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Kinetic evaluation of anthracene biodegradation by *Bacillus velezensis* and mathematical model fitting under substrate inhibition conditions

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) such as anthracene are persistent environmental pollutants that accumulate in ecosystems and contribute to harmful air pollution, posing risks to human health. Due to its significance, anthracene must be removed using nontoxic methods such as bioremediation. This study focuses on degrading anthracene with *Bacillus velezensis* and investigating the kinetics of this process. The bioremediation potential of *B. velezensis* in degrading anthracene under aerobic conditions was explored through batch experiments conducted at varying anthracene concentrations (100–600 ppm). The bacterium exhibited rapid biomass growth and efficient degradation at 100 ppm, while growth was inhibited at higher concentrations due to substrate toxicity. GC-MS analysis identified 9,10-anthracenedione as the primary intermediate, which was further broken down into carbon dioxide and water at 100 ppm. Phytotoxicity assays confirmed this intermediate was nontoxic to the bacterium and phyllosphere. The degradation kinetics deviated from the Michaelis–Menten model at high concentrations, indicating the influence of substrate inhibition. Among several models evaluated, the Wayman and Tseng model showed the best statistical fit, with a high adjusted R^2 value (0.95191) and a low RMSE (0.0019), indicating accurate predictions. Data interpolation improved model performance, providing a more reliable framework for predicting anthracene degradation by *B. velezensis*.

KEYWORDS

Anthracene; bioremediation; inhibition; kinetics; toxicity

Introduction

Air pollution is among the most serious health and environmental issues in the world, usually becoming more severe as countries industrialize and move from low- to middle-income status. It is a source of many of the leading causes of death worldwide, including stroke, heart disease, lung cancer, and respiratory illnesses like asthma (Babatola 2018). As countries industrialize and progress from low to middle income status, death rates may initially increase due to factors, such as environmental pollution and poor working conditions (Shi 2022). However, with continued economic growth, death rates typically decline due to improved healthcare, cleaner environments, and better overall living standards. To help mitigate the negative health effects of air pollution, the World Health Organization (WHO) has established specific guidelines for acceptable exposure levels in the form of its Air Quality Guidelines (AQG) (WHO, 2006). Several air pollutants cause adverse effects on humankind, such as Ozone, $P_{2.5}$, P_{10}, NO_x, and Polycyclic Aromatic Hydrocarbons (PAHs). Among these pollutants, only a few research studies were carried out about PAHs.

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The United States Environmental Protection Agency (US EPA) listed 16 high-priority pollutants in 1982. Among them, anthracene holds a special place since it is a prominent PAH which is fused with 03 benzene rings and it directly affects all living beings (Van Damme and Du Prez 2018). Anthracene was initially identified in 1832 following its extraction from coal tar and from volatile compounds formed during the incomplete combustion of organic compounds in protein-rich foods (Bendouz et al. 2017). Anthracene is moderately toxic and carcinogenic to living beings, and it leads to genotoxicity (Dadrasnia et al. 2020; Rengarajan et al. 2015).

Previous researchers experimented with several chemical and physical methods to remove anthracene-like PAH from the environment (Kamińska et al. 2019; Lee, Hosomi, and Murakami 1998; Liu et al. 2006). However, during these chemical and physical methods, some produced intermediates can be toxic to the environment (Woo et al. 2009). To minimize the generation of toxic intermediates, researchers emphasize the development of eco-friendly and sustainable methodologies. Bioremediation is considered the most effective option for removing PAHs, such as anthracene from the environment without releasing toxic intermediates (Akinola 2022; Vasudevan, Gayathri, and Krishnan 2018; Zeng et al. 2021). It is a process that removes pollutants from the environment using biological systems, such as bacteria, fungi, algae, and others (Dharmasiri et al. 2025; Dharmasiri, Undugoda, Nilmini, Nugara, et al. 2023; Yuvraj 2022).

