistic equation with the lowest relative error.

1. INTRODUCTION

Cell growth of bacteria is an important factor when cultivating it to produce a valuable product. To ensure healthy cell growth, optimum growth conditions for the specific bacteria must be known to control the growth condition in the lab scale or even the commercial scale. This is because the development of the cell growth can cost much labour, time, and money. Growing the cells is considered time consuming and laborious without automation (Mühlegger, 2023) and incur high manufacturing cost due to substrate used

and subsequent downstream processing (Macedo et al., 2020). With that, modelling and computer simulation method can help to reveal the growth mechanisms to some extent. Moreover, the modelling and computer simulation method is much efficient and economic. The growth of bacteria requires network of metabolic reactions that can greatly affect the cell growth and its production in a non-linear way (Lin et al., 2017). The effect of various culture conditions such as agitation speed, temperature, carbon and nitrogen sources on the growth of the *L. lactis* NZ9000 were done experimentally by Ibrahim et al. (2010). The accessibility of the modelling and simulation of the cell growth are very high. This is because there are many mathematical models and method that can simulate the growth of bacteria such as the exponential, logistic and Monod equations. In this paper, the simulation of the effect of different growth conditions such as the temperature effect, agitation speed, carbon and nitrogen sources were solved using Euler's method and MATLAB software. Different growth cell models were used and their numerical results were compared with experimental data from Ibrahim et al. (2010). The percentage relative error was used to express the accuracy of the simulated data to the experimental data.

2. THEORY

Several mathematical models were used to simulate the effect of temperature, agitation speed, carbon and nitrogen sources on the cell's concentration. The models are the exponential growth, logistic growth, and Monod equation.

2.1 Cell Growth Model

In active growth, bacterial cells will increase in number by growing and then physically subdividing. It is practically accurate to say that each cell grows for a specific interval time and divides in half. The interval time heavily depends on the conditions of the bacterial growth, for example the temperature and the availability of nutrients. If the growth conditions are kept almost at constant so that the interval time can remain constant over several generations, the culture is said to be in a balanced growth state (Hagen, 2010). The growth rate of a batch culture under exponential phase is generally follow the first order kinetic model where the growth rate is proportional to the microbial mass in the system as in Eq. (1).

$$\frac{dX}{dt} = \mu X \tag{1}$$

where dX/dt is the bacterial growth rate (g/L/h), X is cell biomass concentration (g/L), and μ is the specific growth rate constant (h^{-1}).

2.2 Logistic Growth

Verhulst in 1838 was the first to established logistic growth models (Charlebois & Balazsi, 2019). The logistic equation considers only the biomass concentration while disregarding the substrate utilisation. It also characterises growth in terms of carrying capacity as in Eq. (2).

$$\frac{dX}{dt} = kX\left(1 - \frac{X}{X_m}\right) \tag{2}$$

where k is the proportionally constant and X_m is the maximum biomass concentration (g/L).

The value of X_m can be calculated from Eq. (3).

$$X_m = X_o + Y_{X/S}S_o \tag{3}$$

where X_o is the initial cell biomass concentration, Y is the growth yield coefficient (g of cell biomass/ g of substrate) and S_o is the initial substrate concentration (g/L).

2.3 Monod Equation

The Monod equation is a widely used mathematical model that describe the dependence of a microorganism's growth rate to the concentration of a limiting source as in Eq. (4) (Zentou et al., 2019).

$$\mu = \mu_{max}\left(\frac{S}{K_S + S}\right) \tag{4}$$

where μ_{max} is the maximum specific growth rate (h^{-1}), S is the concentration of the growth limiting substrate (g/L), and K_S is the substrate concentration at half of the maximum growth rate.

