Golamari Siva Reddy et al /J. Pharm. Sci. & Res. Vol. 10(5), 2018, 1223-1228

ISSN:0975-1459
Journal of Pharmaceutical Sciences and Research
www.jpsr.pharmainfo.in

# Kinetic Measurements for *Achromobacter Xylos* GSR-21 During Biosurfactant Production in Two-phase system and developing a Double-exponential model for viable cell profile [34]

**Golamari Siva Reddy[1], Botlagunta Mahendran[2] and Ronda Srinivasa Reddy[3*]**

[1,2,3] *Centre for Bioprocess Technology, Department of Biotechnology, Koneru Lakshmaiah Education Foundation (Deemed to be UNIVERSITY), Green fields, Vaddeswaram, Guntur, Andhrapradesh, india-522502.*

**Abstract:**

Biosurfactants are microbial substances which impact surface strain. The active investigation was completed for *Achromobacter xylos* GSR21during biosurfactant generation in a two-stage fluid clump maturation framework. The most extreme glycolipid fixation (Pmax) and the yield of biosurfactant per biomass (YP/X) in a 5-L bioreactor containing mineral salt medium (MSM) with expansion of 1% (v/v) vegetable oil as carbon source medium were observed to be around 21.9 g.L-1 and 3.2 g.g-1, individually. Already detailed active models in fluid frameworks, three-parameter Gompertz, Luedeking-Piret and Mercier conditions, exhibited sufficient decency of-fit (r2 >0.95) to active information recorded for biomass development, nitrate utilization and glycolipid arrangement amid maturation. A twofold exponential condition was created to show time-course information of reasonable cells announced as state shaping units (CFUs). This proposed condition could precisely foresee the test information with r2 = 0.967 and give an effective articulation to plot microbial cell check design. Low estimations of the standard blunder of gauge (SEE) and chi-square (x2) affirmed the satisfactory level for the exactness of fit. The 95% certainty interims for motor parameters in every nonlinear model showed a thin range mimicking the accuracy of estimation. This motor examination may work productively in the outline and scaling-up of the coming fluid natural bunch bioreactors which are to be connected to the creation of biosurfactants.

**Key words:** Batch Fermentation, Kinetic behaviour, Modelling, Biosurfactant, Two-Phase system.

## 1. INTRODUCTION

Biosurfactants are significant microbial mixes with an extensive variety of surface-dynamic highlights. They might be utilized as frothing, wetting, solubilizing, antiadhesive and antimicrobial specialists, emulsifiers, invulnerable controllers and resistant modulators. They are potential possibility for some business applications in the oil, pharmaceutical, biomedical, microbial improved oil recuperation and nourishment handling [1-3]. Among vital surfactant gatherings, glycolipid delivered by *Achromobacter xylos* strains have been the most broadly examined. The glycolipid generation by Achromobacter xylos under various conditions that improved creation yield, have shown that the quality and amount of the delivered glycolipids are affected by a few elements, for example, nitrogen and particle fixation, pH, temperature, air circulation rate and the idea of carbon source. Nonetheless, an absence of exhaustive impression of the glycolipid creation energy and conduct of Achromobacter xylos cells under controlled conditions at the bioreactor scale and the nonappearance of individual similar examinations are among the key issues in regards to lacking productivities and practical generation of these biosurfactants [5]. A few examinations have demonstrated that the higher yields of glycolipid are acquired when Achromobacter xylos is developed on water immiscible substrates as carbon sources, when contrasted with the miscible ones [6-8]. By the by, dynamic models have just been introduced for glycolipid creation with water solvent carbon sources, however most as of late Medina-Moreno et.al. Recommended a model to portray the microbial generation of biosurfactant utilizing oleic corrosive as a substrate immiscible in water [4]. Howere both Monod write and Sigmoidal models, for example, calculated and gompertz have already been connected to biomass energy [9-12]. Williams and Luedeking-Piret created substrate utilization conditions which have been broadly utilized as a part of bacterial aging [13-14]. A few conditions have been already settled to display microbial creation [15-17]. The present work proposed to screen glycolipid generation by Achromobacter xylos utilizing mineral salt medium (MSM) with expansion of 1% (v/v) vegetable oil as carbon source in a controlled bioreactor framework. Also, it concentrated on the motor displaying of microbial creation and development at the bioreactor scale. In this examination, 3-parameter Gompertz and Leudeking-Piret conditions utilized by Chavez-Parga et al. [14] were connected to display the time-course example of biomass development and substrate use, separately. Moreover, time-course profile of glycolipid creation was demonstrated by the condition of Mercier et al. [15]. Besides, a novel active model which was not tended to by past works was determined for demonstrating the reasonable cell design after some time, which depended on a twofold exponential condition. Building up a model for the time-course profile of feasible Achromobacter xylos cells involves awesome significance in showing upward pattern of glycolipid while biomass development stops and reasonable cell checks drop.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals, Microorganism and Maintenance:

All chemicals were obtained from HiMedia Leading Biosciences (Secunderabad, Telangana, India).Mineral salt medium was given from microbiology research facility professional (KLEF).Vegetable oil was given from srirama chain stores (Vijayawada, India). The Achromobacter xylos GSR21 (JQ746488) strain, initially segregated from soil tests, was utilized all through this examination. The bacterial cells were kept up as glycerol societies (70:30 (v/v) blend of naturally developed cells and 0.85% (v/v) glycerol arrangement in water at - 70°C.