Bacteria have been frequently studied by researchers for the bioremediation of anthracene (Amini, Tahmourespour, and Abdollahi 2018; Marajan 2020; Taha et al. 2021). Extensive research has been conducted on the degradation of anthracene by *B. velezensis* (Abd-Elsalam et al. 2009; Mandree et al. 2021; Safitri et al. 2019; Soumeya, Allaoueddine, and Hocine 2022); however, only a few studies have focused on the kinetics of anthracene degradation by *B. velezensis*. Studying the growth kinetics of bacteria at various concentrations of PAHs and selecting the most suitable model for their degradation can help assess their potential effectiveness in bioremediation. Although various models have been developed to simulate PAH degradation, there is no consensus on the most appropriate model for specific conditions.

This research investigates the biodegradation of anthracene by *B. velezensis* and evaluates its kinetic behavior under various environmental conditions. Several mathematical models are applied to identify the most suitable framework for describing the degradation process, enabling the prediction of degradation rates, and estimating the time required for anthracene removal from contaminated environments. Optimization of parameters, such as temperature, pH, and nutrient availability is also considered to enhance bioremediation efficiency (Sosa-Martínez et al. 2021). Selecting a suitable kinetic model is crucial for validating experimental data and improving the design of effective bioremediation strategies.

The significance of this research focused on the kinetic evaluation of anthracene degradation by *B. velezensis* under substrate inhibition conditions, which has received limited attention in the existing literature. While *B. velezensis* is known for its potential in PAH degradation, few studies have addressed its degradation kinetics in detail or assessed the applicability of different kinetic models. This study contributes to closing that gap by providing a comparative analysis of model performance, offering a deeper understanding of the biodegradation mechanism, and informing the design of optimized bioremediation approaches for PAH-contaminated environments.

Materials and methods

Enrichment, growth, and maintenance of anthracene-utilizing bacterium

The strain *B. velezensis strain Y9* (Accession number MN190156) was isolated from the phyllosphere, the total above ground parts of a plant of urban areas in Colombo, Sri Lanka. It was stored in the culture bank at $4\,^{\circ}C \pm 1\,^{\circ}C$ in the Department of Biosystems Technology, University of Sri Jayewardenepura, Sri Lanka. This *B. velezensis* bacterium was used in this research experiment to study the kinetics

approach using different concentrations of anthracene.

Acclimatization of culture and inoculum development

The acclimatization of the isolate was performed separately in a Bacto Bucshnell-Hass (BBH) ($MgSO_4$ – 0.200 gL^{-1}, $CaCl_2$ – 0.020 gL^{-1}, KH_2PO_4 1.000 gL^{-1}, K_2HPO_4 1.000 gL^{-1}, NH_3NO_3 1.000 gL^{-1}, $FeCl_3$ 0.050 gL^{-1}, Final pH at 25 °C, 7.0 ± 0.2 medium (Dharmasiri et al. 2019)) prepared using anthracene as a sole carbon source. The 3-d starved *B. velezensis* was slowly enriched and acclimatized in the BBH with substrate concentrations of anthracene: 100, 200, 300, 400, 500, and 600 ppm. The inoculated medium was incubated at 30 °C for 7 d. Then the samples were analyzed each and every day for degradation of anthracene and cell growth.

Measurement of cell growth and determination of dry weight of cells

The remaining anthracene and the produced intermediates were extracted with acetone (Thermo Fisher Scientific, Waltham, MA): hexane (Merck, Darmstadt, Germany) (1:1) solvent system, and the aqueous layer was withdrawn and filtered through a previously weighted filter paper (Whatman, diameter 125 mm, pore size 11 µm). The filter paper was put into the oven at 60 °C for 8 h and left to cool in the desiccator at room temperature. It was reweighed until a constant dry weight was achieved. The dry weight of the cells was determined from the weight difference of the filter paper before and after the constant weight. Colony-forming units (CFU/mL) were counted daily for seven consecutive days using Nutrient Agar (NA) medium (HIMEDIA, Mumbai, India).

Analysis of anthracene degradation by high-performance liquid chromatography (HPLC)

Previously extracted organic layer (Hexane) was evaporated by using the rotary evaporator (Model RE-301, Biobase, Jinan, China). The rotary evaporated residue was reconstituted in 1 mL of acetonitrile (Merck, Germany, HPLC grade, assay 99.8%) and the solution was filtered by using 0.2 µm nylon syringe filter and analyzed using the HPLC (Agilent, C-18 column, 4.6 mm, 100 mm, Santa Clara, CA) equipped with a diode array detector. The mobile phase for anthracene at a flow rate of 1.0 mL min^{-1} was acetonitrile: deionized water 90:10 mixture, and the detector was set at 254 nm for compound detection. The recovery percentage of anthracene (96.76%) was recorded in HPLC.

