Dynamic Modelling of Bioconversion of Domestic Wastewater Sludge for Cellulase Enzyme

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Abstract A mathematical modeling of bioconversion of domestic wastewater sludge for cellulase enzyme production was constructed based on the ideal batch mode. The mathematical models was based on the mass balance equation and focused particularly on the biomass, substrate and product rate of reaction. The biomass, substrate and product considered in this project were glucosamine, reducing sugar and cellulase enzyme respectively. The optimal conditions of pH, temperature, inoculum size, substrate concentration, co-substrate concentration and agitation speed were maintained during the simulation. The MATLAB simulation has been done by using modified Monod equation and modified kinetic equations for biomass, substrate and product. The results showed that higher product yields would be achieved when optimal conditions of parameters were held constant, the time constraints was 10 days and 95% of confidence level had been achieved which determined the range of validity of k's values for these models. This study also involved the quantitative analysis and qualitative analysis graphically for validation of models.

Keywords Bioconversion · Wastewater · Enzyme

1 Introduction

Every year, Indah Water Konsortium (IWK) in Malaysia has produces around 3.8 million cubic meters of wastewater sludge in the wastewater treatment plants. The sludge volume is expected to increase to 7 million cubic meters by the year 2020 which contribute vast amount of money for management cost in the future approximately more than RM 1 billion [1].

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It is believed that cellulose exists in the domestic sludge in few percentages but lignocellulosic components are very resistant to biodegradation [2]. Bioconversion of domestic wastewater sludge refers to biodegradation by specific microorganism for enzymes or other metabolite production which in this case, cellulose can be degrade for cellulase enzyme production by fungi called *Trichoderma harzianum.* Cellulase enzyme is recently used in the pulp, dye, paper, textile, and biodegradation of toxic wastes and in chemical industries. Thus, the cellulase production from the domestic wastewater sludge offers potentially low cost alternatives with the abundant availability of material source [3].

Mathematical modeling is an effective method used widely in science and engineering. The mathematical models and methods provide a rigorous, systematic, and quantitative description of various real-world phenomena such as physical, chemical and biological. The mathematical modeling helps to understand and analyses the important qualitative features of these phenomena, to organize and process data and to design and develop complex engineering system. A good model can identify possible future problem areas in engineering activity and predict the side effects of recommended engineering solutions in the long run [4]. Modeling in bioengineering is devoted to applied mathematical modeling in the rapidly growing field using biotechnology which industries can replaced chemical-based manufacturing processes with bioengineering processes that offers less expensive, faster and safer for the people and environment. This give advantages as biotechnology plays an essential roles in the food production such as yeast to make bread rise, bacteria used in yogurt and enzymes to make cheese. In addition, the biotechnology increasingly used to design a desirable protein or enzyme from the available natural source and can applied on a larger industrial scale [4]. However, the study of mathematical modeling of bioconversion of domestic wastewater sludge for ligninase and cellulase enzyme production is still new. Thus, this paper will propose a suitable mathematical modeling for a product-oriented process of bioconversion that enhanced the understanding of some mechanisms in studied process, to test the sensitivity of system parameters and to optimize system behaviour.

2 Methodology

The model builds in this project mainly for batch mode operation of reactor. Batch reactors are simplest type of mode of reactor operation. In this mode, the reactor is filled with medium and the bioreactor is allowed to proceed. When the fermentation has finished, the contents are emptied for downstream processing. The reactor is then cleaned, re-filled, re-inoculated and the bioreactor process starts again. In a batch bioreactor process, the biomass will start growing from initial concentration (about zero) and often after a short lag period until the substrate will finish. According to the above description, the zero input and the zero output to the system characterize the batch process. Like many other operated reactors, volume of a batch

reactor is usually kept constant. In order to build mathematical modeling that resemble with the actual phenomena or biological process, many assumptions have to be made to offer good models that can simulate the biological reaction within certain conditions or constraints. The construction of this model can be initiated from the simple microbial kinetics for batch fermentation process. In this project, each group members are assigned for mathematical models for production of different enzymes. The assigned project is to identify the mathematical models for the bioconversion of domestic wastewater sludge for cellulase enzyme production.

