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Mathematical Modeling of Molybdenum Reduction to Molybdenum Blue by *Burkholderia* sp. Strain Dr.Y27 and Model Selection Using the MOORA Method

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ABSTRACT

The microbial detoxification process of molybdenum reduction to molybdenum blue directly correlates with bacterial development during Mo-blue production. The reduction process can be modeled mathematically to determine essential kinetic parameters, which include the specific Mo-blue production rate and theoretical maximum Mo-blue generation, as well as the possible effects of high molybdenum concentrations on the lag phase of reduction. Applying linearization techniques including natural logarithmic transformations remains common, but these methods deliver imprecise results that produce only approximate values for specific parameters such as the specific growth rate or specific Mo-blue production rate. This research introduced a complete range of nonlinear growth and models to study Mo-blue production from Burkholderia sp. strain Dr.Y27. The research incorporated nine growth models, including the modified Gompertz and modified Logistic. The modified logistic model demonstrated the highest fit to the Mo-blue production curve of Burkholderia sp. strain Dr.Y27 based on multiple statistical performance criteria which included root-mean-square error (RMSE), Marquardt percent standard deviation (MPSD), adjusted coefficient of determination $(adjR^2)$, bias factor (BF), accuracy factor (AF), Bayesian information criterion (BIC), Hannan-Quinn criterion (HQC), and the corrected Akaike information criterion (AICc). The Multi-Objective Optimization by Ratio Analysis or MOORA approach based on Ratio Analysis was applied to enhance model selection and is the first method used to find the best model for primary modeling of bacterial growth or Mo-blue production rate. The fitted model generated essential parameters to serve as a solid foundation for developing additional models that describe how environmental variables and substrate concentrations affect Mo-blue production.

INTRODUCTION

Some regions throughout the world, including the Black Sea, Tyrol in Austria, and Tokyo Bay, have reported heavy metal pollution with molybdenum levels in the hundreds of parts per billion [1]. In addition, terrestrially, it has been recognized as a significant pollutant in sewage sludge pollution that poses a health hazard [2]. Alloying agents, anti-freeze components for automotive engines, corrosion-resistant steel, and molybdenum disulfide, a lubricant, are just a few of the many uses for molybdenum that contribute to these pollutions. Even at low concentrations (a few parts per million), molybdenum is extremely poisonous to cows and other ruminant [3,4]. To date, quite a number of Mo-reducing bacteria have been isolated, and most of these bacteria were isolated locally [5–13], with the exception of a few [14–17]. Historical assessments have shown that molybdenum presents lower toxicity levels to humans and other organisms when compared to mercury, selenium, and chromium. This perspective has clarified the lack of research on molybdenum reduction as a detoxification method. The scientific community has conducted limited research about metabolic and microbiological pathways that convert molybdenum into less accessible compounds such as molybdenum blue.

New toxicological research has revealed new insights on molybdenum toxicity. Research indicates that molybdenum produces substantial biological effects when present at concentrations as low as a few parts per million. Exposure to

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molybdenum has been proven to cause reproductive problems in aquatic animals, including catfish and spermatogenesis dysfunction. Mice in mammalian models demonstrated similar results to molybdenum exposure, which caused developmental toxicity, stopped embryogenesis, and decreased reproductive criteria [18,19]. These risks of molybdenum contamination in disturbed ecosystems require remediation strategies. Bacterial molybdenum bioreduction produces molybdenum blue, a safe compound that should be considered a vital bioremediation method for reducing molybdenum toxicity in terrestrial and aquatic ecosystems.