Table 1. Variables and relevant descriptions for the best-fitting mathematical model.

Variables	Description
Concentration	Anthracene concentration
Time	The time frame in which the data were gathered
Dry cell Biomass	The dry weight of *B. velezensis*
Colony counts	Live cells in dry cell biomass
Area e4	The peak area under HPLC
Standard area e4	The standard peak area of samples under HPLC

The degradation rates (r) of anthracene by bacteria were determined based on the following derived Equation (1), reflecting the kinetics of the degradation process.

$$C_t = C_o e^{-rt} \tag{1}$$

Selection of the best-fitting mathematical model

Bacterial growth patterns during degradation were analyzed based on fluctuations in dry biomass. Table 1 lists the variables included in the experimental data set.

After collecting the data, several variables related to the investigation of anthracene degradation by *B. velezensis* were created based on the dataset. These variables are listed below.

Specific growth rate (µ)

$$\boldsymbol{\mu = \frac{ln(x) - ln(x_0)}{\Delta t}} \tag{2}$$

$x = Dry\ cell\ biomass$

$x_0 = Dry\ cell\ biomass\ at\ time\ t = t$

$\Delta t = Time\ difference$

Remaining concentration(S)

$$\boldsymbol{S = \frac{A_s}{A_{sd}} \times S_{sd}} \tag{3}$$

$S = Remaining\ Concentration$

$A_s = Area\ of\ sample$

$A_{sd} = Area\ of\ standard$

$S_{sd} = Concentration\ of\ standard$

Degradation rate (R_D)

$$R_D = \frac{ln(S_0) - ln(S)}{\Delta t} \quad (4)$$

$R_D = Degradation\ Rate$

$S_0 = Substrate\ Concentration\ at\ time\ t = 0$

$S = Substrate\ Concentration\ at\ time\ t = t$

Degradation (removal efficiency) (D)

$$D = \frac{A_{sd} - A_s}{A_{sd}} \times 100\% \quad (5)$$

$D = Degradation\ efficiency$

$A_{sd} = Standard\ area\ of\ sample$

$A_s = Area\ of\ sample$

This study investigated anthracene degradation by *B. velezensis* and evaluated growth kinetics using various mathematical models. Simpler models were preferred based on the principle of Occam's Razor, provided they fit the data well and aligned with known biochemical mechanisms. Statistical significance was assessed using appropriate tests (*F*-test, chi-squared), and model fit was evaluated using *R*-squared, adjusted *R*-squared, and reduced chi-squared values.

Three independent growth measurements were collected under identical conditions. Duplicate entries were removed to improve accuracy, and the remaining values were used to calculate representative growth rates, reducing variability. Growth data at varying anthracene concentrations were analyzed using SPSS version 26 (IBM Corp., Armonk, NY). One-way ANOVA revealed significant differences ($p < 0.05$), and descriptive statistics highlighted trends across concentrations.

Multiple kinetic models, including Monod's model, Haldane's model, and Wayman and Tseng's model (substrate inhibition models), were fitted using non-linear regression with the statistical software OriginPro (2023). Three models closely matched the experimental data, providing reliable tools for predicting degradation rates and guiding bioremediation strategies in anthracene-contaminated environments.

By-products analysis by gas chromatography mass spectrometry (GC-MS)

The acetonitrile solution was gravity filtered by using a 0.2 µm nylon syringe filter and analyzed using GC-MS (Agilent 5977B autosampler) for the identification of the produced by-products (Ikram et al. 2022; Dharmasiri, Undugoda, Nilmini, Pathmalal, et al. 2023). [Parameters: Capillary column – HP-5MS (30 m × 0.25 mm, with 0.25 µm film), Injection volume – 1 µL, Inlet temperature – 280 °C, Carrier gas - Helium, 1 mL/min, Injection mode - Split less, Temperature program – 40 °C (1 min) → 120 °C (35 °C/min) → 160 °C (10 °C/min) → 300 °C (5 °C/min, hold for 10 min), Mass Selective detector – (MS) Agilent 5977 A, Ionization mode – Electron ionization (EI), Transfer line temperature – 280 °C, Ion source temperature – 230 °C, Quadrupole temperature – 150 °C, Electronic energy – 70 eV, Scan mode – SCAN, and Solvent delay – 4 min].