MATLAB^{TM} is a software package used for high performance numerical computation and visualization to solve application of mathematical and computer modeling and simulation [5]. It provides an interactive environment with hundreds of built-in functions for technical computation, graphic and animation. It also provides easy extensibility with its own high-level programming language. The name MATLAB stands for MATrix LABoratory. In this study, MATLAB 7.0 was used to simulate specifically the rate of glucosamine, reducing sugar and cellulase activity by using module of ode45.

2.1 Modeling Procedure

There are two method of model reduction which is the first situation when a number of different candidate models are available and the best model can be selected from them while the second situation occurs when a reference model available and the model could be simplified for certain purpose [6]. In this project, the first method has been selected where the model equation has been developed by available models that resemble the system of bioconversion of domestic wastewater sludge to produce enzyme cellulase.

2.2 Phases of Model

According to Jeppsson [6], the modeling construction can be divided to three phases, which is Phase 1 for identification, Phase 2 for mathematical model formulation and construction and Phase 3 for verification and validation. Phase 1 has been subdivided to functional specification of the process, setting the modeling objectives and selected a model type and system characterization. The functional specification of the process refers to a quantitative understanding of the structure and the parameters describing the process are required. The modeling objectives are decided that may concern about model purpose, system boundaries, time constraints and accuracy of the model developed. While Phase 2, consist of mathematical model formulation and construction according to a generic modeling methodology where many attempts have been made to apply systems approach to the development of a modeling methodology, which have the major concern on system characterization

and model construction. In Phase 3, the process involves with the verification and validation of model to obtain the appropriate model at the end of the project. It is clear that model verification and model validation are closely related and require an iterative procedure

In this model development, analytical method of calculation for $\mu_{\max}$, K_s, $Y_{p/x}$ and $Y_{x/s}$ were calculated by previous study using experimental data [3], which was held as constant parameters in the model. Even though the above-mentioned parameter was held constants in each run or trial of MATLAB coding, the values are within the range of boundaries that indicates validity of constant parameters (Figs. 1 and 2).
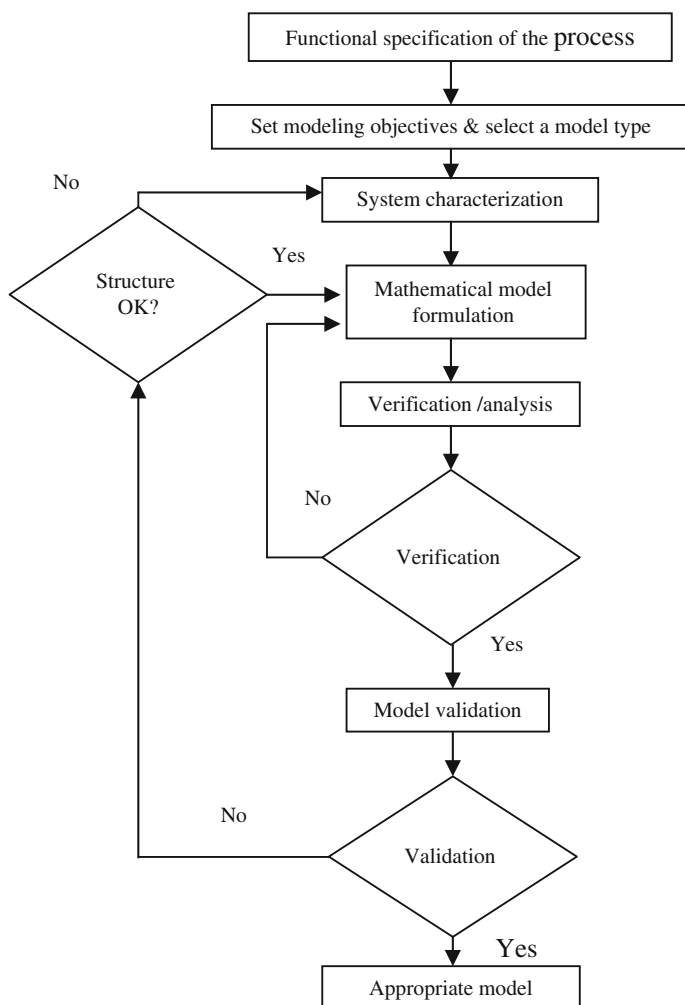
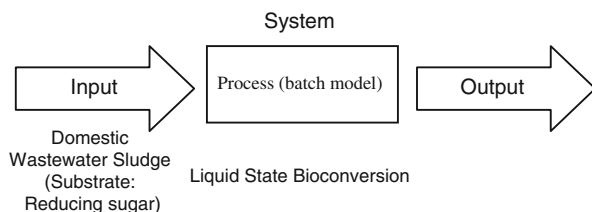**Fig. 1** A generic modeling methodology [6]

