https://doi.org/10.31436/iiumecp.v1i1.3011

MODELING OF *E. COLI* GROWTH, GLUCOSE CONSUMPTION, AND RECOMBINANT COLLAGEN-LIKE PROTEIN FORMATION KINETICS

ABEIR HUSSEIN MOHAMED GAMEIL, FARIDAH YUSOF*, AZLIN SUHAIDA AZMI, NOOR ILLI MOHAMAD PUAD

Dept. of Chemical Engineering and Sustainability,
International Islamic University Malaysia, Kuala Lumpur, Malaysia

**Corresponding author: yfaridah@iium.edu.my*

ABSTRACT: Mathematical modeling of the kinetics of fermentation processes is crucial to understand complex microorganism behavior and identify problems. To investigate growth kinetics, as well as glucose utilization and recombinant collagen-like protein formation kinetics, *E. coli* BL21 (DE3) harboring a collagen-like protein from *Rhodopseudomonas palustris* (RPCLP) was cultivated in M9-casamino acids medium containing glucose (10 g/L). OD_{600} and a glucose assay kit were used to monitor the biomass concentration and glucose depletion, respectively. The RPCLP concentration was measured via a fluorometric assay. Results show that the maximum biomass achieved was 8.25 g/L, whereas the maximum RPCLP yield was 2 mg/L. Growth data was then fitted into unstructured models- Monod model and logistic model. The Luedeking-Piret model was used for the substrate utilization and product formation data. Kinetic parameters were determined by nonlinear regression and ANOVA analyses were done to determine the significance of each fit. While the growth data fitted both the Monod and the logistic model well, with significant coefficients of determination (R^2 values 0.96 and 0.99, respectively), the Luedeking-Piret models did not represent the substrate utilization and product formation well (correlation coefficients ≤ 0.7). The study suggests that the production is mixed-growth associated and modification of the model is required.

KEYWORDS: *Fermentation Kinetics, Kinetic Modelling, Escherichia coli, Recombinant Protein, Collagen.*

1. INTRODUCTION

Interest in alternative sources of collagen has risen over the past several decades, primarily due to the demand for collagen in various sectors and in part due to disadvantages with the current sources, namely bovine and porcine sources. Collagens are a family of proteins that are ubiquitous in the mammalian body and are indispensable due to their numerous uses in the body. Many species of bacteria have been discovered to produce collagen-like protein sequences, and the production of recombinant collagen-like proteins from a few selected species has been studied at the shake flask culture level, using recombinant *E. coli*. The current literature has supported the viability of recombinant bacterial collagen-like protein production as a sustainable source of Halal, vegan, and non-immunogenic collagen-like peptides. To our best of knowledge, none of these studies have considered fermentation kinetics. Prior to any scale-up attempts, the laboratory scale production must first be characterized via a kinetic study to investigate and mathematically model *E. coli* growth kinetics, as well as substrate utilization

https://doi.org/10.31436/iiumecp.v1i1.3011

and recombinant collagen-like protein formation kinetics. Mathematical modeling of the kinetics of fermentation processes sheds light on the metabolic regulation of the microbe and in process control. Furthermore, it has techno-economic and physiological implications in the reactor design and optimization of operation conditions. By understanding the growth kinetics of the microbe of interest, the fermentation process can be designed to achieve optimum product concentration. In this study, a collagen-like protein from *R. palustris* (RPCLP) has been successfully expressed in recombinant *E. coli*. This recombinant collagen-like protein production aims to yield Halal, high purity, non-immunogenic, collagen-like proteins that could be modified to suit various applications and cater to the ever-growing collagen demands of the pharmaceutical, biomedical, and food industries in a sustainable manner. To facilitate its scaleup, insight into the fermentation kinetics is necessary. Therefore, the objective of this work was to evaluate the data using unstructured models and estimate the values of relevant kinetic parameters.

2. MATERIALS AND METHODS

Strains, Medium and Batch fermentation

Genetically engineered *E. coli* BL21 (DE3) carrying pColdII (Takara Bio) containing a collagen like protein-coding gene from *R. palustris*, an ampicillin-resistance gene, and temperature-sensitive promoter. The *E. coli* was cultivated in a complex M9-casamino acid medium that had been optimized for *R. palustris* collagen-like protein (RPCLP) expression using *E. coli* BL21 (DE3). This medium consists of D-glucose (10 g/l), 1X M9 salts, casamino acids, trace elements, magnesium sulfate, thiamine, calcium chloride, and ampicillin (50 µg/ml).

