

Modeling Biodegradation of Polyethylene Terephthalate Involving the Growth of Factor Escherichia Coli Bacteria

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ABSTRACT

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A design of a Polyethylene Terephthalate (PET) waste biodegradation system using Escherichia Coli (E-Coli) bacteria in a whole-cell biocatalyst system. E-Coli bacteria will produce LC-Cutinase enzyme on the cell surface so PET can be broken down into Ethylene glycol and Terephthalate acid. With the help of Reductase and Dehydrogenase enzymes, a chemical reaction occurs that converts Ethylene glycol into Malate. Through the chemical reaction process, it is guaranteed that Ethylene glycol does not explain the environment but can be used as an energy source for the growth of E-Coli bacteria. Thus E-Coli can grow faster, so the more bacteria, the more PET that can be broken down quickly.



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1. Introduction

For more than 50 years, the production and consumption of plastic in the world have continued to increase, resulting in increased environmental pollution and creating severe risks to nature and humans. In 2017, 438 million tons of plastic were produced worldwide and used as containers or packaging in everyday life [1]. Its light and durable nature make it a versatile material used to make various tools in our lives. However, plastic is one of the materials that are not environmentally friendly because it takes a very long time to decompose [2].

In Indonesia, plastic is used as drinking water packaging or plastic bottles. With the production and consumption of bottled drinking water (AMDK) from year to year continuing to increase, in 2021, the production of bottled drinking water reached 29,4 billion liters. In 2022 the production of bottled water reached 30,87 billion liters which grew by 4-5% [3].

The type of plastic used as raw material for plastic bottles is Polyethylene Terephthalate (PET). Although PET recycling has increased from 7% to 30% in recent years, most plastic bottles are disposed of in landfills and continue to the environment [4].

A PET waste degradation system design using *Escherichia Coli* (E-Coli) bacteria in a whole-cell biocatalyst system. Bacteria will produce LC-Cutinase enzyme on the cell surface so plastic bottles can be broken down into Ethylene glycol and Terephthalic acid. Ethylene glycol can be used as an energy source for bacterial growth with the help of reductase and dehydrogenase enzymes [5]. E-Coli bacteria can utilize compounds from PET degradation as nutrients for their growth. Therefore, the authors made a model involving the growth factor of E-Coli bacteria in the PET biodegradation system. The model made will help understand the mechanism of the biodegradation process that takes place and know the effect of the number of bacteria on the degradation time of PET.

2. Research Methods

The PET biodegradation model involves a regulation module, reporter module, degradation module, conversion module, and growth of E-Coli bacteria. [5]. In this case, the growth of E-Coli bacteria is divided into two stages. The first stage is that the bacteria are allowed to grow by consuming nutrients, and the second stage is that the bacteria consume nutrients and/or malate to grow.

The assumptions and mathematical models of the PET biodegradation process involving bacterial growth follow:

2.1 Assumptions

The assumptions used in the PET biodegradation process involving bacterial growth are:

- a. Observations for one unit cell
- b. There is no change in temperature, pH, and pressure during the process
- c. Bacteria have started to express LC-Cutinase and Inclusion Body (in very small amounts) before being reacted with PET.
- d. Incorporate bacterial growth factors
- e. The mechanism of the regulation module is considered to be the same so that it produces the same LC-Cutinase for all bacteria
- f. LC-Cutinase in the cell membrane does not affect LC-Cutinase in the cell
- g. Meets the quasi-equilibrium on the Michaelis-Menten kinetic theory, namely $k_1; k_1 \gg k_2$
- h. Meets the quasi-steady state on Michaelis-Menten kinetic theory, namely $[E] \ll [S]$
- i. The total number of enzymes remains constant according to the Michaelis-Menten kinetic theory, $[E]_{tot} = E + ES$
- j. 1 bacterial colony equals 18000 bacterial cells
- k. Regulation, reporter and conversion modules are assumed to be the same in all bacteria
- l. Nutrients consumed evenly on all bacteria
- m. Malate is produced evenly on all bacteria
- n. Bacteria can grow if there are nutrients and/or malate
- o. Bacteria prefer to consume nutrients than malate as an energy source