### 2.2 Inoculum Preparation:

To set up an inoculum, a 1 mL test from the glycerol stock culture of Achromobacter xylos GSR21was immunized into 7 mL of LB soup [19] in a 50 mL-bird of prey tube and hatched overnight at 30°C, with shaking at 200 rpm. From that point, an aggregate volume of 1 mL was exchanged to 50 mL of LB soup in a 250 mL-shake flagon and kept overnight at 30°C and 200 rpm. Cell pellets were first gathered by centrifugation of the way of life medium at 2,147×g for 20 min and afterward suspended in a predefined volume of mineral salt medium (MSM) with expansion of 1% (v/v) vegetable oil [8] to achieve an optical thickness of (OD ) of 1. Suitable volumes of the seed societies filled in as inoculum for beginning up the bunch development in the bioreactor.

### 2.3 Culture Medium Composition and Cultivating Conditions:

All developments were done at 30°C of every a 5-L blended tank bioreactor containing 3.5 (l) of MSM and 2.5% (v/v) inoculum. The bioreactor was outfitted with a coordinated procedure control framework for temperature, pH, pO2 and wind stream. Frothing was controlled physically by raising and bringing down the air

circulation and fomentation rates conversely. To control the froth level and abstain from flooding the bioreactor, air circulation rate was lessened from 1 to 0.5 volume of air for each volume of fluid per min (vvm) thus unsettling rate was raised from 250 to 300 rpm, to adjust for drop in the broke down oxygen (DO) and keep it higher than 10%. Without a doubt, an additional impeller with rectangular cutting edges calculated at 45°, was mounted on a typical pivot with the mixing impeller situated at a separation of around 8 cm from, to break the froth that was created. In addition, one drop of antifoam was included when froth volume filled all the void spaces over the fluid level in the bioreactor.

**2.4 Sampling and Analytical Methods**

At certain time interims, tests were pulled back for disconnected examination of bacterial development, biosurfactant creation and nitrate arrangement. Feasible cell checks (CFU per milliliter) were evaluated by plating the fitting bacterial serial weakenings onto LB agar and brooding the plates at 37°C for 24 h. The way of life suspension was blended enthusiastically with n-hexane 1:1 (v/v) and centrifuged (4,830×g, 4°C, 30 min) to isolate the biomass, watery and natural dissolvable [5]. The natural dissolvable stage was evacuated and the cell pellets were washed once with physiologic serum arrangement centrifuged (4,618×g, 4°C, 30 min) and dried till steady weight. Nitrate fixations were resolved in the fluid stage by applying the marginally altered technique for Jagessar and Sooknundun [20], in which ammonium hydroxide arrangement was supplanted with 12N potassium hydroxide arrangement [21]. To keep away from obstruction by nitrite amid nitrate investigation, nitrite focus levels ought to be kept beneath 0.2 mg.L . Consequently, nitrate focuses were estimated in appropriate serial weakenings of the watery stage whose nitrite fixation was checked by the Ivanov spectrophotometric strategy utilizing a blend of sulfanilic corrosive and 1-naphthylamine [22]. Glycolipids were measured in the watery stage utilizing a corrosive precipitation and dissolvable extraction technique that utilized ethyl acetic acid derivation [23]. All measures were led in triplicate. As needs be, the information introduced here are the number-crunching midpoints of no less than three recreates and the mistake bars speak to the standard deviations.

**2.5 Data Processing and Mathematical Model Description**

Trial information were fitted to the proposed models to evaluate the motor model parameters utilizing MATLAB 7.5 by limiting nonlinear slightest squares bend fitting. A numerical joining order in MATLAB, known as ode45, was connected to unravel the differential conditions utilizing the Runge-Kutta strategy. In spite of the fact that the calculated model has been generally utilized for cell mass development energy, it didn't function admirably in this examination. A change of the established capacity that was presented by Gompertz for populace development, in 1825 [24] and the 3-parameter Gompertz condition utilized by Chavez-Parga et al. [14], were connected to scientifically display the biomass development as the accompanying articulation (Eq.1);

$$\frac{dX}{dt} = kX^{-\mu t} - aX \qquad (1)$$

Where $X$ is the biomass concentration (g.L$^{-1}$ ), $k$ and $a$ are kinetic parameters relating to initial specific growth rate (h$^{-1}$) and growth inhibition (h$^{-1}$), respectively. $\mu$ implies the specific growth rate (h$^{-1}$) and $t$ denotes the time (h). Nitrate consumption by *Achromobacter xylos GSR21* was interpreted by the Luedeking-Piret differential equation that neglects the amount of substrate used for product formation [14], The equation is given below as Eq.2:

$$\frac{dN}{dt} = -\frac{1}{R_{X/N}}\frac{dX}{dt} - m_N X \qquad (2)$$

Where $N$ is nitrate concentration (g.L$^{-1}$), $R_{X/N}$ refers to ratio of biomass to nitrogen and $m_N$ represents the maintenance coefficient (h$^{-1}$) indicating the amount of nitrate needed for energy, or maintenance requirements.