Mo-reduction by bacterium has benefited from nonlinear modeling using various primary models such as Logistic [20,21], Gompertz [21,22], Richards [21,23], Schnute [21], Baranyi-Roberts [24], Von Bertalanffy [25,26], Buchanan three-phase [27] and more recently Huang model [28] to gain useful reduction parameters that can be further modeled using secondary models such as Haldane, Teissier, Aiba, Yano and Monod to name a few. Previous modeling efforts to fit molybdenum reduction rates in primary models [29-32]-as well as in other xenobiotic transformation processes [33-37], whether primary or secondary-have predominantly relied on manual consensus approaches based on error functions, particularly the adjusted coefficient of determination (adjusted R²) [38] that accounts for model complexity, corrected Akaike Information Criterion (AICc) [39,40], Bayesian Information Criterion (BIC) [41], Hannan and Quinn's Criterion (HQ) [42] to name a few.

Although widely used, manual consensus selection is naturally susceptible to subjective bias, stressing the need for more methodical and objective model selection techniques. The Multi-Objective Optimization by Ratio Analysis (MOORA) method is one such tool; it provides a strong framework for simultaneously assessing several factors during decision-making. MOORA is included in the larger category of Multi-Criteria Decision-Making (MCDM) approaches, which also comprises well-known techniques like the Analytic Hierarchy Process (AHP) [43], Technique for Order Preference by Similarity to Ideal Solution (TOPSIS) [44], Preference Ranking Organization Method for Enrichment Evaluation (PROMETHEE) [45] and Weighted Sum Model (WSM) [46,47]. With its direct ratio-based approach that aggregates normalized performance values, the MOORA method eliminates the need for subjective preference assignments or complex iterative calculations, making it more efficient and suitable for small datasets compared to the other methods [48,49]. In this study, we present the first application of the MOORA method for selecting the best-fitting primary model in the field of bioreduction and biodegradation, using time-based Mo-blue production (molybdenum reduction) by the bacterium as a case study.

MATERIALS AND METHODS

Growth and maintenance of *Burkholderia* **sp. Strain Dr.Y27** The bacterium was a previously isolated Mo-reducing bacterium [50]. The maintenance and growth of the bacterium were carried out on either liquid medium or on solid agar, both in low phosphate medium (pH 7.0). The medium contained glucose (1%), (NH4)2SO4 (0.3%), NaCl (0.5%), yeast extract (0.05%), MgSO4.7H2O (0.05%), Na2MoO4.2H2O (0.242%) and Na2HPO4 (0.071% or 5 mM) (Abo-Shakeer et al., 2013). Glucose must be autoclaved separately.

Preparation of resting cells for molybdenum reduction characterization

Using a static microplate (microtiter) test with resting bacterial cells, Mo-blue synthesis was tracked at different sodium molybdate concentrations as before [51]. Resting cells were collected from a 1 L overnight culture cultured in High Phosphate Medium (HPM) at room temperature with agitation at 150 rpm on an orbital shaker. The only difference between HPM and Low Phosphate Medium (LPM) was the phosphate concentration, which was kept at 100 mM in HPM. Centrifugation at 15,000 × g for 10 min recovered cells, which were then washed many times to remove leftover phosphate and resuspended in 20 mL of LPM without molybdenum.

At 600 nm, the cell suspension was set to an optical density of about 1.00. Higher sodium molybdate concentrations significantly hindered the reduction process [50]. Each well of a sterile microplate received an aseptic 180 μ L of the prepared cell solution dispensed into it. Each well received 20 μ L of sodium molybdate at different concentrations, drawn from a stock solution, to start Mo-blue production. Room temperature incubation was then followed with sterile gas-permeable sealing tape (Corning® microplate) sealing the microplate. Using a BioRad Microtiter Plate Reader (Model 680, Richmond, CA), absorbance at 750 nm was recorded at specified time intervals. The specific extinction coefficient of 11.69 mM⁻¹ cm⁻¹ at 750 nm was used to quantify Mo-blue production in the microplate format, the maximum available filter wavelength for the plate reader [52].