2.2 Mathematical Models

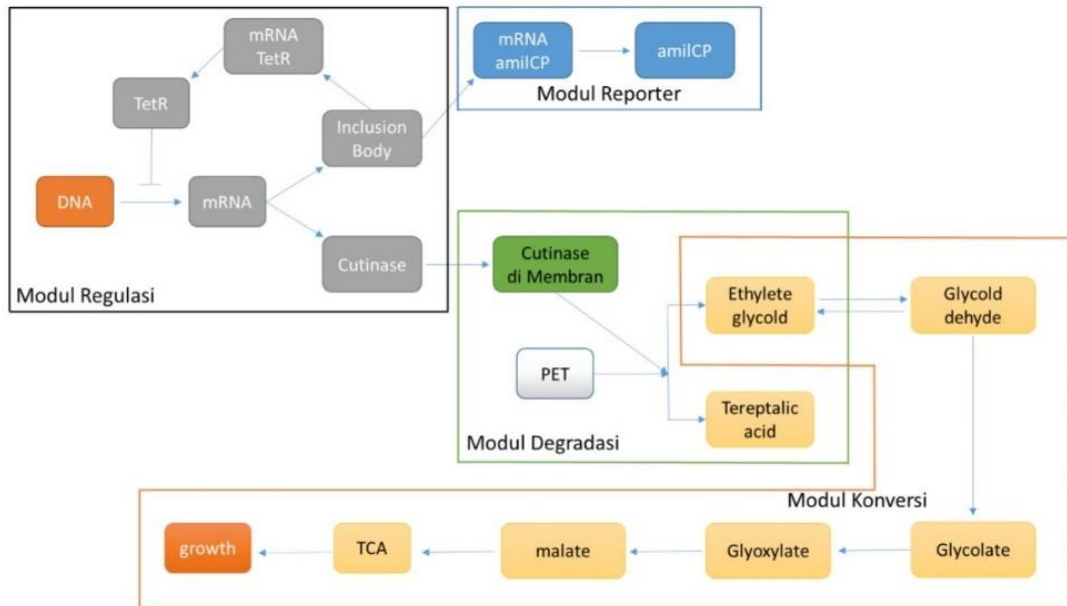


Figure 1. Sistem Biodegradasi PET

Figure 1, explains that mRNA is produced from the DNA transcription process, after being translated into the LC-Cutinase or Inclusion Body enzyme. For example, x mRNA is translated into LC-Cutinase, while the remaining $(1-x)$ mRNA is translated into Inclusion Body, with $0 < x < 1$. Next, the Inclusion Body activates TetR mRNA production, which is then translated into TetR. This TetR enzyme will suppress or stop the DNA transcription process. Cells will stop producing the LC Cutinase and Inclusion Body enzymes so that the Inclusion Body concentration will decrease as well as the TetR and TetR mRNA concentrations. As a result, the mRNA transcription process that produces LC-Cutinase will increase until, at a specific time, the inclusion body begins to appear, and the above cycle repeats itself. Likewise, the AmilCP and AmilCP mRNAs are affected by the production of the Inclusion Body. When the Inclusion Body concentration increases, the AmilCP and AmilCP mRNA concentrations also increase.

In the degradation module, LC-Cutinase in the cell membrane is obtained from the diffusion process of LC-Cutinase inside the cell in the regulation module. Meanwhile, there is a carrying capacity of LC-Cutinase in the membrane, so there is competition for LC-Cutinase in the cell against the production of LC-Cutinase in the cell membrane. Based on experiments in the laboratory, PET is inserted at 360 minutes. The reaction of PET and LC-Cutinase produces Ethylene glycol and Terephthalic acid.

The conversion module describes the metabolic process of Ethylene glycol, which is to simplify the Ethylene glycol compound with a larger molecule into Malate, a smaller molecule consumed by cells as an energy source. The PET decomposition process involves total LC-Cutinase occurring in the cell membrane, while the Glycolaldehyde to Malate process occurs in the cell.

The growth process of E-Coli bacteria in PET biodegradation is made into two stages. In the first stage the bacteria are allowed to grow until the end of the stationary phase, which is the phase where the rate of bacterial growth is balanced with the rate of death. At this stage, the bacteria are also given nutrients to grow. In the second stage, at 360 minutes or the end of the stationary phase, 50 mg of PET was added. At this stage, the PET biodegradation process produces malate as an energy source. The malate produced is in the bacterial cell and is immediately used for growth. The bacteria here consume nutrients and/or malate as a source of energy for growth.