The kinetics of glycolipid formation was based on the equation suggested for lactic acid production [16] and is presented below as Eq. 3:

$$\frac{dP}{dt} = P_r P(1 - P/P_{max}) \qquad (3)$$

Where $P$ is the biosurfactant concentration (g.L$^{-1}$), $P_{max}$ shows maximum concentration of biosurfactant (g.L$^{-1}$) and $P_r$ is the ratio of the initial volumetric production rate ($r$ ) to the initial product concentration $P_o$(h ).

**2.6 Developing a Double-exponential Model for Viable Cell Kinetics:**

Albeit both feasible cells and biomass are of a similar kind, the pattern in suitable cell checks was mostly not at all like the biomass profile. The pattern in cell mass (Fig. 1) seemed to have expanded exponentially, like that portrayed by the Gompertz condition. The feasible cell design exhibited in Figure1 can be separated into two phases. The primary stage, communicating cell development demonstrated an always expanding bend and the second one showing cell lysis, spoke to a continually decrease with no evident S-shape. Accordingly, a solitary exponential capacity of time couldn't satisfy the time-course profile of feasible cell tallies demonstrating a non-monotonic example after some time. Be that as it may, scientific demonstrating of feasible cell energy is a testing issue in numerical science and empowers building up another numerical strategy. With a specific end goal to conquer this issue, feasible cell active information were fitted to a twofold exponential articulation of the frame appeared in Eq. (4), by a slightest squares method, utilizing MATLAB:

$$\frac{dc}{dt} = k_g c e^{-\sigma_g t} + k_l c e^{-\sigma_l t} - a_c c \qquad (4)$$

Where $c$ is the viable cell count (cell/mL), $k_g$ and $k_l$ are kinetic parameters pertaining to initial specific growth in cell number and lysis rates (h$^{-1}$), respectively. $\sigma_g$ and $\sigma_l$ are the specific growth in cell number and lysis rates (h$^{-1}$),respectively and $a_c$ *is* a kinetic parameter relating to cell count inhibition (h$^{-1}$).

**2.7 Statistical Analysis:**

Quantitative assessments were done to check the legitimacy of the proposed models. Standard mistake of gauge (SEE) and chi-square (x2) were evaluated to mind the consistency between the test dynamic information and relating model-anticipated esteems [25]. Certainty interims were considered to look at the unwavering quality of the assessed parameters. The MATLAB worked in capacities were utilized to perform measurable examination.

The standard blunder of gauge, as a measure of mistake amid expectation, was utilized to show how precisely every condition predicts applicable esteems. This is outlined in Eq. 5 [25-26]:

$$SEE = \sqrt{\frac{\sum_{i=1}^{n}(M_{i,exp} - M_{i,pre})^2}{d_f}} \qquad (5)$$

Where $M_{i,exp}$ and $M_{i,pre}$ are experimental and model predicted values at the i$^{th}$ data, respectively. $n$ is the number of data points and $d_f$ is the degree of freedom or the number of independent variables in the regression model.

The chi-square ($x^2$) test was applied to determine the goodness of fit between the observed and calculated data. Chi-square is the sum of the squared difference between experimental (*exp*) and predicted data (*pre*) divided by the predicted data in all possible categories [25]. The mathematical equation is given as Eq. 6 [25, 27]:

$$X^2 = \sum_{i=1}^{n} \frac{(M_{i,exp} - M_{i,pre})^2}{M_{i,pre}} \qquad (6)$$

To meet the model robustness, $X^2$ should not exceed the corresponding critical value shown by $X_C^2$ , as presented in Eq.7 [25]:

$$X_C^2 = X_{1-\alpha}^2(n - 1 - \theta) \qquad (7)$$

Where n is number of data points, $\theta$ and α number of model parameters and significance level, respectively. Assuming that the

level of significance (α) is 0.05 and with respect to number of data points in this study (17), $X_C^2$ calculated by $X_{0.95}^2(16-\theta)$ regarding the number of parameter for each model. The confidence intervals of the best-fit values of nonlinear regression were provided through the MATLAB statistics toolbox. The confidence intervals reflect the uncertainty associated with the parameter estimate or in other words, the precision of a parameter estimate. A 95% confidence interval means that the true value of the fitting parameter has a 95% probability of falling within the confidence interval [24].