Fitting of the data

Using nonlinear regression based on the Marquardt approach, which minimizes the sum of squared residuals, growth data were fitted to nonlinear equations (**Table 1**). CurveExpert Professional software (Version 1.6) was used for this study. The best fit is obtained in this iterative method by minimizing the difference between predicted and observed values. The program lets manual and automated entry of initial parameter estimates. A four-data point steepest ascent search produced the maximum specific growth rate (μ_m) or Mo-blue production rate. The x-axis intercept of the projected line from the sharpest ascent was used to find the lag phase duration (*l*). Taking the last data point signifying the plateau period allowed one to estimate the asymptotic value (*A*).

Statistical analysis

The following tests for statistical discrimination or error functions were utilized in this study: HQ (Hannan and Quinn's Criterion) [42], Bias Factor (BF), Accuracy Factor (AF) [53], root-mean-squared error (RMSE), adjusted coefficient of determination (R^2) [38], corrected Akaike Information Criterion (AICc) [39,40], Marquardt's percent standard deviation (MPSD) [54–56] and Bayesian Information Criterion (BIC) [41]. In general, *n* is the total number of observations, *Obi* and *Pdi* are the predicted and observed values, and *p* is the total number of parameters of the model [57].

RMSE was calculated using the following formula;

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (Pd_i - Ob_i)^2}{n-p}}$$
(Eqn. 1)

BF and AF were calculated using the following formula;

$$Bias \ factor = 10 \left(\sum_{i=1}^{n} log \frac{(Pd_i/Ob_i)}{n} \right)$$
(Eqn. 2)
Accuracy factor = $10 \left(\sum_{i=1}^{n} log \frac{|(Pd_i/Ob_i)|}{n} \right)$ (Eqn. 3)

5)

AICc was calculated using the following formula;

$$AICc = 2p + n \ln\left(\frac{RSS}{n}\right) + \frac{2(p+1)+2(p+2)}{n-p-2}$$
(Eqn. 4)

BIC was calculated using the following formula;

$$BIC = n \ln \left(\frac{RSS}{n}\right) + k \ln(n)$$
 (Eqn.

HQC was calculated using the following formula;

$$HQC = nln\left(\frac{RSS}{n}\right) + 2kln(ln n)$$
 (Eqn. 6)

Adjusted coefficient of determination (R^2) was calculated using the following formula;

$$\begin{array}{l} Adjusted \ (R^2) = 1 - \frac{1}{S_r^2} & (Eqn. \ 7) \\ Adjusted \ (R^2) = 1 - \frac{(1-R^2)(n-1)}{(n-p-1)} & (Eqn. \ 8) \end{array}$$

MPSD was calculated using the following formula;

$$MPSD = 100 \sqrt{\frac{1}{n-p} \sum_{i=1}^{n} \left(\frac{ob_i - Pd_i}{ob_i}\right)^2}$$
(Eqn. 9)

Table 1. Mo-blue production models used in this study.

Model p Equation $y = \frac{A}{1 + exp\left[\frac{4\mu_m}{A}(\lambda - t) + 2\right]}$ Modified Logistic 3 $y = Aexp\left\{-exp\left[\frac{\mu_m \cdot e}{4}(\lambda - t) + 1\right]\right\}$ Modified Gompertz
$$\begin{split} y &= A \left\{ 1 + vexp(1+v)exp\left[\frac{\mu_m}{A}(1+v)\left(1+\frac{1}{v}\right)(\lambda - t)\right] \right\} \end{split}$$
Modified Richards $y = \left(\mu_m \frac{(1-\beta)}{\alpha}\right) \left[\frac{1-\beta exp(\alpha\lambda+1-\beta-\alpha t)}{1-\beta}\right]^{\frac{1}{\beta}}$ Modified Schnute 4
$$\begin{split} y &= N_0 + \mu_m t + \frac{1}{\mu_m} ln(e^{-\mu_m t} + e^{-h_0} - e^{-\mu_m t - h_0}) \\ &- ln \left[1 + \frac{e^{\mu_m t + \frac{1}{\mu_m} ln(e^{-\mu_m t} + e^{-h_0} - e^{-\mu_m t - h_0})}{e^{(A - N_0)}} \right] \end{split}$$
Baranvi-Roberts $y = k \left[1 - \left[1 - \left(\frac{A}{k} \right)^3 \right] exp^{-\left(\frac{\mu_m t}{3k} \right)^{\frac{3}{3}}} \right]$ Von Bertalanffy 3 $y = A + \mu_m - \ln(e^A + (e^{\mu_m} - e^A)e^{-\mu_m B(t)})$ $B(t) = t + \frac{1}{\alpha}\ln\frac{1 + e^{-\alpha(t-\lambda)}}{1 + e^{\alpha\lambda}}$ Huang $Y = N_0$, IF X < LAGBuchanan 3 $Y = N_0 + K(X - \lambda)$, IF $\lambda \le X \ge X_{MAX}$ Three-phase Y = A. IF $X > X_{MAX}$ linear model $y = A - \frac{(A - \beta)}{1 + (\mu_m t)^{\delta}}$ Morgan-Mercer-Flodin (MMF) 4