From the statement above, a mathematical model is built for the concentration rate of LC-Cutinase and the Inclusion Body.

$$\frac{dC}{dt} = \frac{\alpha_c M}{1+I} - \gamma_c C \quad (1)$$

$$\frac{dI}{dt} = \frac{\alpha_i M I}{1+I} - \gamma_i I \quad (2)$$

The mathematical model for the concentration rates of mRNA and mRNA TetR follows Hill's equation. Meanwhile, the concentrations of TetR, AmilCP mRNA, and AmilCP followed the addition (production) and reduction (degradation) model so that a mathematical model could be made for the concentration rates of mRNA, TetR mRNA, TetR, AmilCP, and AmilCP mRNA.

$$\frac{dM}{dt} = \frac{\alpha_m}{1 + \left(\frac{R}{K}\right)^n} - \gamma_m M \quad (3)$$

$$\frac{dMr}{dt} = \frac{\alpha_{mr} I}{K_{mr}^n + I^n} - \gamma_{mr} Mr \quad (4)$$

$$\frac{dR}{dt} = \alpha_r Mr - \gamma_r R \quad (5)$$

$$\frac{dMcp}{dt} = \alpha_{mcp} I - \gamma_{mcp} Mcp \quad (6)$$

$$\frac{dCP}{dt} = \alpha_{cp} Mcp - \gamma_{cp} CP \quad (7)$$

The growth model of LC-Cutinase in the membrane was modeled by a logistic model, namely

$$\frac{dCmb}{dt} = \alpha_{mb} C \left(1 - \frac{Cmb}{L}\right) - \gamma_{mb} Cmb \quad (8)$$

By using the rate of the Michaelis-Menten reaction [6], the reaction process for the **Ethylene glycol** reaction is modeled as follows.

$$\frac{dP}{dt} = \frac{k_2 [Cmt][P]}{k_p + [P]}, \quad Cmt = Cmb * tb \quad (9)$$

$$\frac{dEg}{dt} = \frac{k_2 [Cmt][P]}{k_p + [P]} - \frac{\frac{v_{max2}^{for}[Eg]}{k_{Eg,f}} - \frac{v_{max2}^{back}[Ga]}{k_{Ga,b}}}{1 + \frac{Eg}{k_{Eg,f}} - \frac{Ga}{k_{Ga,b}}} \quad (10)$$

$$\frac{dGa}{dt} = \frac{\frac{v_{max2}^{for}[Eg]}{k_{Eg,f}} - \frac{v_{max2}^{back}[Ga]}{k_{Ga,b}}}{1 + \frac{Eg}{k_{Eg,f}} - \frac{Ga}{k_{Ga,b}}} - \frac{v_{max3}[Ga]}{k_{Ga} + [Ga]} \quad (11)$$

$$\frac{dGc}{dt} = \frac{v_{max3}[Ga]}{k_{Ga} + [Ga]} - \frac{v_{max4}[Gc]}{k_{Gc} + [Gc]} \quad (12)$$

$$\frac{dGx}{dt} = \frac{v_{max4}[Gc]}{k_{Gc} + [Gc]} - \frac{v_{max5}[Gx]}{k_{Gx} + [Gx]} \quad (13)$$

Furthermore, a mathematical model was built for the growth rate of E-Coli Bacteria in Nutrition and Malate as follows.

$$\frac{dMal}{dt} = \frac{v_{max5}[Gx]}{k_{Gx} + [Gx]} - \gamma_{mal} Mal \quad (14)$$

$$\frac{dB}{dt} = \alpha_{b1} BN + \alpha_{b2} 18000 B Mal - \gamma_{b3} B \quad (15)$$

$$\frac{dN}{dt} = -\alpha_n \alpha_{b1} BN \quad (16)$$

The variables and parameters used in the above model with parameter values selected and obtained from the literature derived from experimental results are shown in the following Table 1.