## 3. Results And Discussion

**3.1 Kinetic Behavior in the Batch System:** glycolipid creation by Achromobacter xylos GSR21was achieved three times, in a 5-L bioreactor framework utilizing mineral salt medium (MSM) with expansion of 1% (v/v) vegetable oil as carbon source. The regular time course profile of nitrate utilization, cell development and glycolipid creation are portrayed in Figure 1. In light of these outcomes, the majority of the nitrate was devoured amid the initial 50 h of development when microscopic organisms developed exponentially and produced a biomass of 5.9 g.L-1 (Fig. 1). From that point, cells entered stationary stage however out of the blue cell mass uncovered a developing example with a delicate slant after 110 h and took after a level and steady pattern after 140 h. It was combined with a gentle lessening in the quantity of suitable cells and soak increment in glycolipid creation between 110 h and 140 h, amid the development time frame. This marvel appears to have started from cell lysis and arrival of biosurfactant and different parts into the way of life medium. Generous glycolipid generation was identified toward the finish of the exponential period of development, as likewise showed by Nitschke et al. [28]. This may be followed back to the nonattendance of nitrate particles, achieving a predefined carbon-to-nitrogen proportion [29] and additionally accomplishing the high cell thickness which is an essential factor for glycolipid articulation by Achromobacter xylos through the majority detecting system [30-31]. Glycolipid creation proceeded up to 168 h of development and in this manner climbed inconspicuously to a most extreme of around 21.9 g.L-1, after 180 h. outwardly, after 24 h of development, froth began to show up, which appeared uncongested at to start with, however progressively advanced into the reduced frame with the advance in development, prompting a flooding circumstance in the bioreactor framework in spite of the activity of the mechanical froth breaker. To control the intemperate froth and keep the bioreactor from flooding, a drop of antifoam was added to the framework at roughly 28, 92 and 119 h of development, when froth ascended in the aging vessel.

**Table 1A: Model parameters estimated by minimizing nonlinear least squares curve fitting of kinetic data for *Achromobacter xylos GSR21*growing in MSM[a]**

| Biomass Growth | | | | Nitrate concentration | | |
|---|---|---|---|---|---|---|
| k | μ | a | $r^2$ | R | M | $r^2$ |
| 0.945 | 0.207 | 0.004 | 0.997 | 3.012 | 0.004 | 0.985 |

**Table 1B: Model parameters estimated by minimizing nonlinear least squares curve fitting of kinetic data for *Achromobacter xylos GSR21*growing in MSM[a]**

| Glycolipid Concentration | | | Viable cell counts | | | | | |
|---|---|---|---|---|---|---|---|---|
| $p_r(h^{-1})$ | $P_{max}$ | $r^2$ | $k_g$ | $a_g$ | $k_l$ | $a_l$ | $a_c$ | $r^2$ |
| 0.102 | 21.9 | 0.975 | 23.54 | 0.039 | -23.31 | 0.023 | -0.0099 | 0.967 |

**3.2 Determination of Kinetic Parameters:** Figure 2 (a-d) presents the observed data as well as the predicted values calculated by Eqs. (1) - (4) for ***Achromobacter xylos GSR21***growing in MSM medium.

Trial information were fitted to important models utilizing the nonlinear minimum squares relapse. The relapse parameters were delineated in Table 1A&1B, which demonstrate that the scientific models portray the trial information sensibly, with r2 of 0.997 and 0.967 for biomass development and nitrate utilization, individually. The Rx/N estimation of 3.275 g.g-1, as evaluated by Eq.2 was equivalent to the tentatively ascertained estimation of R_(X⁄N)=ΔX/ΔN(3.14 g.g^(- 1)) where X and N are contrasts between the underlying and last biomass and nitrate focuses, individually. A low upkeep coefficient (mN) of 0.001 h-1 showed that the vitality of substrate digestion which isn't associated with biomass combination was little, hence prompting a high cell thickness amid nitrate consumption. Figure 2c confirmed that the Mercier display depicted the time course profile of glycolipid creation well, with a r2 of 0.975, yet slight contrasts were additionally clear at prior circumstances. Thinking about the outcomes in Figure1, most extreme glycolipid focus (Pmax) was 21.9 g.L-1 , which came very near 21.8 g.L-1 , as anticipated by the Mercier condition. The yield factor in connection to biomass was tentatively gotten as roughly 3.2 g.g-1 utilizing the Y_(P⁄x)=ΔP/ΔX condition and the outcomes appeared in Figure1, where P and X speak to contrasts between the underlying and last item and biomass fixations, individually. Aging by Achromobacter xylos GSR21in SOM in a 5-L bioreactor demonstrated a high biosurfactant yield when contrasted with YP/X esteems detailed in past works [32-33]. The twofold exponential model was found in all cases to give a uniquely superb fit to the feasible cell motor information (Fig. 2d), with r2=0.967.

**Table 2: Validating measures estimated by statistical analysis of kinetic models parameters for *Achromobacter xylos GSR21*growing in MSM[a]**

| Models | SEE | $x^2$ | robustness | parameters | 95% Confidence level | |
|---|---|---|---|---|---|---|
| | | | | | lower bound | Upper bound |
| Biomass concentration | 0.412 | 0.9154 | 22.9 | k | 0.86 | 1.0303 |
| | | | | μ | 0.1065 | 0.3078 |
| | | | | a | -0.0009 | 0.009 |
| Nitrate consumption | 0.205 | 0.4514 | 24.9 | R | 2.885 | 3.14 |
| | | | | m | -0.0009 | 0.009 |
| Glycolipid production | 2.69 | 6.478 | 23.9 | pr | 0.0404 | 0.1654 |
| | | | | pmax | 19.949 | 23.951 |
| Cell count | 0.457 | 0.0718 | 17.8 | kg | 20.898 | 26.197 |
| | | | | ag | 0.0292 | 0.0489 |
| | | | | kl | -30.63 | -15.99 |
| | | | | al | 0.01801 | 0.02811 |
| | | | | ac | -0.0911 | 0.0712 |

"a" Parameters were identified in nomenclature

"α" is the level of significance=0.05

**3.3 Kinetic Model Validation:** Standard mistakes of gauge demonstrating low esteems in Table 2 cleared the relative integrity of attack of the proposed models. The consequences of the chi-square (x2) investigation for various proposed models delineated in Table 2 were beneath the applicable basic chisquare (X_C^2 ). Thusly, there was no critical distinction between the trial information and the ones produced by the models.