Note:

A= Microorganism growth upper asymptote;

 N_0 = Microorganism growth lower asymptote;

 u_m = maximum specific microorganism growth rate or Mo-blue production rate; v= affects near which asymptote maximum growth occurs.

 $\lambda = \text{lag time}$ e = exponent (2.718281828)

t = sampling time

 α,β,k,δ = curve fitting parameters

 h_0 = a dimensionless parameter quantifying the initial physiological state of the reduction process. For the Baranyi-Roberts model, the lag time (λ) (h⁻¹) or (d⁻¹) can be calculated as $h_0=\mu_m$

For modified Schnute, A = m/a

Application of Multi-objective Optimization by Ratio Analysis (MOORA) in Modeling

For the modeling exercise's multi-criteria decision-making (MCDM), we used MOORA, since the best models typically have a combination of error function superiority. Through the simultaneous evaluation of numerous performance criteria, this approach makes it easier to identify the ideal model [58,59]. As a first step in the process, the decision matrix was standardized to make it easier to compare various performance measures. The following equation must be used for normalization because the units and magnitudes of these measurements can vary.:

$$X'_{ij} = \frac{x_{ij}}{\sqrt{\sum_{i=1}^{n} x_{ij}^{2}}}$$
(Eqn. 10)

Where X_{ij} is the original value of the j^{ih} metric for the i^{ih} model, and X^{i}_{ij} is the normalized value.

Ratio System Analysis

The aggregated normalized numbers were subsequently calculated using a ratio technique. Using the following formula, we added up the advantageous criteria ($adjR^2$) and removed the non-beneficial criteria (the remaining error functions) or those that should be minimized. Here, we use a cost function for the error function bias factor (BF) or CBF=|1-BF| whilst the cost function for the error function accuracy factor (AF) or CAF=AF-1.

$$Y_i = \sum_{beneficial} X'_{ij} - \sum_{non-beneficial} X'_{ij}$$
(Eqn. 11)

Where Y_i is the final score for the *i*th model. It is recommended to use weighted ratios in cases when some criteria are considered more important than others. There is currently no evidence in the literature to support the recommendation to incorporate Weighted Ratios because there is no consensus on which error functions mentioned above are more important. Finally, models are ranked according to their total performance ratings. A higher score meant that the performance was better. The decisionmaking criteria were as follows: the model with the highest value was deemed the most optimal. Using this approach, we were able to compare kinetic models systematically and objectively, which helped us find the model that performed the best across all of our performance parameters.

RESULTS AND DISCUSSION

For decades, researchers have relied on linear regression to ascertain the slope of a growth curve or xenobiotics transformation parameters after manually estimating the nearly linear portion of the curve. An improved approach would be to use a nonlinear regression growth model to characterize the whole dataset and then use the model to estimate μ_m , λ , and A [60]. Theses parameters can then be used for further secondary modeling like Monod, Haldane, Aiba, and Teissier [7,61].