Table 1. Variables

Variables	Description	Unit
<i>M</i>	mRNA concentration	μM
<i>C</i>	<i>LC-Cutinase</i> concentration	μM
<i>I</i>	<i>Inclusion Body</i> concentration	μM
<i>Mr</i>	mRNA TetR concentration	μM
<i>R</i>	mRNA TetR concentration	μM
<i>Mcp</i>	mRNA AmilCP concentration	μM
<i>CP</i>	AmilCP concentration	μM
<i>Cmb</i>	Concentration of <i>LC-Cutinase</i> in the membrane	μM
<i>P</i>	PET concentration	<i>mg</i>
<i>Eg</i>	<i>Ethylene glycol</i> concentration	μM
<i>Ga</i>	<i>Glycolaldehyde</i> concentration	μM
<i>Gc</i>	<i>Glycolate</i> concentration	μM
<i>Gx</i>	<i>Glyoxylate</i> concentration	μM
<i>Mal</i>	<i>Malate</i> concentration	μM
<i>B</i>	Many of Bacteria	<i>koloni</i>
<i>N</i>	Lots of Nutrients	<i>mg</i>

Table 2. Parameters

Parameters	Description	Values	Reference
K	Activation coefficient for mRNA	1	
K_{mr}	Activation coefficient for mRNA TetR	0.7	
n	Hill coefficient	3	
α_m	Maximal expression level of the promotor of mRNA	0.08	
α_c	Maximal expression level of the promotor of <i>LC-Cutinase</i>	0.001	
α_i	Maximal expression level of the promotor of <i>Inclusion Body</i>	0.005	
α_{mr}	Maximal expression level of the promotor of mRNA TetR	0.073	
α_r	Maximal expression level of the promotor of TetR	0.1	
α_{mcp}	Maximal expression level of the promotor of mRNA AmilCP	0.97	
α_{cp}	Maximal expression level of the promotor of AmilCP	0.97	
γ_m	Degradation rate of mRNA	0.005	
γ_c	Degradation rate of <i>LC-Cutinase</i>	0.5	
γ_i	Degradation rate of <i>Inclusion Body</i>	0.009	
γ_{mr}	Degradation rate of mRNA TetR	0.03	
γ_r	Degradation rate of TetR	0.03	
γ_{mcp}	Degradation rate of mRNA AmilCP	1.07	
γ_{cp}	Degradation rate of AmilCP	1.07	
α_{mb}	LC-Cutinase production rate in membrane	0.0001 min^{-1}	
γ_{mb}	LC-Cutinase degradation rate in membrane	0.5 min^{-1}	
L	Carrying capacity of <i>LC-Cutinase</i> in membrane	$0.08 \mu\text{M}$	
k_2	Constant rate of Conversion	30.9 min^{-1}	[7]
v_{max2}^{for}	Maximum rate for forward reaction	$26 \mu\text{min}^{-1}\text{mg}^{-1}$	[8]
v_{max2}^{back}	Maximum rate for reverse reaction	$5.7 \mu\text{min}^{-1}\text{mg}^{-1}$	[9]
v_{max3}	Maximum rate of <i>Glycolaldehyde</i> reaction	$19 \mu\text{min}^{-1}\text{mg}^{-1}$	[10]
v_{max4}	Maximum rate of <i>Glycolate</i> reaction	$0.15 \mu\text{min}^{-1}\text{mg}^{-1}$	[11]
v_{max5}	Maximum rate of <i>Glyoxylate</i> reaction	$31.8 \mu\text{min}^{-1}\text{mg}^{-1}$	[12]
k_p	Concentration for PET reaction	$2100 \mu\text{M}$	[7]
$k_{Eg,f}$	Concentration for forward reaction <i>Ethylene glycol</i>	$7000 \mu\text{M}$	[8]
$k_{Ga,b}$	Concentration for <i>Glycolaldehyde</i> reverse reaction	$480 \mu\text{M}$	[9]
k_{Ga}	Concentration for <i>Glycolaldehyde</i> reaction	$380 \mu\text{M}$	[10]
k_{Gc}	Concentration for <i>Glycolate</i> reaction	$40 \mu\text{M}$	[11]
k_{Gx}	Concentration for <i>Glyoxylate</i> reaction	$50 \mu\text{M}$	[12]
γ_{mal}	Degradation rate of <i>Malate</i>	0.0025 min^{-1}	
tb	Total bacteria per cell	$\frac{18000sel}{koloni} B(koloni)$	
α_{b1}	Bacteria growth rate by Nutrition	$0.003 \text{ min}^{-1}\text{mg}^{-1}$	
α_{b2}	Bacterial growth rate by Malate	$0.0013 (\mu\text{M})^{-1}\text{min}^{-1}$	
α_n	The rate of nutrient consumption by bacteria	$0.0013 \text{ min}^{-1}\text{koloni}^{-1}\text{mg}$	
γ_{b3}	Natural death rate	0.001 min^{-1}	

3. Results And Discussion

The results and discussion will discuss the analysis and simulation models by showing a equilibrium point and stability analysis. The stability analysis of the conversion model is carried out separately from the model regulation, because the model does not directly affect the results of the analysis on the model regulation. The analysis results on the model regulation, namely the equilibrium point, stability analysis, dynamic behavior, parameter analysis, and initial value behavior, can be seen in [13] [14]. At the same time, what is discussed is model conversion analysis. Then a numerical simulation will be carried out to explain the results that have been obtained.