Combining Eq. (1) and Eq. (4) yields Eq. (5). To relate the biomass and substrate concentrations during the batch growth, the rate limiting substrate, S can be substituted as Eq. (6) (Kargi, 2009) into Eq. (5) to give Eq. (7).

$$\frac{dX}{dt} = \left(\frac{\mu_{max}S}{K_S + S}\right)X \tag{5}$$

$$S = S_o - \frac{X - X_o}{Y_{X/S}} \tag{6}$$

$$\frac{dX}{dt} = \frac{\mu_m\left(Y_{x/s}S_0 + X_0 - X\right)}{\left(K_s Y_{x/s} + Y_{x/s}S_0 + X_0 - X\right)}X \tag{7}$$

2.4 Euler's Method

In 1768, Leonhard Euler developed a method to prove that the initial value problem had a solution, (Greenspan, 2008). It is a numerical method called Euler's method with the following basic idea. Definition of a derivative is given as Eq. (8).

$$\frac{dy}{dx} = y'x = \lim_{h \to 0}\frac{f(x+h) - f(x)}{h} \tag{8}$$

For small $h > 0$, then Eq. (7) implies that a reasonable difference quotient approximation for $y'(x)$ is given by Eq. (9) and Eq. (10).

$$y'x = \frac{f(x+h) - f(x)}{h} = F(x,y) \tag{9}$$

$$f(x+h) = f(x) + hF\big(x, y(x)\big) \tag{10}$$

For small $h > 0$, then equivalently, Eq. (9) can be rewritten as Eq. (11).

$$y(x+h) = y(x) + hF\big(x, y(x)\big) \tag{11}$$

Eq. (10) enables one to approximate $y(x+h)$ in terms of $y(x)$ and $F\big(x, y(x)\big)$, and in explicit recursive form as in Eq. (12).

$$y_{i+1} = y_i + hF(x_i, y_i), i = 0, 1, 2, \ldots\ldots, n-1 \tag{12}$$

Thus, one can calculate the current value y_{i+1} based on the previous value y_i by knowing the step size h, the rate $\frac{dy}{dx}$ and the initial value y_0.

3.   METHODOLOGY

The microbial growth was simulated using experimental data by Ibrahim et al. (2010) with initial values of X_i and S_o were 0.3 g/L and 10 g/L, respectively. Meanwhile, the values for μ_{max} and $Y_{X/S}$ are tabulated in Table 1 and the K_S value for Monod equation was taken as 4.2 h^{-1} according to Ziadi et al. (2010). This simulated data was then compared with X_{max} from Table 1.

The three microbial growth models were used (Eq. (1), (2), and (7)) for simulation and they are in the form of differential equations. These equations are difficult to solve precisely because of their complexity. However, it can be solved numerically using Euler's method. Thus, Eq. (1), (2), and (7) were written in the form of Eq. (12):

Exponential:
$$X(i+1) = X(i) + dt * \mu * X(i) \tag{13}$$

Logistic:
$$X(i+1) = X(i) + dt * \mu * X(i) * (1 - X(i)/X) \tag{14}$$

Monod:

$$X(i+1) = X(i) + dt * X(i) * (\mu * (Y_{X/S} * S_O X_O - X(i))/(K_S * Y_{X/S} + Y_{X/S} * S_O + X_O - X(i))) \tag{15}$$

A numerical computation is required to solve Eq. (13), (14), and (15), so MATLAB (R2021a) source codes were written as shown in Fig. 1. Finally, the accuracy of the models was measured as percentage percent relative error (Eq. 16) of the maximum cell concentration X_{max} between the experimental and simulation results.

$$Relative\ Error\ (\%) = \left| \frac{measured\ value - expected\ value}{expected\ value} \right| \times 100\% \tag{16}$$