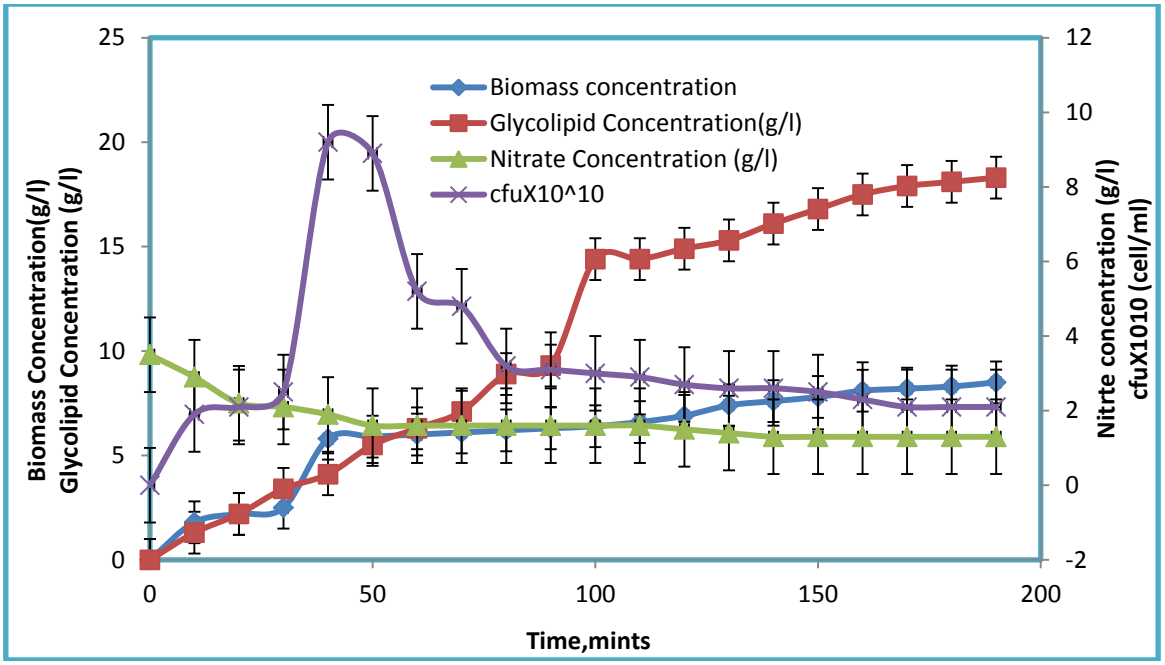


**Figure 1: Time-course profile of biomass growth, viable cell counts, nitrate consumption and glycolipid production during *Achromobacter xylos GSR21*growing in MSM. Results represent the average of three independent experiments.**