Fig. 2 Schematic diagram for cellulase enzyme production

2.3 Model Structures

The available model which best describe the bioconversion of domestic wastewater sludge for cellulase enzyme was Monod equation and general kinetic models for biomass, substrate and product that has been modified where this system has been identified as closed system (batch mode reactor). Valid coefficients and parameters were added, which then compared to the experimental graph in order to indicate the appropriate modified Monod equation and kinetic models.

2.3.1 Monod Equation

Based on the literature review on the cellulase activity that has been produced by *Trichoderma harzianum* in the batch mode operation [3], the mathematical reaction rate models that fitted the general characteristics of bioconversion process was known as Monod equation [7] was used as general model that undergone certain modification with boundaries as shown below:

$$\mu = \frac{\mu_{\max} S}{K_s + S} \tag{1}$$

where μ = specific growth rate, $\mu_{\max}$ = maximum specific growth rate (h^{-1}), S = limiting substrate concentration (g/l), K_s = saturation constant (equivalent to substrate concentration when $\mu_{\max}/2$).

2.3.2 General Kinetic Model

Until today, there have been no reports on mathematical modeling of the kinetics of cellulase production by *Trichoderma harzianum* in batch fermentations. It is logical to use the mathematical models that have been used to describe batch fermentations for process control as a basic equation and was modified to indicate the process control of liquid state bioconversion of domestic wastewater sludge for cellulase enzyme production. The series of equations describing a general kinetic system for cellulase production is as follows:

$$\text{Biomass } X(t) \colon \frac{dX}{dt} = \mu \cdot X \tag{2}$$

$$\text{Product } P(t)\colon \frac{dP}{dt} = \mu \cdot X \cdot Y_{px} \tag{3}$$

$$\text{Substrate } S(t)\colon \frac{dS}{dt} = -\frac{\mu \cdot X}{Y_{xs}} \tag{4}$$

where μ = specific growth rate (h^{-1}), X = biomass concentration (g/l), Y_{px} = moles of product formed per unit mole substrate consumed, Y_{xs} = moles of biomass produced per unit mole of substrate consumed.

3 Results and Discussion

3.1 Mathematical Equation for Cellulase Enzyme Production by Liquid State Bioconversion

The modified Monod equation and kinetic models of biomass, substrate and product described the liquid state bioconversion of domestic wastewater sludge to produce cellulase enzyme within valid range. The parameters were held constants while the dimensionless parameters (the k's values) were varied to identify the best-fit models and pattern of graph. The following equations were modified Monod and kinetic models:

3.1.1 Modified Monod Equation

$$\mu = \frac{\mu_{\max} \cdot S.X}{K_s.X + S} \tag{5}$$

where μ = specific growth rate (per day), $\mu_{\max}$ = maximum specific growth rate (per day), S = concentration of substrate (mg/ml), X = concentration of biomass (mg/ml), K_s = saturation constant of the substrate (mg/ml).