Table 1. Experimental data

Variables		μ_{max} (h⁻¹)	$Y_{X/S}$ (g/g)	X_{max} (g/L)
Temperature	27 °C	0.59	0.58	3.22
	33 °C	0.41	0.74	2.74
	37 °C	0.42	0.66	2.81
Agitation Speed	50 rpm	0.46	0.58	2.43
	100 rpm	0.65	0.57	2.93
	150 rpm	0.41	0.64	2.60
	200 rpm	0.75	0.66	2.82
	250 rpm	0.71	0.59	2.73
Carbon Sources	Glucose	0.52	0.57	2.57
	Sucrose	0.62	0.56	2.23
	Lactose	0.24	0.37	0.79
Nitrogen Sources	Yeast Extract	0.58	0.56	3.14
	Peptone	0.58	0.56	3.18
	NH₄Cl	0.34	0.35	1.23
	(NH₄)₂SO₄	0.31	0.39	1.25
	Urea	0.14	0.14	0.41

Source: Ibrahim et al. (2010)

```
function exp_speed50()

% Effect of agitation speed on the growth of Lactoccus lactis
% Ibrahim et. al (2010)

% Parameters
u = 0.58 ; % specific growth rate h-1 at 50 rpm

% Initial Value
x0 = 0.3 ;       % Initial cell concentration (g/L)
XVec(1) = x0 ;  % Store initial value in solution vector

% Time information
dt = 0.5 ;        % Time step hr
TFinal = 4 ;      % Final simulation time hr
t=0 ;             % Initialize time to zero
tVec(1) = 0;      % store first time-value

% Solve equation/ March forward in time
i = 1 ;    % counter
while t < TFinal

    % Euler's time-step
    XVec(i+1) = XVec(i) + dt*u*XVec(i);

    % Store time-value
    tVec(i+1) = t + dt;

    % Update counter and time
    t = t+dt ;
    i = i+1 ;

end
plot(tVec,XVec,'.');
hold on ;
save ('exp_speed50.mat')
```

Fig. 1. Example of MATLAB codes to simulate the effect of agitation speed on microbial growth using exponential equation.

Source: Author's own data

4.   RESULTS

4.1   Effect of Temperature on Cell Growth

In this study, the effect of temperature for *L. lactis* NZ9000 has been simulated at 27 °C, 33 °C, and 37 °C according to the experimental data by Ibrahim et al. (2010). Fig. 2 shows the effect of temperature on cell growth with different equations. Meanwhile, Table 2 shows the comparison of cell growth and accuracy with different models for temperature effect. The highest cell concentration was 6.26 g/l at 27 °C as predicted by Monod equation but the relative error was high. Similarly, the relative error for maximum cell concentration predicted by exponential equation was also high except at temperature 37 °C.

Even though the relative error was the lowest at 37 °C which is 4.42% but the cell concentration predicted was not the highest. Note that the highest cell concentration from experimental data was 3.22 g/L at 27 °C. It was shown that the highest cell concentration predicted by logistic equation was 3.44 g/L at 27 °C with the lowest relative error, 6.40%. Thus, we conclude that in terms of accuracy of the models, the logistic equation was able to simulate the closest maximum cell concentration as compared to experimental data.
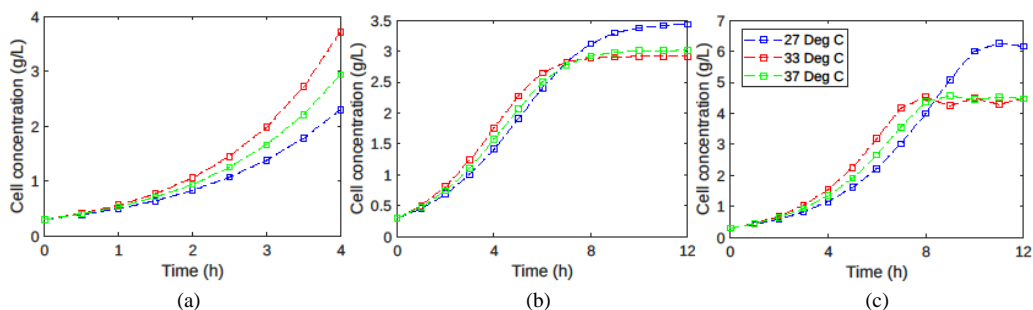
| (a) | (b) | (c) |