Phytotoxicity assay for the by-products of anthracene

A single bacterial strain was initially cultured in 25 mL of BBH broth and incubated at 30 °C for 3 d. Following incubation, the bacterial culture in the amount of 100 µL was inoculated in 50 mL of BBH broth supplemented with 100 ppm anthracene and incubated at 30 °C for 6 d. After incubation, the culture was centrifuged at 10,000 rpm for 10 min, and the supernatant obtained was utilized to check phytotoxicity in *Vigna radiata* (dicot plant) through measuring seed germination according to Ekanayake and Manage (2020). Distilled water was used to wash mung bean seeds before treatment and placed in Petri dishes with sterile, moist filter paper and six seeds per dish. Anthracene-treated samples were exposed to BBH broth supernatant-free natural sunlight between 06:00 and 18:00 h at a temperature of 29 °C. There was a control group that was treated with BBH broth supernatant without anthracene. The experiment was carried out in triplicate.

Results and discussion

Extensive research studies have been conducted regarding the PAH degradation capability of *B.*

velezensis during the past decade because of their high adaptability (Safitri et al. 2019; Sultana et al. 2021). According to these experimental findings, the researchers concluded that *B. velezensis* is a perfect biological agent to remove anthracene from the natural environment. In this study, the preliminary results revealed that this bacterium had an immense ability to degrade anthracene into nontoxic levels.

The dry cell biomass results (Figure 1(a)) revealed that up to 400 ppm concentration, the bacterium exhibited a strong exponential growth, where the bacteria divided and increased their cell biomass. The dry cell biomass at 100 ppm concentration of anthracene reached its highest weight within 24 h, and it was maintained with slight variations until the 6th day by balancing live and dead cells. Moreover, comparing the degradation rates (Figure 1(b)) over the incubation period at an anthracene concentration of 100 ppm reveals that the degradation rate entered an exponential phase within one day, and then a stationary phase that lasted until the second day. Afterward, the degradation rate was drastically increased until the 5th day, and it again showed a clear secondary exponential phase. After the 5th day, the degradation was maintained stationary phase while obtaining the highest dry cell biomass.

As shown in Figure 1, the microbial growth entered to exponential phase within one day and concluded by the fourth day at both 100 ppm and 200 ppm concentrations of anthracene. It clearly explains that within four days, *B. velezensis* can consume anthracene and multiply by itself without toxicity at below 200 ppm concentration of anthracene. After the fourth day, no color variations were observed in Figure 1(a), indicating the stability in the cell biomass. At 200 ppm of anthracene concentration, the degradation rate showed an exponential growth within the first day while increasing the dry cell biomass and then at the 2nd day it exhibited a stationary phase, then again on the 3rd day the degradation rate slightly increased and showed a second exponential phase with the highest dry cell biomass. By the 4th day, the degradation rate again showed a stationary phase. In addition, during the 5th day, it exhibited an exponential phase