Inoculum (2.5 ml, OD_{600} ~ 3-5) was transferred into 250 ml unbaffled Erlenmeyer flasks containing 50 ml M9-casamino acid medium, supplemented with ampicillin (50 µg/ml). The flasks were cultured at 37 ℃ temperature and 250 rpm agitation speed. Sampling was done every 30 minutes until induction, and then hourly until the end of the fermentation (24 hours). Induction was carried out at the mid-log phase by lowering the temperature to 20 ℃, increasing the agitation speed to 300 rpm, and adding IPTG solution to attain a final concentration of 1 mM [1]. The cells were harvested via centrifugation at 4696 x g, at 4°C, for 30 minutes (Heraeus Multifuge X1R, Thermo Scientific, USA), and cell pellets were stored at -20 ºC. Cell pellets were resuspended with ice-chilled lysis buffer (20 mM sodium phosphate pH 7.4, 0.5 M sodium chloride) and lysed by sonication using 5-minute burst cycle, with 0.5 second pulse, at 40% amplitude (Labsonic M, Sartorius, Germany) [1]. The resultant lysed cells were clarified via centrifugation at 12, 000 rpm, 4°C, for 30 minutes (Heraeus Multifuge X1R, Thermo Scientific, USA).

Analysis Methods

Cell density was analyzed turbidometrically at 600 nm optical density (OD_{600}) using a spectrophotometer (Biophotometer, Eppendorf, Germany). Cell wet weight (CWW) and dry cell weight (DCW), in g/l, were also determined using established calibration curves. A colorimetric glucose assay kit (Cell Biolabs Inc, USA) was used to determine the glucose concentration during fermentation. A glycine-based, fluorometric collagen assay was used to determine collagen-like protein concentration as described by Yasmin and colleagues, measured at $\lambda_{\text{excitation/emission}}$ = 375/465 nm [2].

https://doi.org/10.31436/iiumecp.v1i1.3011

Modeling and Statistical Analysis

Unstructured batch kinetic models, based on either Monod model of growth and the logistic model of growth, combined with Luedeking-Piret equations for substrate utilization and product formation were employed in this study [3, 4]. These models are hereafter referred to as the Monod-Luedeking Piret (MLP) and the Logistic-Luedeking Piret (LLP), respectively. Unsegregated and unstructured models based on the Monod equation are often used to describe the overall substrate-dependent cell growth and fermentation kinetics in batch, fed-batch, or continuous microbial processes [5]. As such, the simple Monod model was selected. Sometimes, information on the limiting carbon substrate is not available, due to use of complex media, and in such cases, substrate-independent growth kinetics can be determined by applying models like the Logistic model or modifications to the Monod model such as the Contois equation [6]. To consider the effect of substrate consumption, the logistic model of cell growth was chosen for comparison. The Monod model for specific growth rate, μ, is expressed as follows:

$$\mu = \mu_{max} \frac{S}{Ks+S} \quad (1)$$

where μ_{max} is the maximum specific growth rate, S is substrate (glucose) concentration (g/L), and K_S is the half-saturation constant for glucose.

The logistic model equation is:

$$\mu = k\,(1 - (\frac{X}{Xmax})) \quad (2)$$

$$\frac{\Delta X}{\Delta t} = \mu . X \quad (3)$$

The Luedeking-Piret model equations for rates of substrate consumption ($\frac{\Delta S}{\Delta t}$) and product formation ($\frac{\Delta P}{\Delta t}$) are:

$$\frac{dS}{dt} = -m\frac{dX}{dt} - n.X \quad (4)$$

$$\frac{dS}{dt} = -\frac{1}{YXS}\frac{dX}{dt} - \frac{1}{YPS}\frac{dP}{dt} - k_e X \quad (5)$$

$$\frac{dP}{dt} = \alpha\frac{dX}{dt} + \beta X \quad (6)$$

where X represents biomass concentration (g/L), P is collagen-like protein concentration (g/L), α is the growth associated collagen-like protein production constant, β is the non-growth associated collagen-like protein production constant, m is growth associated glucose consumption constant (g g^{-1}), and n is the non-growth associated glucose consumption constant (g g^{-1} g^{-1}), k_e is the maintenance coefficient for cells (g/g.h), Y_{XS} is the yield of cell mass from substrate , Y_{PS} is the yield coefficient of product from substrate, k is initial specific growth rate (h^{-1}), and X_{max} is the maximum cell concentration (g/L).