Fig. 1 shows that after roughly 50 hours of static incubation, the bacterium's Mo-blue production reached its maximum, following a sigmoidal pattern that began with a lag phase of around 15 hours. Nine distinct models were fitted using the Moblue production overtime profile (Figs. 2 to 10). Fig. 11 displays the visually acceptable fitting that resulted from the fitting. The improved logistics model outperformed the others, achieving the best results in terms of adjusted R^2 , RMSE, and AICc. The model's AF and BF values, which were near 1.0, were also excellent (Table 2). The modified logistic model was confirmed as the best model upon further analysis using MOORA (Table 3).

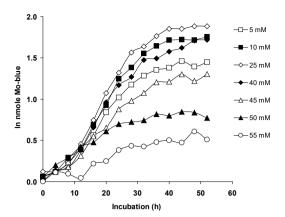


Fig. 1. Time series graphs showing the natural log-transformed values for Mo-blue synthesis of different sodium molybdate concentrations.

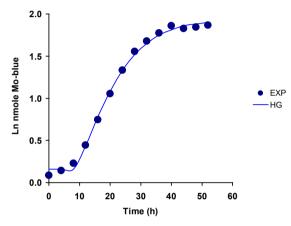


Fig. 2. The Mo-blue production curve at 25 mM of sodium molybdate fitted to Huang (HG),

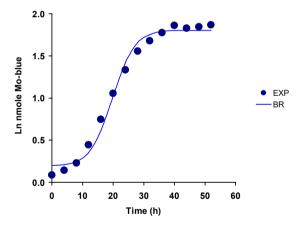


Fig. 3. The Mo-blue production curve at 25 mM of sodium molybdate fitted to Baranyi-Roberts (BR).

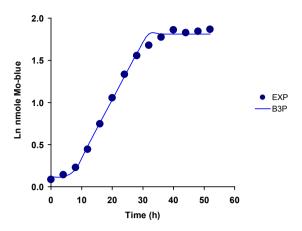


Fig. 4. The Mo-blue production curve at 25 mM of sodium molybdate fitted to Buchanan-three phase (B3P).

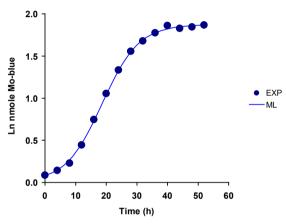


Fig. 5. The Mo-blue production curve at 25 mM of sodium molybdate fitted to modified Logistics (ML).

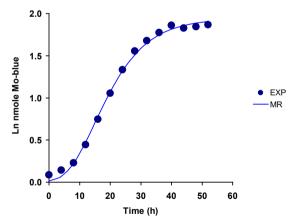


Fig. 6. The Mo-blue production curve at 25 mM of sodium molybdate fitted to modified Richards (MR).

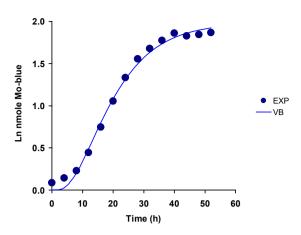


Fig. 7. The Mo-blue production curve at 25 mM of sodium molybdate fitted to von Bertalanffy (VB).

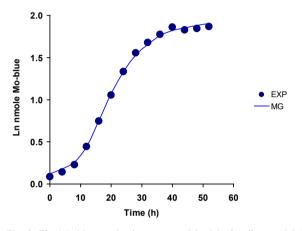


Fig. 8. The Mo-blue production curve at 25 mM of sodium molybdate fitted to modified Gompertz (MG).

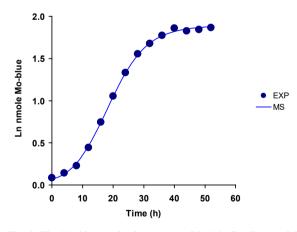


Fig. 9. The Mo-blue production curve at 25 mM of sodium molybdate fitted to modified Schnute (MS).