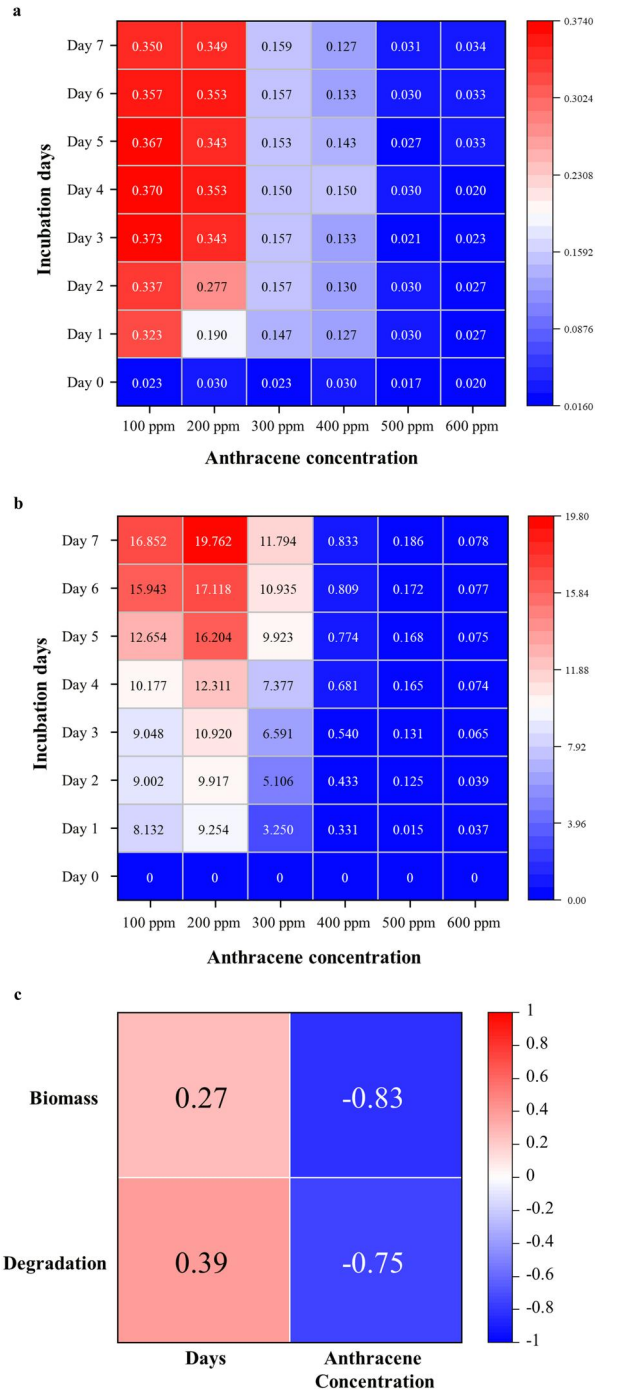

Figure 1. Heat map correlation for *B. velezensis* between (a) biomass variation for different concentrations of anthracene and incubation period, (b) degradation ability for different concentrations of anthracene and incubation period, and (c) Pearson correlation of *B. velezensis* biomass and degradation ability with different anthracene concentrations and incubation days.

and afterwards again a stationary phase on the 6th day while obtaining the highest anthracene degradation percentage. Having numerous stationary phases in a single degradation rate plot can be justified that this *B. velezensis* degrades

200 ppm of anthracene while tolerating its toxicity (Egbewale et al. 2025). This bacterium balanced its death and live cell ratios by tolerating and adapting to the high anthracene concentrations. At a concentration of 200 ppm, the maximum dry cell biomass did not reach the level observed at 100 ppm, indicating a potential inhibitory effect at the higher concentration.

However, as for the medium concentrations of 300 ppm and 400 ppm, this bacterium takes two days to reach the exponential phase. After 2 d, the dry cell biomass showed no variations in color change, and this clarifies that there was no new cell growth (Figure 1(a)). At an anthracene concentration of 300 ppm, the degradation rate remained in the stationary phase for the first 2 d. Following this lag period, an exponential degradation phase was observed, lasting until the fifth day, after which the system entered a stationary phase. Notably, the dry cell biomass remained constant from the second day onward. In contrast, at 400 ppm anthracene, the degradation rate exhibited a delayed response, with a slight exponential phase emerging only after 5 d. Despite this delay, the dry cell biomass was maintained at its maximum level from the second day throughout the experiment.