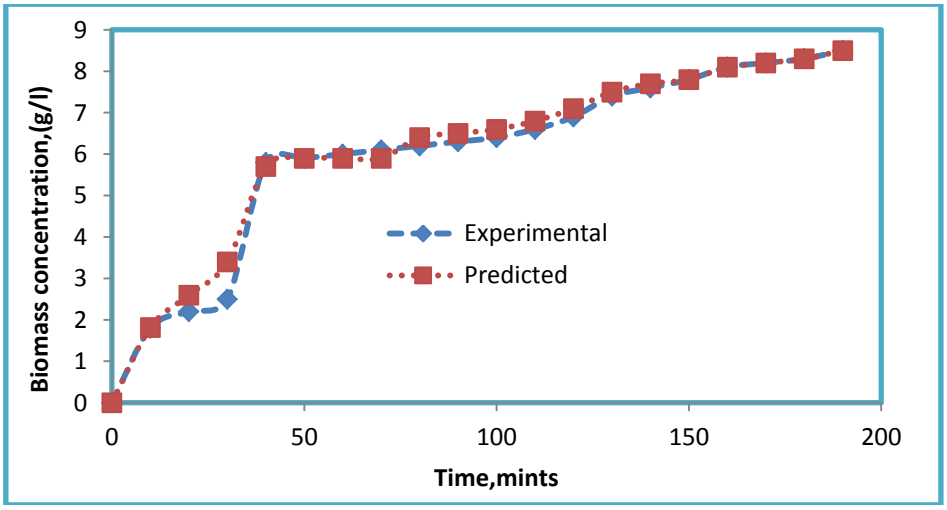


**Figure 2A: Representation of experimental and predicted data for biomass growth**

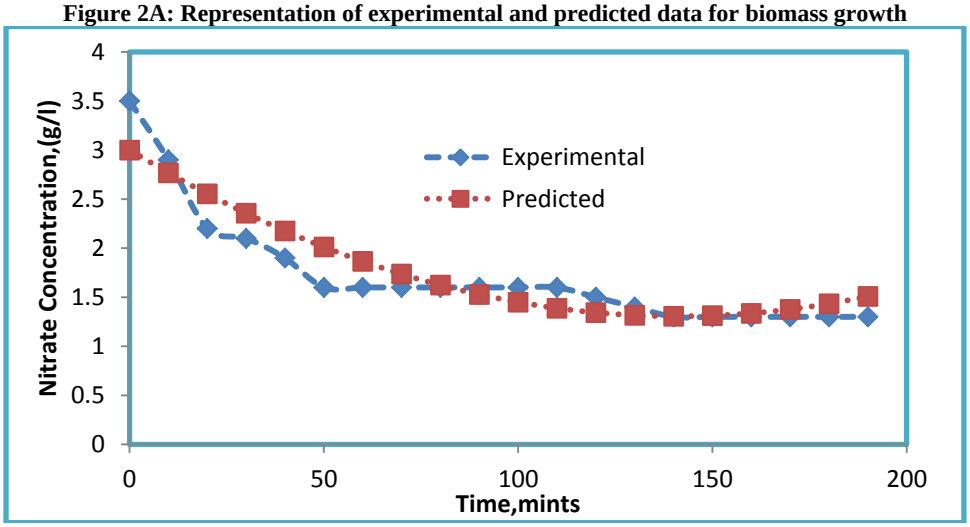


**Figure 2B: Representation of experimental and predicted data for Nitrate consumption**

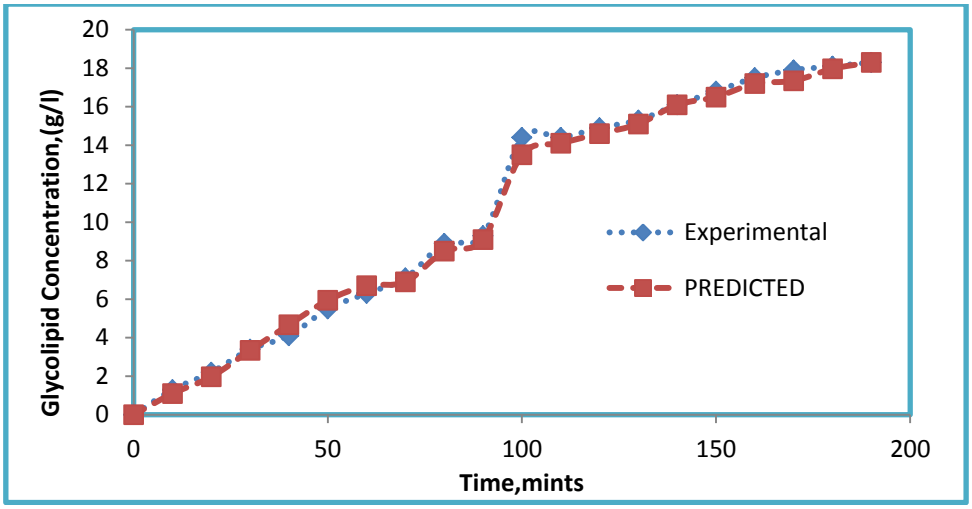


**Figure 2C: Representation of experimental and predicted data for glycolipid production**

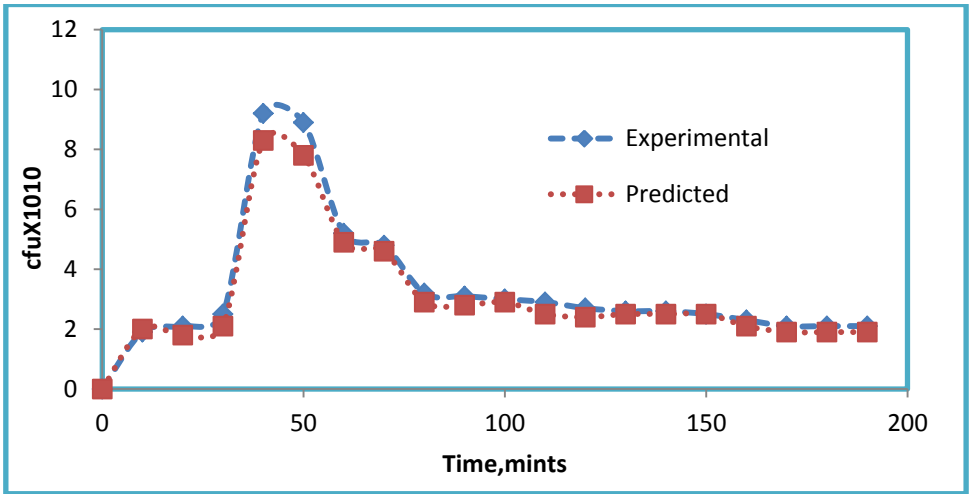


**Figure 2D: Representation of experimental and predicted data for viable cell counts during *Achromobacter xylos GSR21*growing in MSM**

The thin certainty interims for the evaluated show parameters exhibited in Table 2 meant the low vulnerability of fitting parameter esteems. It was inferred that these qualities and model expectations are not extremely touchy to the specific example utilized for building the model. The width of the certainty interim is a measure of the vulnerability about the situation of the genuine estimation of the assessed parameter and a wide certainty interim implies that information don't characterize that parameter extremely well. Therefore, the tight widths at high certainty level are alluring. The limitation of the got certainty interims rendered the parameters to lie in the assessed certainty locales, with 95% possibility. Correlation between the outcomes given in Tables 1A, 1B and 2 demonstrated that the computed estimations of the model parameters fell in the certainty zone.