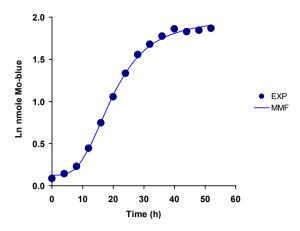


Fig. 10. The Mo-blue production curve at 25 mM of sodium molybdate fitted to Morgan-Mercer-Flodin (MMF).

Table 2. Statistical analysis of the various fitted models.

Model	р	MPSE	RMSE	R^2	adR^2	AICc	BIC	HQC	BF	AF
Huang	4	4.600	0.046	1.00	0.995	-65.426	-80.37	-83.16	1.001	1.007
Baranyi-Roberts	4	9.103	0.091	0.99	0.981	-46.313	-61.26	-64.05	0.993	3 1.009
Mod Gompertz	3	3.016	0.030	1.00	0.998	-82.971	-93.50	-95.59	1.001	1.006
Buchanan-3-phase	3	5.339	0.053	1.00	0.994	-66.974	-77.50	-79.60	0.995	5 1.008
Mod Richard	4	4.788	0.048	1.00	1.00	-64.307	-79.25	-82.04	0.829	0 1.007
Mod Schnute	4	2.230	0.022	1.00	0.999	-85.695	-100.64	-103.43	1.000	1.004
Mod Logistics	3	2.079	0.021	1.00	0.999	-93.380	-103.91	-106.00	1.000	0 1.003
on Bertalanffy	3	6.266	0.063	0.99	0.992	-62.495	-73.02	-75.12	1.002	2 1.009
MMF	4	3.119	0.031	1.00	0.998	-76.307	-91.25	-94.04	1.000	0 1.007
Note:										

p no of parameters

Table 3. MOORA ranking of the error functions.

Model	Cost function	Rank
Modified Logistics	-124.19	1
Modified Schnute	-113.29	2
Modified Gompertz	-100.15	3
MMF	-92.60	4
Huang	-71.73	5
Modified Richard	-69.89	6
Buchanan-3-phase	-69.20	7
on Bertalanffy	-62.01	8
Baranyi-Roberts	-44.89	9

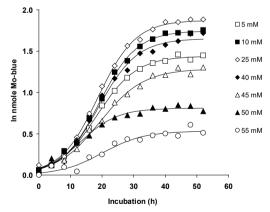


Fig. 11. The Mo-blue production curves for various concentrations of sodium molybdate fitted using the modified logistics model.

MOORA uses a decision matrix incorporating several performance metrics to rank models. Each criterion is given a weight, and models are ranked according to their normalized performance ratings. In contrast to traditional model selection, which could prioritize a high R^2 value even in the face of significant SSE deviations, MOORA considers several frequently contradictory indications to guarantee a comprehensive assessment [58,59,62–64].

The MOORA method has not been applied previously to evaluate and rank primary or secondary growth models of bacteria. The MOORA method is a strong decision-making tool that enables simultaneous evaluation of multiple criteria to rank different alternatives. MOORA provides thorough performance assessments of alternatives through ratio analysis, enabling decision-makers to make informed choices while understanding the trade-offs between competing objectives [59]. The approach has been widely applied in finance, engineering, and environmental management but not in microbial growth modeling. The primary growth models describe microbial growth as a function of time by focusing on growth rate and lag time, but secondary growth models incorporate environmental factors such as temperature, pH, and water activity to affect these parameters.

The application of MOORA to rank microbial growth models with multiple objectives remains unexplored despite its proven success in solving complex multi-objective problems. The Analytic Hierarchy Process (AHP) and the Technique for Order of Preference by Similarity to Ideal Solution (TOPSIS) are two other multi-criteria decision-making (MCDM) techniques that are commonly used for similar purposes. The Analytic Hierarchy Process (AHP) enables systematic evaluation of options through the hierarchical organization of decision issues. TOPSIS ranks alternatives by measuring their geometric distance from an ideal solution and enables trade-offs across criteria, thus providing a realistic decision-making modeling technique [65].