3.1 Conversion Model Analysis

To make it easier to analyze the stability of the conversion model, the parameter values in Table 2 are substituted into equations (9)-(14).

3.1.1 Equilibrium points

From the mathematical model that has been formed in equation (9)-(14) we get a fixed point.

$$(P = 0, Eg = 0, Ga = 0, Gc = 0, Gx = 0, Mal = 0) \tag{17}$$

Where Cmt in equation (9) is constant. Furthermore, the point still **exists** because it is a real and non-negative number

3.1.2 Stability of the equilibrium

The Jacobian matrix of the system of equations (9)-(14), which has been substituted for the equilibrium point value (17), is

$$J = \begin{pmatrix} -0.0147 * Cmt & 0 & 0 & 0 & 0 & 0 \\ 0.0147 * Cmt & -0.0037 & 0.0119 & 0 & 0 & 0 \\ 0 & 0.0037 & -0.0619 & 0 & 0 & 0 \\ 0 & 0 & 0.05 & -0.0038 & 0 & 0 \\ 0 & 0 & 0 & 0.0038 & -0.636 & 0 \\ 0 & 0 & 0 & 0 & 0.636 & -0.0025 \end{pmatrix} \quad (18)$$

Similar to the stability analysis of the regulatory model, stability can be seen from the eigenvalues at a equilibrium point. The eigenvalues of the matrix (18) are as follows.

$$\lambda_1 = -0.0147 * Cmt, \lambda_2 = -0.0025, \lambda_3 = -0.00297, \lambda_4 = -0.00375, \lambda_5 = -0.0626, \lambda_6 = -0.636$$

Because all the eigenvalues obtained are negative real values, this conversion model is **stable**.

3.2 Model Simulation

A simulation model of all equations solved simultaneously obtained the following results. Numerical simulation results on model and reporter regulation with parameter values in Table 3 and initial values given according to biological conditions where the initial concentration of model and reporter regulation is feasible to be zero (except for inclusion bodies in small amounts), because DNA has not yet transcribed mRNA. Simulation results with initial values ($I=0.00001, C=0, M=0, Mr=0, R=0, Mcp=0, CP=0$) as shown in the following figure.

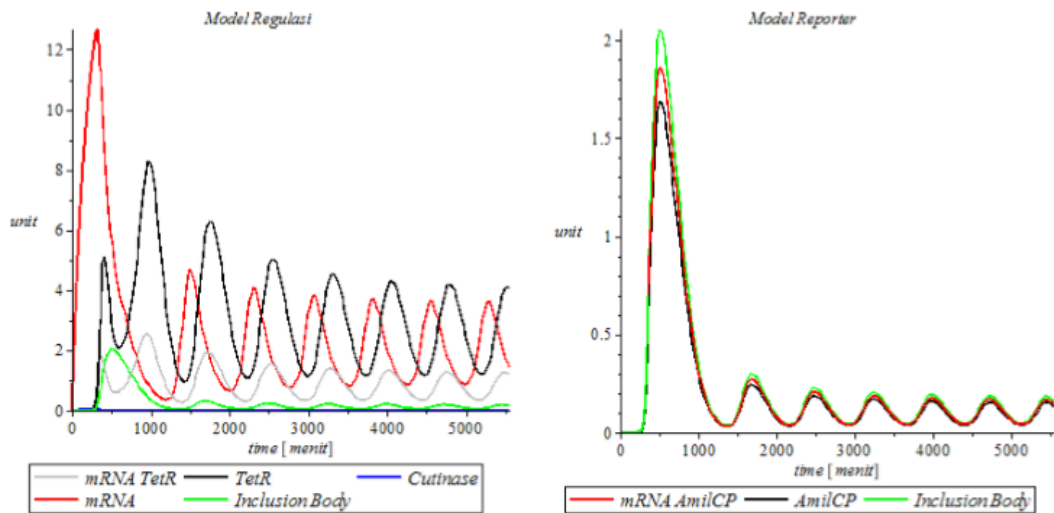


Figure 2. Regulation model simulation results and reporter

It can be seen from Figure 2, that for the regulatory model when the concentration of LC-Cutinase increases, inclusion bodies begin to appear. When the concentration of the inclusion body begins to grow, TetR mRNA appears, followed by TetR. When TetR began to appear, mRNA decreased because TetR stopped the transcription process from DNA to mRNA. If the mRNA drops, then the same thing happens to LC-Cutinase. The Inclusion Body will also decrease when the mRNA contains, and so will the TetR mRNA. The decrease in TetR will cause the mRNA to rise again, and this situation will repeat itself.