Initial estimation of kinetic parameters and yield constants was accomplished using Microsoft Excel software, wherein experimental data was keyed in and the OD_{600} values were plotted against time. The slope of the resulting linear regression line was taken as μ_{max} [5]. These values were then used as initial guesses for solving the ordinary differential equations. MATLAB software (R2022a, MathWorks, USA) was used to generate plots of experimental data and pass initial values of kinetics parameters into sets of ordinary differential equations which were solved by using the *ode45* function and the Levenberg-Marquardt method of the *lsqcurvefit* function. The coefficient of determination (R^2) was then estimated to evaluate the

https://doi.org/10.31436/iiumecp.v1i1.3011

accuracy of the estimated parameters achieved by fitting the experimental data to the kinetic models [6].

3. RESULTS AND DISCUSSION

The pattern of growth of the recombinant *E. coli* follows typical bacterial growth curve composed of a short lag phase, a log phase of exponential increase in biomass, and a stationary phase. Similar growth patterns were observed in protein-producing fermentations employing recombinant *E. coli* [3]. The profile of substrate concentration over time shows a sharp decrease in substrate concentration after inoculation. The product concentration profile with time shows accumulation of product, but the increase is inconsistent with expected results. Induction of the protein expression with IPTG starts at the mid-log phase, after hours of inoculation, which means that product accumulation should gradually increase after the induction. However, this is not reflected well in the product concentration curve (refer to Fig. 1-2). The kinetic parameters were approximated by the respective models as in Table 1.

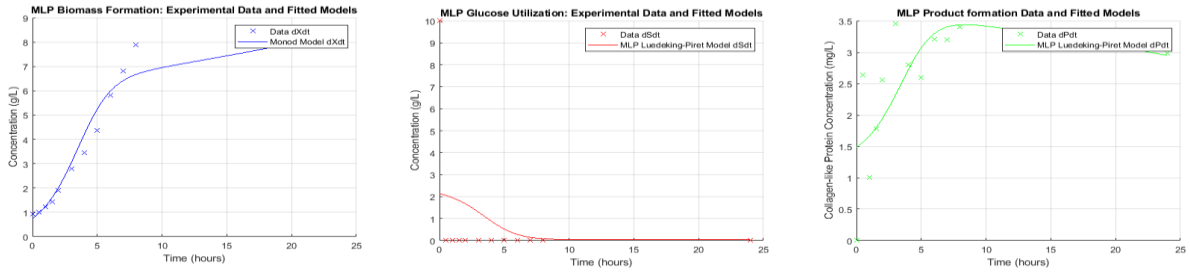

Fig. 1 Monod-Luedeking Piret (MLP) model experimental data and fitted models.

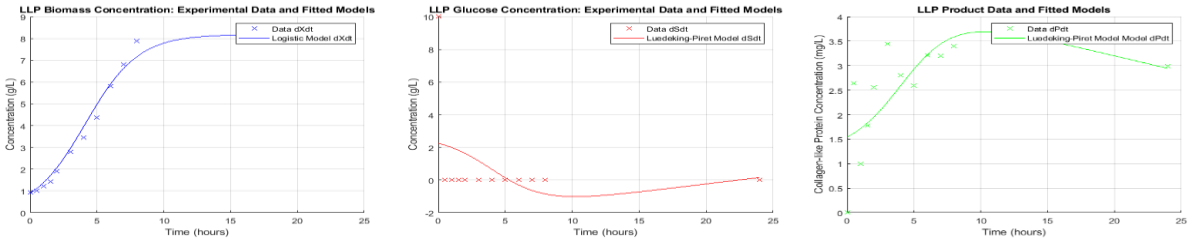

Fig. 2 Logistic-Luedeking Piret (LLP) model experimental data and fitted models.