3.1.2 Modified Kinetic Models

$$\frac{dX}{dt} = \mu \cdot X - K_1 \cdot X \tag{6}$$

$$\frac{dS}{dt} = -\left(\frac{\mu \cdot X}{Y_{xs}}\right) \cdot K_2 + \frac{K_3}{S} \tag{7}$$

$$\frac{dP}{dt} = \mu \cdot X \cdot Y_{px} \cdot K_4 - K_5 \cdot X - K_6 \cdot P \tag{8}$$

where μ = specific growth rate (per day), X = concentration of glucosamine (mg/ml), S = concentration of reducing sugar (mg/ml), P = concentration of cellulase activity (FPU/ml), Y_{xs} = moles of biomass produced per unit mole of substrate consumed, Y_{px} = moles of product formed per unit mole of biomass formed, and K_i = dimensionless constants where i = 1, 2, 3, 4, 5, 6.

The model equations have been developed according to the principles of mass balance. The following are assumptions developed in mathematical modeling formation:

1. Ideal batch mode operation is considered at steady state
2. Co-substrate are not considered in the mathematical modeling
3. The mathematical modeling was valid within range of the following physical parameters:

3.2 The Simulation Result

The conditions for simulations such as μ_{max}, Y_{xs}, Y_{px}, K_s, the initial conditions and the constants were determined as shown in Table 1:

According to the graph plotted for each trial, Trial 1 provides the most significant result as shown in Fig. 3 compared to Trial 2 which varies in constant parameters by using the lower range of k's values and Trial 3 which varies in constant parameters by using the upper range of k's values. The plot of Trial 1 resembles the rate of production of glucosamine, reducing sugar and cellulase activity which was simulated by modified Monod equation and kinetic models as shown before. The mathematical model developed was on the basis of general mass balance equation and one of the major objectives in this study was to achieve maximal production of cellulase enzyme as illustrated in Fig. 3.

The Monod equation was used because it resembles the interaction of glucosamine, reducing sugar and cellulase activity in the bioreactor. As shown in Eq. (5), the specific growth rate was mainly influenced by the maximum specific growth rate, the concentration of biomass and substrate and the saturation constant of the substrate, which was determined from experimental data. As illustrated in

Table 1 The values of parameters and constants that varied in Trial 1, Trial 2 and Trial 3

| Trial | μ_{max} | Y_{xs} | Y_{px} | K_s | Initial conditions (mg/ml) | | | Constants | | | | | |
					X	S	P	K_1	K_2	K_3	K_4	K_5	K_6
Trial 1	0.12	0.0119	5.9725	0.6	1.112	9.025	0	0.01	0.157	0.6	12.13	3.5	1.37
Trial 2	0.12	0.0119	5.9725	0.6	1.112	9.025	0	0.009	0.122	0.5	11.55	3.2	1.32
Trial 3	0.12	0.0119	5.9725	0.6	1.112	9.025	0	0.03	0.16	0.7	13.21	3.7	1.39
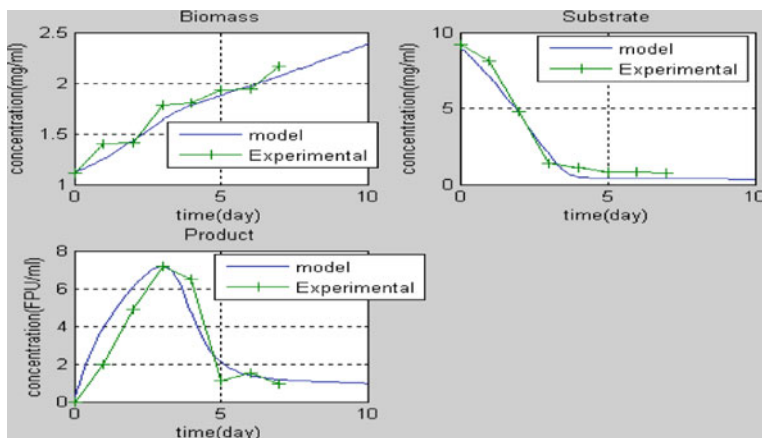
Fig. 3 Graph of biomass, substrate and product in Trial 1