At higher concentrations, such as 500 ppm and 600 ppm, a prolonged lag phase was observed lasting up to the fourth day, and the dry cell biomass continued to increase with slight variations until the sixth day. It can be clarified that the *B. velezensis* bacterium demands its finest growth to survive and adapt to the growth conditions; however, the survivable possibility was low in these high concentrations. Endospore formation was the finest tactic that the *Bacillus* genus (*B. velezensis*) exhibited toward adverse conditions, such as high concentrations (Safitri et al. 2019). Except for this *B. velezensis,* other species in the *Bacillus* species, such as *B. subtilis* (Salamat, Lamoochi, and Shahaliyan 2018), *B. cereus* (Bibi et al. 2018), and *B. pumilus,* can also degrade anthracene into nontoxic levels at below 200 ppm concentrations (Bibi et al. 2018; Marzuki et al. 2021; Salamat, Lamoochi, and Shahaliyan 2018) within 6 d. At 500 ppm anthracene concentration, the plot exhibited a degradation rate varying below 0.5 up to the 5th day, and after that, a slight exponential phase was obtained, and on the 6th day, the degradation rate showed a stationary phase while maintaining the dry cell biomass under 0.05 mg. In the 600-ppm anthracene concentration plot, there was a slight degradation rate varying below 0.5, and the dry cell biomass was also observed under 0.05 mg for the whole 6 d. All these results revealed that these higher concentrations of anthracene are toxic to the bacterium itself, while the optimum concentration of anthracene was recognized as 100 ppm for *B. velezensis*.

Overall, according to Figure 1(c), the biomass showed a weak positive correlation with anthracene concentration and a strong negative correlation with incubation days. Similarly, the degradation ability had a moderate positive correlation with anthracene concentration and a strong negative correlation with incubation days. These results indicate that while higher anthracene concentrations slightly increased biomass and moderately enhanced degradation ability, both parameters significantly declined over extended incubation periods.

The plotted graph of degradation rate versus concentration (Figure 2) follows the linear equation $y = -0.0029x + 1.7126$, with a coefficient of determination (R^2) of 0.9867. This linear relationship deviates from the typical Michaelis – Menten kinetic model due to substrate inhibition caused by the toxic effects of high anthracene concentrations. Kinetic models have previously been applied to anthracene degradation using various microbes, such as *Pseudomonas stutzeri* (Velayutham 2019). According to Rahimi et al. (2022), the Michaelis – Menten constant (K_m) and maximum degradation rate (V_{max}) for anthracene removal by *Alkalibacillus salilacus* were

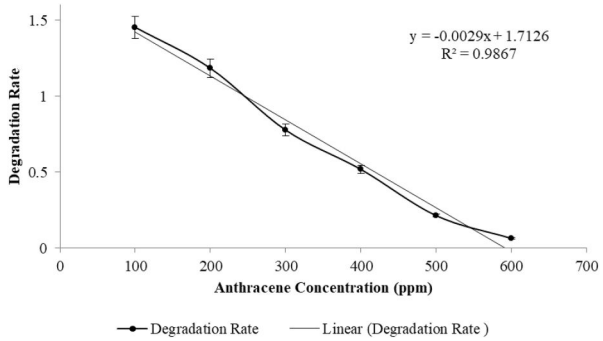

Figure 2. Anthracene degradation rate by *B. velezensis* with different concentrations (100, 200, 300, 400, 500, and 600 ppm).

reported as 0.114 μM and 0.546 μmol $h^{-1} mg^{-1}$, respectively. *Bacillus subtilis* has also been tested for anthracene degradation, achieving 99% removal within 5 d, and its kinetics were similarly studied (Abdelhaleem et al. 2019). However, based on the findings of this study, the Michaelis–Menten model is not applicable in cases where substrate inhibition occurs.

Further analysis of *B. velezensis* growth patterns was done by applying various mathematical models that were evaluated using curve-fitting techniques and non-linear regression analysis. An evaluation of three distinct models was conducted: Monod's model, Haldane's model, and Wayman and Tseng's model (Table 2). The primary criterion for evaluation was their capacity to converge with our experimental data. Table 3 presents the model parameters, goodness-of-fit statistics, and performance metrics for each model. According to Table 3, the Wayman and Tseng model remains the bestfitting mathematical model for investigating the anthracene-degrading ability of *B. velezensis*. It exhibits a high adjusted R^2 value (0.95191), indicating a strong fit to the data, and a low RMSE (0.0019), indicating accurate predictions. However, the threshold concentration (S_0) is negative. This means, this bacterium can break down anthracene to nontoxic levels, and even after it has completely broken-down anthracene, it can still survive by degrading the nontoxic by-product (9,10-Anthracenedion). Although the Haldane model has a relatively high Adj. R^2 value (0.86119), the standard errors for the parameters are very high, which suggests that the model may not be reliable for predicting the anthracene degradation ability of *B. velezensis*. The Monod model has a lower Adj. R^2 value (0.66941) compared to the Wayman and Tseng model, and the negative value for K_s is not biologically meaningful. This suggests that the Monod model may not be the best fit for the data.