Fig. 2. Effect of temperature on cell growth with (a) exponential (b) logistic and (c) Monod equations

Source: Author's own data

Table 2. Comparison of cell growth and accuracy with different model for temperature effect

		Temperature (°C)		
		27	**33**	**37**
Simulated X_{max} (g/L)	Exponential	2.30	3.72	2.94
	Logistic	**3.44**	2.92	3.02
	Monod	6.26	4.53	4.57
Relative error (%)	Exponential	40.00	26.3	4.42
	Logistic	**6.40**	6.16	9.30
	Monod	48.55	39.58	38.45
Experimental X_{max} (g/L)		3.22	2.74	2.81

Source: Author's own data

4.2 Effect of Agitation on Cell Growth

Fig. 3 shows the effect of agitation on cell growth with different equations. The agitation speeds that were tested are at 50 rpm, 100 rpm, 150 rpm, 200 rpm and 250 rpm. It was shown that the highest cell growth was observed at agitation of 100 rpm with the maximum cell concentration of 2.92 g/L using the logistic equation. Meanwhile, the highest concentrations of 2.94 g/L and 7.85 g/L were attained at agitation of 200 rpm as simulated by the exponential and Monod equations, respectively. On the other hand, the maximum cell concentration from experimental data was 2.93 g/L when agitated at 100 rpm. Thus, the logistic equation provided the lowest percentage error of 0.33% as compared to the experimental data as shown in Table 3.
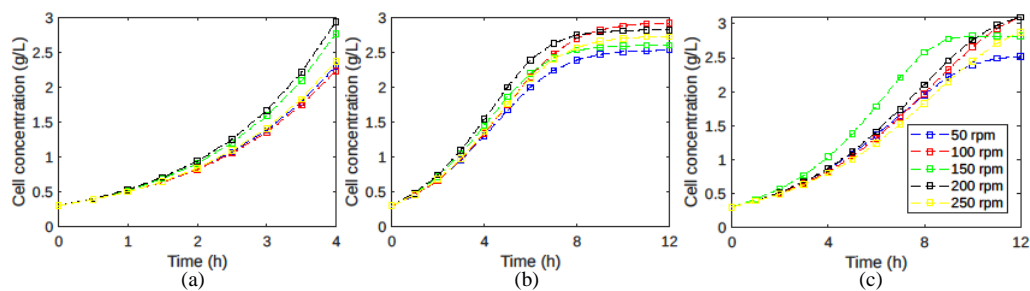
Fig. 3: Effect of agitation on cell growth with (a) exponential (b) logistic and (c) Monod equations

Source: Author's own data

Table 3. Comparison of cell growth and accuracy with different model for agitation effect

		Agitation speed (rpm)				
		50	100	150	200	250
Simulated X_{max} (g/L)	Exponential	2.30	2.23	2.77	2.94	2.37
	Logistic	2.53	**2.92**	2.60	2.82	2.72
	Monod	2.52	3.10	2.82	3.10	2.88
Relative error (%)	Exponential	5.19	31.38	5.97	3.99	15.05
	Logistic	4.37	**0.33**	0.05	0.04	0.19
	Monod	4.10	5.49	4.52	9.08	5.37
Experimental X_{max} (g/L)		2.42	2.93	2.60	2.82	2.73