Predicted data showing in figures (2a-2d) were calculated by using the equations (1-4), respectively. Results represent the average of three independent experiments.

## CONCLUSION

The outcomes (Pmax, YP/X) acquired for Achromobacter xylos GSR21growing in MSM (in a 5-L bioreactor) under controlled conditions, showed this strain as a solid biosurfactant maker when contrasted with the already revealed glycolipid makers. The proposed models were prepared to do precisely foreseeing the test comes about relating to cell development, nitrate usage and glycolipid generation, with r2 >0.95. Time course profiles of suitable cell tallies were effectively displayed utilizing the twofold exponential approach, with r2= 0.967. The SEE and chi-square investigations uncovered a fairly fantastic decency of fit between the proposed models and the watched information. The width of the built certainty interims were restricted and exhibited that the genuine estimations of the fitting parameters would have a likelihood of 95% to lie in certainty area. All in all, all numerical conditions were measurably adequate to portray the dynamic examples for Achromobacter xylos GSR21under the conditions specified previously. Additionally studies ought to be done to build up the conditions stretching out parameters to indicate the mass transport impacts and interface associations.

### Authors' Contributions

GSR and BM conceived the study. GSR carried out the laboratory analysis. GSR, BM, and RSR participated in the study design and coordination and drafting of the manuscript. All authors read and approved the final manuscript.

### Acknowledgments

The authors are grateful to Sri. K Satyanarayana Garu, president, K L University for supporting this research work. The authors are also thankful to Prof. L S S Reddy, Vice Chancellor for giving permission and Dr. K. Srinivasulu, HOD-BT for his encouragement during this work.

## Competing Interests

The authors declare that they have no competing interests.