Table 1 Estimated Kinetic Parameters of Respective Models

Model	μ_{max} **[/h]**	K_S **[g/L]**	Y_{XS} **[g X/g S]**	Y_{PS} **[g P/ g S]**	α **[g P/g X]**	β **[g P/g X h]**	k_e **[g S/g X h]**	X_{max} **[g/L]**	k **[h^{-1}]**
MLP	1.75	4.82	2.69	0.02	0.0004	-0.000009	-0.00483	-	-
LLP	-	-	1.80	0.02	0.00037	-0.000008	-0.01224	8.18311	0.507

Of the two models studied, it can be observed from Figure 1 that biomass concentration is best described by the Monod model. Even though the Monod-Luedeking Piret and Logistic-Luedeking Piret substrate utilization and product formation models studied did not fit the experimental data well, the Monod model for biomass accumulation fitted the data very well (Figure 1). This is further supported by the Monod kinetic parameter values of μ_{max} = 1.75 and K_S = 4.82 g/l, which were acceptable, compared to those reported in the literature for *E. coli* [7]. As for the logistic model (Figure 2), it can be observed that it fits the experimental data for growth

https://doi.org/10.31436/iiumecp.v1i1.3011

but was unable to attain a good fit for substrate utilization and product formation. Factors such as hydrodynamics, oxygen partial pressure, agitation speed, pH, and temperature can affect the fermentation process by a large margin. While the growth data fitted both the Monod and the logistic model well, with significant coefficients of determination (R^2 values 0.96 and 0.99, respectively), the Luedeking-Piret models did not represent the substrate utilization and product formation well (correlation coefficient ≤ 0.7).

4. CONCLUSION

Both the Monod model and logistic model provided a good fit of the growth experimental data. The growth-associated and non-growth associated constants, α and β, suggest that the production is mixed growth associated, and as the Luedeking-Piret model was not a good fit for the product formation and substrate consumption, further modification of the model is required. As there is limitation to the control of parameters such as pH and oxygen partial pressure, in the shake flask culture, it is recommended that the kinetics are evaluated at the 2 L bioreactor culture for comparison. Overall, the kinetic parameters obtained provide useful indicators for the fermentation process optimization and its scale-up.

ACKNOWLEDGEMENT

This study is funded by the Ministry of Higher Education, Malaysia, via the Transdisciplinary Research Grant Scheme (TRGS/1/2018/UIAM/01/1/3). We would like to express our gratitude towards the Institute of Halal Research and Training (INHART), IIUM, for the use of the INHART Lab facility.

REFERENCES

[1] Xu C, Yu Z, Inouye M, Brodsky B, Mirochnitchenko O. (2010) Expanding the family of collagen proteins: recombinant bacterial collagens of varying composition form triple-helices of similar stability. Biomacromolecules, 11(2), 348-356.

[2] Yasmin H, Kabashima T, Rahman MS, Shibata T, Kai M. (2014) Amplified and selective assay of collagens by enzymatic and fluorescent reactions. Sci Rep, 4, 4950.

[3] Ariff AB, Nelofer R, Abdul Rahman RNZR, Basri M. (2015). Kinetics and modelling of batch fermentation for the production of organic solvent tolerant and thermostable lipase by recombinant E. coli Turkish Journal of Biochemistry, 40(4).

[4] Garnier A, Gaillet B. (2015). Analytical solution of Luedeking-Piret equation for a batch fermentation obeying Monod growth kinetics. Biotechnol Bioeng, 112(12), 2468-2474.

[5] Doran PM. (2013) Bioprocess Engineering Principles. Academic press, Waltham, MA.

[6] Ali MK, Serge H, Nawel O, Radia C, Noreddine, KC. (2017). Kinetic models and parameters estimation study of biomass and ethanol production from inulin by Pichia caribbica (KC977491). African Journal of Biotechnology, 16(3), 124-131.

[7] Limoes S, Rahman SF, Setyahadi S, Gozan M. (2018) Kinetic study of Escherichia coli BPPTCC-EgRK2 to produce recombinant cellulase for ethanol production from oil palm empty fruit bunch. IOP Conf. Ser.: Earth Environ. Sci., 141 012016.