Source: Author's own data

4.3   Effect of Carbon and Nitrogen Sources on Cell Growth

L. lactis NZ9000 were grown on glucose, sucrose or lactose as a carbon source in an experiment. The simulation findings in Fig. 4 show that exponential and logistic equations predicted the experimental data less accurate than Monod equation. Logistic equation managed to predict the concentration of 2.95 g/L whereas exponential equation estimated at 2.23 g/L using glucose as the carbon source. The accuracy of the estimated maximum concentration was 12.95% for logistic equation and 15.24 % for exponential equation as shown in Table 4. On the other hand, the maximum cell concentration of 2.52 g/L with 1.85% relative error is the closest concentration predicted by using Monod equation.
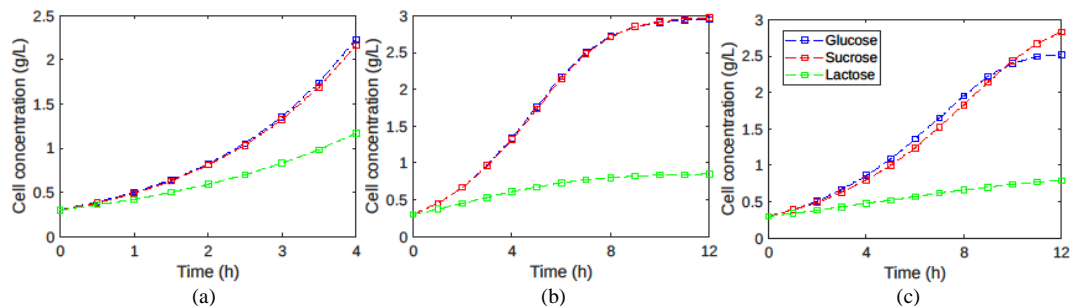
Fig. 4. Effect of carbon source on cell growth with (a) exponential (b) logistic and (c) Monod equations

Source: Author's own data

Table 4. Comparison of cell growth and accuracy with different model for carbon source effect

		Carbon source		
		Glucose	Sucrose	Lactose
Simulated X_{max} (g/L)	Exponential	2.23	2.16	1.17
	Logistic	2.95	2.97	0.85
	Monod	**2.52**	2.83	0.793
Relative error (%)	Exponential	15.24	3.16	32.37
	Logistic	12.95	24.97	7.15
	Monod	**1.84**	21.26	0.42
Experimental X_{max} (g/L)		3.22	2.57	2.23

Source: Author's own data

Besides the carbon sources, the support of nitrogen sources such as yeast extract, peptone, ammonium chloride, ammonium sulphate, and urea on the cell growth of *L. lactis* NZ9000 was well simulated with Monod equation. This is evidenced by the equation's low relative error when compared to exponential and logistic equations as tabulated in Table 5. Cell growth was highly dependent on peptone as the nitrogen source as predicted by Monod and logistic equations, similar to experimental result by Ibrahim et al. (2010). The maximum cell concentrations were 3.42 g/L and 3.46 g/L, respectively, while the actual maximum cell concentrations from the experiment were 3.14 g/L and 3.18 g/L for Monod and logistic equations, respectively. Overall, it was observed that Monod equation was able to simulate the closest maximum cell concentration to the experiment with relative error ranging from 0.97% to 9.10%.
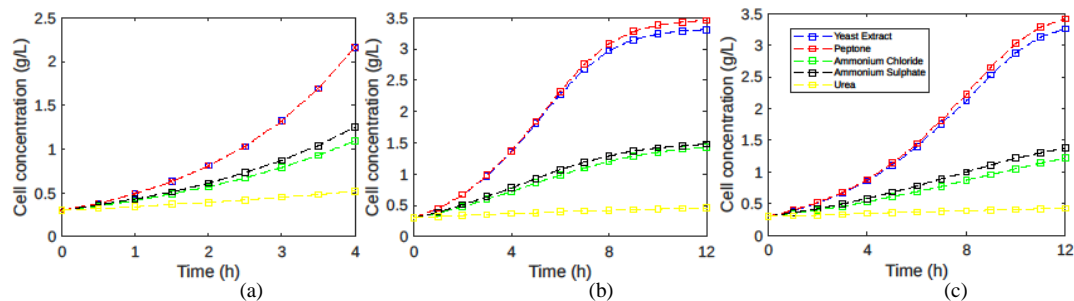
Fig. 5: Effect of nitrogen source on cell growth with (a) exponential (b) logistic and (c) Monod equations

Source: Author's own data

Table 5. Comparison of cell growth and accuracy with different model for nitrogen source effect