Including a variety of variables in the model selection process improves objectivity and guarantees clear and repeatable results. MOORA is a perfect tool for predictive microbiology research because it can balance several statistical variables, such as error functions, model complexity penalties, and performance accuracy. This work showcasing the modified Logistics as the best model is a first because MOORA has not been used for bacterial growth or the ranking of the Mo-blue production model. To develop a more thorough and impartial model selection procedure in bioreduction investigations, future studies should investigate the integration of MCDM frameworks with conventional error functions.

The logistic model represents one of the earliest mathematical models which simulates microbial growth dynamics. The model was created to describe population growth under resource constraints, but microbiologists have adapted it for microbial systems because it effectively represents sigmoidal growth curves. The logistic model applied in microbiology actually represents three identifiable growth phases that include initially the lag phase. This is then followed by exponential growth and then stationary phase. The equation contains three essential variables which include maximum specific growth rate (μ_m), lag time (λ) and maximum population size or carrying capacity (A_{max}) that represents environmental constraints or nutrient depletion. The model shows an accurate representation of declining growth rates because populations face increased competition for scarce resources when approaching A_{max} .

The model serves as a fundamental tool in microbial kinetics because it requires minimal parameters and remains open to modification. The logistic model provides biologically meaningful values which serve as inputs for advanced secondary modeling systems. A differential equation provides the growth rate or Mo-blue production rate based on the model as follows;

$$\frac{dA}{dt} = \mu_m A \left(1 - \frac{A}{A_{\text{max}}} \right)$$
(Eqn. 12)

The highest specific growth rate or Mo-blue production rate is μ_m , the starting population density or bacterial cell number (CFU/ml), or maximum Mo-blue produced at time t. Amax is the highest bacterial cell number (CFU/ml) or Mo-blue production at the stationary phase. This is commonly referred to as the environment's carrying capacity. In the logictics model, the term $1-A/A_{max}$ suppresses the growth rate at large bacterial cell numbers (CFU/ml) or or Mo-blue production. The growth rate is unaffected when the term is converted to nearly one by a reduced A value, which is frequently observed during the lag phase. The growth rate at the stationary phase is nearly zero when the value of A approaches Amax at high Mo-blue production or bacterial cell number (CFU/ml), which changes the term to nearly zero. The curve that results is sigmoidal. It is Gibson et al.'s groundbreaking work [66] that modified the logistic model to be used to fit bacterial growth data as follows:

$$\log A = a + c \left[1 + \exp(-b(t-m))\right]$$
(Eqn. 13)

The exp is an exponential function and *a*, *b*, *b*, and *m* are model parameters. The logistics model is routinely used alongside the modified Gompertz model to fit the growth of microorganisms [67–71], which indicates the model versatility. The parameters maximum Mo-blue production rate (μ_m), lag time (λ) and maximal Mo-blue production (Y_{max}) are obtained from the nonlinear regression modeling.

CONCLUSION

The research findings demonstrate that the modified logistic model provides the most accurate representation of Mo-blue production by the studied bacterium throughout the time period. The $adjR^2$, RMSE, and AICc statistical fit criteria enabled the model to represent lag, exponential, and stationary production phases accurately. Implementing MOORA as a multi-criteria decision-making tool during model selection enhanced the objectivity and dependability of model assessment. MOORA provided an extensive evaluation process that moved past traditional single-indicator limitations by assessing multiple statistical measures simultaneously. This research presents the initial recorded application of MOORA for evaluating and ranking bacterial growth and product generation models. The method's successful application in this scenario demonstrates its potential for wider adoption in bioprocess modeling when multiple competing criteria affect model performance. The modeling output, including maximal production rate, lag time and final yield now functions as fundamental parameters for conducting secondary modeling incorporating environmental elements. The research demonstrates both the optimal growth model for Mo-blue production and develops a robust decisionmaking framework that can be applied to future modeling studies in environmental microbiology and biotechnology.

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