The simulation on the reporter model shows the formation of an inclusion body so that the AmilCP and AmilCP mRNA are also formed following the inclusion body pattern. This is because the inclusion body concentration is directly proportional to the AmilCP mRNA concentration. That is when the concentration of Inclusion Body increased, so did the concentration of AmilCP mRNA, followed by an increase in the concentration of AmilCP. In the results of this reporter simulation, it was seen that the bacteria experienced stress when the AmilCP concentration increased and vice versa. This can already represent biological phenomena that produce oscillating simulations.

Bacteria produce LC-Cutinase in the cell on the regulation module, which is then transported to the cell membrane. LC-Cutinase, which is in the cell membrane, will react with PET. The following are the simulation results for LC-Cutinase and total LC-Cutinase in cell membranes.

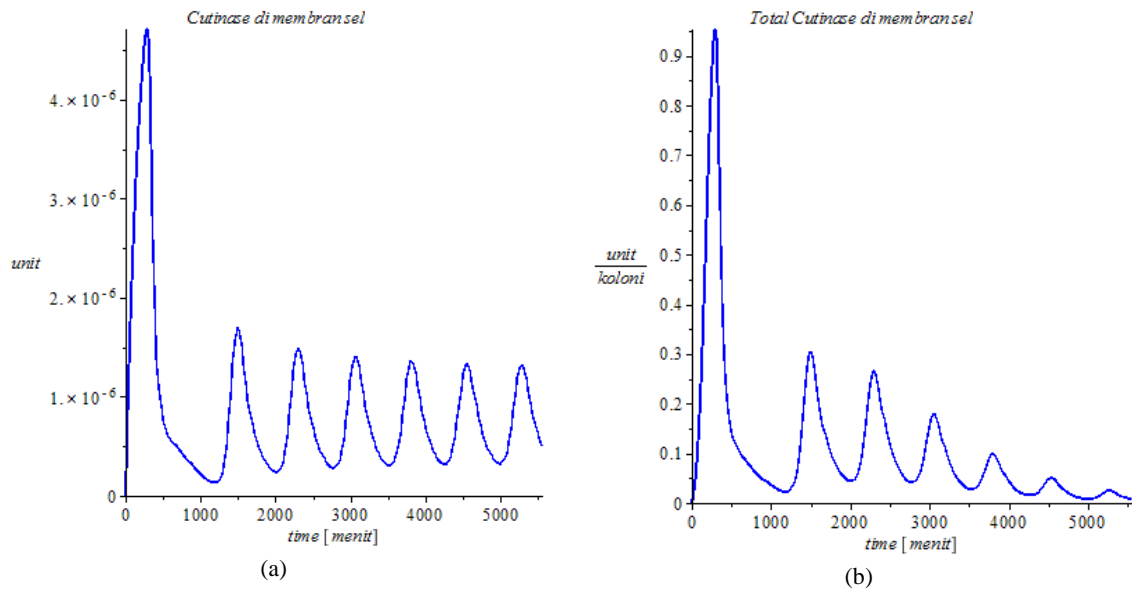


Figure 3. Simulation results (a) LC-Cutinase in the membrane, (b) total LC-Cutinase in the membrane

Figure 3 shows that the simulation results of LC-Cutinase in the membrane follow the pattern of LC-Cutinase simulation results in oscillating cells. However, the concentration of LC-Cutinase in the membrane was not as much as LC-Cutinase in the cells because LC-Cutinase was limited by its carrying capacity. While the assumption of LC-Cutinase in the membrane is the same for each bacterial cell, the results of the total simulation of LC-Cutinase in the membrane are obtained.