## REFERENCES


1. Salihu, A., I. Abdulkadir and M.N. Almustapha, 2009. An investigation for potential development on biosurfactants. Biotechnol. Mol. Biol. Rev., 3(5): 111-117.
2. Muthusamy, K., S. Gopalakrishnan, T.K. Ravi and P. Sivachidambaram, 2008. Biosurfactants: properties, commercial production and application. Curr. Sci., 94(6): 736-747.
3. Desai, J. and I. Banat, 1997. Microbial production of surfactants and their commercial potential. Microbiol. Mol. Biol. Rev., 61: 47-64.
4. Medina-Moreno, S.A., D. Jiménez-Islas, J.N. Gracida-Rodríguez, M. Gutiérrez-Rojas and I.J. Díaz-Ramírez, 2011. Modeling rhamnolipids production by *Pseudomonas aeruginosa* from immiscible carbon source in a batch system. Int. J. Environ. Sci. Technol., 8(3): 471-482.
5. Müller, M.M., B. Hörmann, M. Kugel, C. Syldatk and R. Hausmann, 2011. Evaluation of rhamnolipid production capacity of *Pseudomonas aeruginosa* PAO1 in comparison to the rhamnolipid over-producer strains DSM 7108 and DSM 2874. Appl. Microbiol. Biotechnol., 89(3): 585-592.
6. Gautam, K.K. and V.K. Tiagi, 2006. Microbial surfactants: A review. J. Oleo Sci., 55(4): 155-166.
7. De Lima, C.J.B., E.J. Ribeiro, E.F.C. Sérvulo, M.M. Resende and V.L. Cardoso, 2009. Biosurfactant Production by *Pseudomonas aeruginosa* grown in residual soybean oil. Appl. Biochem. Biotechnol., 152(1): 156-168.
8. Lotfabad, T.B., H. Abassi, R. Ahmadkhaniha, R. Roostaazad, F. Masoomi, H.S. Zahiri, G. Ahmadian, H. Vali and K.A. Noghabi, 2010. Structural characterization of a rhamnolipid-type biosurfactant produced by *Pseudomonas aeruginosa* MR01: Enhancement of di-rhamnolipid proportion using gamma irradiation. Colloids Surf., B, 81(2): 397-405.
9. Okpokwasili, G.C. and C.O. Nweke, 2005. Microbial growth and substrate utilization kinetics. Afr. J. Biotechnol., 5(4): 305-317.
10. Kermanshahi pour, A., D. Karamanev and A. Margaritis, 2006. Kinetic modeling of the biodegradation of the aqueous *p*-xylene in the immobilized soil bioreactor. Biochem. Eng. J., 27(3): 204-211.
11. Juárez, T., L. De, R. De and M.E. Nader-Macías, 2002. Estimation of vaginal probiotic lactobacilli growth parameters with the application of the Gompertz model. Can. J. Microbiol., 48(1): 82-92.
12. Brandam, C., C. Castro-Martínez, M.L. Délia, M.L.F. Ramón-Portugal and P. Strehaiano, 2008. Effect of temperature on *Brettanomyces bruxellensis*: metabolic and kinetic aspects. Can. J. Microbiol., 54(1): 11-8.
13. Williams, F.M., 1967. A model of cell growth dynamics. J. Theor. Biol., 15(2): 190-207.
14. Chavez-Parga, M.C., O. González-Ortega, M.L.X. Negrete- Rodríguez, I.G. Vallarino, I.G.G.G. Alatorre and E.M. Escamilla-Silva, 2008. Kinetic of the gibberellic acid and bikaverin production in an airlift bioreactor. Process. Biochem., 43(8): 855-860.
15. Luedeking, R. and E.L. Piret, 1959. A kinetic study of the lactic acid fermentation: batch process at controlled pH. J. Biochem. Microbiol. Technol. Eng., 1(4): 393-401.
16. Mercier, P., L. Yerushalmi, D. Rouleau and D. Dochain, 1992. Kinetics of lactic acid fermentation on glucose and corn by *Lactobacillus amylophilus*. J. Chem. Technol. Biotechnol., 55(2): 111-121.
17. Rodrigues, L., A. Moldes, J. Teixeira and R. Oliveira, 2006a. Kinetic study of fermentative biosurfactant production by *Lactobacillus* strains. Biochem. Eng. J., 28(2): 109-116.
18. Lotfabad, T.B., M. Shourian, R. Roostaazad, A.R. Najafabadi, M.R. Adelzadeh and K.A. Noghabi, 2009. An efficient biosurfactant-producing bacterium *Pseudomonas aeruginosa* MR01, isolated from oil excavation areas in south of Iran. Colloids Surf., B. 69(2): 183-193.
19. Bertani, G., 1951. Studies on lysogenisis: the mode of phage II liberation by lysogenic *Escherichia coli*. J. Bacteriol., 62: 293-300.
20. Jagessar, R.C. and L. Sooknundun, 2011. Determination of nitrate anion in waste water from nine selected areas of coastal Guyana via a spectrophotometric method. Int. J. Res. Rev. Appl. Sci., 7(2): 203-212.
21. Partovi, M., T.B. Lotfabad, R. Roostaazad, M. Bahmaei and Sh Tayyebi, 2013. Management of soybean oil refinery wastes through recycling them for producing biosurfactant using *Pseudomonas aeruginosa* MR01. World J. Microbiol. Biotechnol.,
22. Ivanov, V.M., 2004. The 125 Anniversary of the Griess Reagent. J. Anal. Chem., 59(10): 1002-1005.
23. Wei, Y.H., C.L. Chou and J. Sh. Chang, 2005. Rhamnolipid production by indigenous *Pseudomonas aeruginosa* J4 originating from petrochemical wastewater. Biochem. Eng. J., 27(2): 146-154.
24. Gompertz, B., 1825. On the nature of the function expressiveness of the law of human mortality and a new mode of determining the value of life contingencies. Philos. Trans. R. Soc. London., 115: 513-585.
25. Hogg, R.V. and E. Tanis, 2010. Probability and Statistical Inference. New Jersey: Prentice Hall.
26. Ghodake, H.M., T.K. Goswami and A. Chakraverty, 2007. Moisture sorption isotherms, heat of sorption and vaporization of withered leaves, black and green tea. J. Food Eng., 78(3): 827-835.
27. Ho, Y.S., W.T. Chiu and C.C. Wang, 2005. Regression analysis for the sorption isotherms of basic dyes on sugarcane dust. Bioresour. Technol., 96(11): 1285-1291.
28. Nitschke, M., S.G.V.A.O Costa and J. Contiero, 2010. Structure and applications of a rhamnolipid surfactant produced in soybean oil waste. Appl. Biochem. Biotechnol., 160(7): 2066-2074.
29. Guerra-Santos, L.H., O. Kappeli and A. Fiechter, 1984. *Pseudomonas* th *aeruginosa* biosurfactant production in continuous culture with glucose as carbon source. Appl. Environ. Microbiol., 48(2): 301-305.
30. Pearson, J.P., E.C. Pesci and B.H. Iglewski, 1997. Roles of *Pseudomonas aeruginosa* las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. J. Bacteriol., 179(18): 5756-5767.
31. Rahman, P., D. Dusane, S. Zinjarde, V. Venugopalan, R. McLean and M. Weber 2010. Quorum sensing: implications on rhamnolipid biosurfactant production. Biotechnol. Genet. Eng. Rev., 27: 159-184.
32. Amani, H., M.R. Mehrnia, M.H. Sarrafzadeh, M. Haghighi and M.R. Soudi, 2010. Scale up and application of biosurfactant from *Bacillus subtilis* in enhanced oil recovery. Appl. Biochem. Biotechnol., 162(2): 510-523.
33. Rashedi, H., E. Jamshidi, M. Mazaheri Assadi and B. Bonakdarpour, 2005. Isolation and production of biosurfactant from *Pseudomonas aeruginosa* isolated from Iranian southern wells oil. Int. J. Environ. Sci. Technol., 2(2): 121-127.
34. Tayebe Bagheri Lotfabad, Shokoufe Tayyebi and Reza Roostaazad,2013. Kinetic Measurements for *Pseudomonas aeruginosa* MR01 During Biosurfactant Production in Two-phase System and Developing a Double-Exponential Model for Viable Cell Profile. World Applied Sciences Journal 22 (6): 809-816, 2013