		Nitrogen source				
		Y. Extract	**Peptone**	**A. chloride**	**A. sulphate**	**Urea**
Simulated X_{max} (g/L)	Exponential	2.16	2.16	1.09	1.25	0.52
	Logistic	3.31	3.46	1.43	1.48	0.46
	Monod	3.26	**3.42**	1.22	1.38	0.43
Relative error (%)	Exponential	42.25	47.10	12.84	0.19	20.46
	Logistic	5.15	8.06	13.91	15.36	11.34
	Monod	3.70	**7.11**	0.97	9.10	3.99
Experimental X_{max} (g/L)		3.14	3.18	1.23	1.25	0.41

Source: Author's own data

5.   DISCUSSION

According to Ibrahim et al. (2010), *L. lactis* NZ9000 took four to six hours to reach maximum cell growth. However, based on simulation, the maximum cell growth was achieved at around eight hours of incubation for Monod and logistic equations. The logistic and Monod models considered resource availability (as carrying capacity or substrate concentration) (Xu, 2019). In experiments, resource limitations might play a more significant role than assumed by the models, thus, cells might reach maximum growth earlier due to resource exhaustion. While for exponential model, the cell growth was simulated up to 4 hours only, thus, the maximum cell growth was taken at 4 hours of incubation. The cell would continuously grow if the incubation time was prolonged to 12 hours since this model did not consider any limiting factors or resource constraints on the cell growth. It was found out that, at 4 hours, the relative error was the lowest for the effect of temperature, thus the time of simulation was standardised up to 4 hours only for exponential equation.

Mathematically, the logistic equation minimises relative error because it captured both exponential growth (when X is small compared to X_m) and the slowing growth as X approaches X_m. Since logistic equation incorporated carrying capacity which could be growth-inhibiting factors (i.e. toxic chemical accumulation, temperature, pH etc.) other than the nutrient level (Wachenheim et al., 2003), thus the equation led to better predictions than the other models.

Based on Monod equation, the growth rate of a microorganism is a function of the concentration of a limiting substrate (Monod, 1949). This model depicted that as substrate concentration increases, the growth rate approaches a maximum value and when substrate concentration is low, microbial growth is limited. Thus, the relative error is minimum because the Monod equation captures the nonlinear relationship between substrate concentration and growth rate.

The accuracy of each model used to simulate cell growth was also affected by the method used to solve the differential equation which is Euler's method. The Euler's method is a straightforward numerical technique for solving ordinary differential equations (ODEs). While it is computationally simple, its accuracy depends on the step size (denoted as dt in Eq. 13, 14 and 15) that was set in the calculation using Matlab (R2021a) (Desta and Mekashew, 2022). Generally, smaller steps provide more accurate results but require more computation. For exponential and logistic equations, a smaller step size generally improves accuracy but for Monod equation, the specific context (e.g., microbial growth rate) needs to be considered to determine an appropriate step size. Thus, step size strategies as suggested by Gülnur & Kemal (2012) can be utilised to increase the accuracy of step size selected to solve the differential equations used in this study.

6. CONCLUSION

Simulation findings showed that the optimal conditions for cell growth were 27 °C, 100 rpm agitation, glucose as the carbon source, and peptone as the nitrogen source. According to the comparative results, the logistic equation accurately predicted cell growth, with percentage errors of 6.40% and 0.33% for the effects of temperature and agitation speed, respectively. However, for carbon and nitrogen supplies, the Monod equation was able to model the most accurate microbial growth, with percentage accuracy of 1.84% and 7.11%, respectively. Thus, it is demonstrated that Euler's method can be used to solve complex differential equations and allow for prediction and simulation from various cell growth models at certain level of accuracy.

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CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

AUTHORS' CONTRIBUTIONS

Siti Humaira Mohd Jasni: Data analysis, formal analysis and investigation; **Zaihar Yaacob**: Writing original draft, software, validation; **Zainatul `Asyiqin Samsu**: Conceptualisation, methodology; supervision, writing- review and editing, and validation.

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