Then at 360 minutes, 50 mg of PET is added. When PET is inserted, a degradation process occurs, which causes PET to decompose. This Decomposable PET involves total LC-Cutinase in the membrane. The results of PET degradation by total LC-Cutinase in the membrane can be seen in the image below.

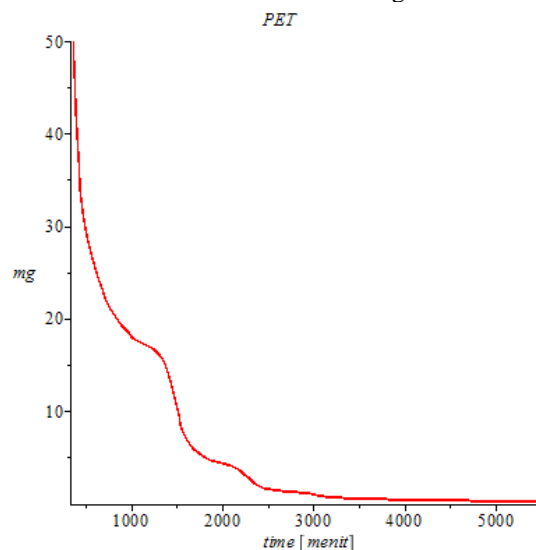


Figure 4. PET degradation simulation results

Figure 4, shows that the PET degradation process occurs rapidly. This is because the total concentration of LC-Cutinase in the membrane peaks in 360 minutes. At that minute, PET is also added so that the degradation process occurs immediately and decomposes PET quickly. However, in the 2000th minute, the degradation process slowed down because the total concentration of LC-Cutinase in the membrane decreased in concentration. The PET degradation process lasts for three days.

Furthermore, the decomposition of PET with the total enzyme LC-Cutinase in the membrane produces Ethylene glycol and Terephthalic acid. With the help of Reductase and Dehydrogenase enzymes, a chemical reaction occurs that converts Ethylene glycol into Malate. The chemical reaction process ensures that Ethylene glycol does not explain the

environment. But consuming Malate can be a source of energy for these bacteria. The simulation results from the Conversion model can be seen in the following figure.