Fig. 3, the experimental plot was compared to the model plot. The formula used was shown in Eq. (6) to simulate the rate of cell growth or glucosamine within 10 days. Based on this equation, the constant, K_1 was multiplied with biomass concentration and subtracted from the rate of cell growth. The constant applied in this formula indicates the kinetics of cell death where deactivation of the culture occurs over a finite period of time depending on the initial number of viable cells and the severity of the conditions imposed. The plotted model of biomass seems to increase within time as stated in the literature review that *Trichoderma* genus had capacities to adapt severe environmental constraints [8]. Thus, the model of rate of glucosamine concentration was confirmed by the experimental result from the previous study.

The graph (Fig. 3) of substrate was plotted on the basis of Eq. (7) that indicates the rate of reducing sugar in the batch culture process. In this model, the substrate uptakes coincide with product formation. When products are formed in energy-generating pathways, product synthesis is an unavoidable consequence of cell growth and maintenance. The Eq. (7), illustrated the substrate taken up for product synthesis and to support growth and maintenance [9]. This statement supported by the addition of constant K_2 and K_3 for maintenance of cell growth and maximal production of cellulase enzyme. As shown in Fig. 3, the maximum synthesis of the cellulase enzyme in optimal conditions was on day 3 and the minimum substrate observed was on day 3 before reaches zero concentration of substrate. Therefore, the rate of reducing sugar concentration was confirmed by the experimental result from the previous study. Based on the Eq. (8), the rate of cellulase activity was influenced mostly by the biomass concentration. This explained that the higher the cell concentration, the higher the cellulase enzyme production. But, the cellulase enzyme formation decreased dramatically after day 3 because of inconsistency of pH that stated in the previous study [3]. Also, Eq. (8) was influenced by the product concentration, which indicates that there was other formation of metabolites, which

simultaneously produced and inhibit the cellulase enzyme. Thus, the model of rate of cellulase activity was confirmed by the experimental result from the study.

3.3 Validation of Mathematical Model

Each simulation of mathematical model according to the trial was compared with the experimental data. Combining the graph of both measured and predicted of each trial did the comparison. It can be observed that the Trial 1 gave the best result.

The combination of each graph of Trial 1, illustrate least percentage error and the pattern of curve shape was approximately the same with the experimental graph. The best-fit conditions of these graphs validated by using optimal physical parameters, constant parameters and within time constraints of 10 days with 95% of accuracy level. The mathematical models after validation could be modify within the range of validity of k's values and parameters such as Y_{xs} and Y_{px} for future studies. The range of validity for Trial 1 is as follows (Table 2):

Table 2 The range of validity for Trial 1

Y_{xs}	Y_{px}	K_1	K_2	K_3	K_4	K_5	K_6
0.0119 ± 0.0001	5.9725 ± 0.0005	0.01 ± 0.0002	0.157 ± 0.001	0.6 ± 0.02	12.13 ± 0.13	3.5 ± 0.08	1.37 ± 0.003

4 Conclusion

Based on the mathematical model development of bioconversion of domestic wastewater sludge to produce cellulase enzyme, the model could be used as an indicator for prediction of maximum production of cellulase enzyme as illustrated before where the maximum cellulase enzyme formation was occurred on day 3. The development of mathematical model in this study was based on the batch culture mode and considered the general mass balance equation that results in modifying Monod equation and general kinetics model. The validity of the constructed mathematical model was bounded by several restrictions such as assumptions was done to simplified the complex reaction to the simplified model, the time constraints was 10 days and the accuracy of confidence level was at 95%. The final model developed in this study was modified Monod equation and modified kinetic models of biomass, substrate and product, which can be used to solve certain problem especially with the accumulation of sludge in the wastewater treatment. The model described the bioconversion process to convert the available source of sludge to give value-added material or substances, which in this case the production of cellulase enzyme for industrial usage in Malaysia. The advantages of model developed, it can reduce the accumulation of sludge cakes, the cost of wastewater treatment in Malaysia and the content of pollutant in the effluent treatment.

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