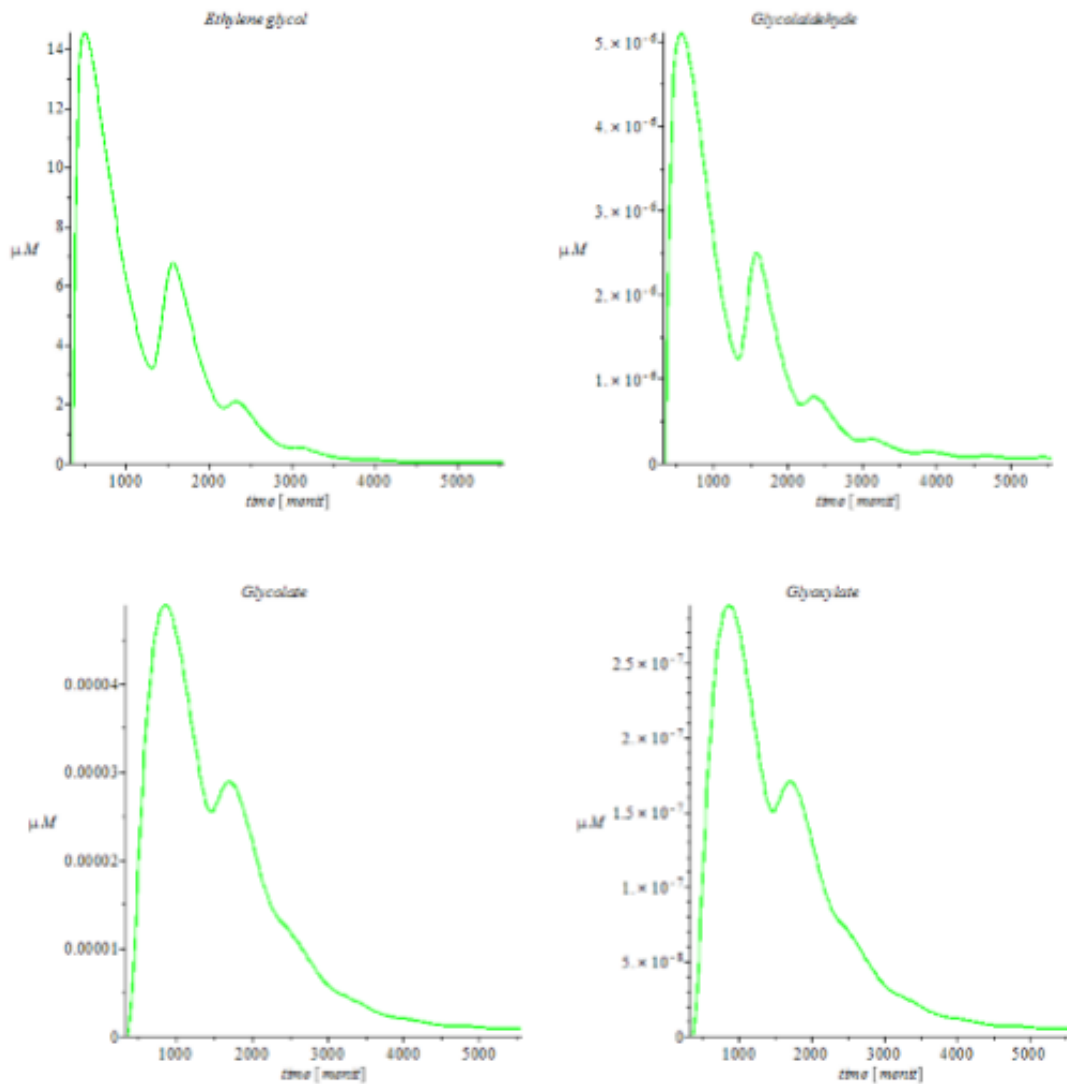


Figure 5. Conversion model simulation results

When the degradation process takes place, the Ethylene glycol formed increases. Furthermore, Ethylene glycol will be converted into Glycolaldehyde in the cells, with the concentration value also increasing. The process of changing Glycolaldehyde to Malate occurs in cells. It is seen that Glycolaldehyde to Malate has increased. This is inversely proportional to PET which has decreased. Because Malate is an energy source is in the cell, it has an impact on the growth of these bacteria.

The growth of E-coli bacteria in PET biodegradation is divided into two stages. In the first stage, bacteria are allowed to grow by consuming nutrients. In the second stage, the new bacteria consume malate. The following is a simulation result of a bacterial growth model that can represent its biological phenomena.

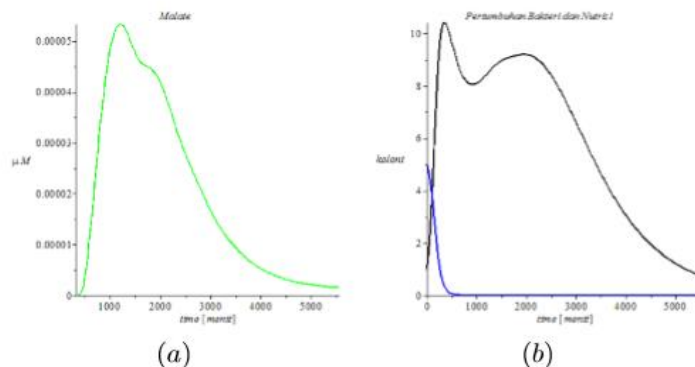


Figure 6. Simulation results (a) Malate, (b) Bacterial growth

In the first stage, the number of bacteria seen from the simulation results increased from the initial minute to the 360th minute. At the 360th minute, the bacteria had reached the peak of growth or at the end of the stationary phase. Likewise, the concentration of nutrients continues to fall because bacteria consume these nutrients.

In the second stage, PET is inserted in the 360th minute, so PET decomposition occurs and produces malate. Bacteria directly use this malate. From the simulation results, the number of bacteria decreased, but in the 1000th minute, the growth occurred again. This is because the concentration of malate increases, so bacteria can use malate as an energy source to grow until, at the 2000th minute, the number of bacteria decreases as a sign that the malate concentration has been used up.

4. Conclusions

Models of the PET biodegradation system and bacterial growth, as well as model analysis and simulations, have been described. Then it can be locked in outline as follows:

- a. The phenomenon in the biodegradation system can already represent the experimental results. This can be seen from the regulatory model, and the reporter produces an oscillating simulation. PET degradation results can show the metabolic processes carried out by bacteria, the change from Ethylene glycol to Malate.
- b. PET reacts with LC-Cutinase in cell membranes, producing malate, which can be used as an energy source for bacterial growth. Thus, bacteria can grow faster. The more bacteria, the more PET can be broken down quickly.

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