Table 2. Converging models for the experimental data.

Model	Equation
Monod's	$\mu = \frac{(\mu_{max} \times S)}{(K_s + S)}$ (6) μ = The specific growth rate (1/h) μ_{max} = The maximum specific growth rate (1/h) S = The concentration of the limiting substrate (mg/L) K_s = The half-saturation constant (mg/L)
Haldane's	$\mu = \frac{(\mu_{max} \times S)}{\left(S + \frac{S^2}{K_i} + K_s + S \times \frac{K_s}{K_i}\right)}$ (7) μ = The specific growth rate (1/h) μ_{max} = The maximum specific growth rate (1/h) S = The concentration of the limiting substrate (mg/L) K_s = The half-saturation constant (mg/L) K_i = substrate inhibition constant
Wayman and Tseng's	$\mu = \frac{(\mu_{max} \times S)}{(S + K_s)} - i \times (S - S_\theta)$ (8) μ = The specific growth rate (1/h) μ_{max} = The maximum specific growth rate (1/h) S = The concentration of the limiting substrate (mg/L) K_s = The half-saturation constant (mg/L) S_θ = Threshold substrate concentration (mg/L) i = Inhibition coefficient

Furthermore, Figure 3 demonstrates that *B. velezensis* is capable of degrading anthracene to a nontoxic level within three days. HPLC analysis (Figure 3) showed that the highest concentration of by-product formation occurred on the third day at a concentration of 100 ppm. Subsequent GC-MS analysis confirmed the by-product as 9,10-anthracenedione (Figure 3). Previous studies have also reported the presence of this compound during anthracene degradation. For instance, Peng et al. (2018) identified 9,10-anthracenedione as an intermediate in the biodegradation of anthracene by *Rhodococcus* sp. Their study also reported the formation of benz[a]anthracene-7,12-dione during the degradation process. Similarly, *Bacillus cereus* has been shown to degrade anthracene into 9,10-dihydroxyanthracene and anthraquinone as part of its biodegradation pathway (Bibi et al. 2018).

When comparing intermediate concentrations over seven days at 200 ppm of anthracene, it was observed that no intermediates remained by the seventh day (Figure 4). As in Figure 4, the intermediate was initiated in its formation and achieved its best concentration on the third day, and lastly, on the seventh day, there were no records of remaining intermediate. Few research studies have been carried out to prove that microbes can degrade PAHs into carbon dioxide and water (CO_2 and H_2O) (Sartoros et al. 2005). Consequently, this study confirms that the formed intermediate was also degraded into nontoxic levels, such as CO_2 and H_2O, by *B. velezensis* on the seventh day.

At an anthracene concentration of 100 ppm, the by-product concentration began to decrease as the degradation rate (Figure 1) entered the exponential phase. This relationship clearly defined that the *B. velezensis* bacterium utilized the produced by-product on the 3rd day and

Table 3. Estimated bio-kinetic parameters of the anthracene degradation ability of *B. velezensis*.

	μmax		K_s		K_I		S_0		I				
Model	Value	Standard error	Value	Standard error	Value	Standard error	Value	Standard error	Value	Standard error	Adj. *R*-square	Chi-square	RMSE
Monod's	0.01402	0.00314	−58.56128	13.79134							0.65941	4.98E-05	0.007053588
Haldane's	0.14701	1576.73491	57.24694	616652.1411	57.18146	613237.5203					0.86119	2.03E-05	0.00450311
Wayman and Tseng's	0.0675	0.00315	70.18236	9.59107			−46.50742	18.80759	8.7714E-5	4.84142E-6	0.95191	3.61241E-6	0.0019

achieved the exponential phase. At an anthracene concentration of 200 ppm, the degradation rate (Figure 1) exhibited exponential phases on the 2nd and 5th days. During these periods, the by-product concentration (Figure 5) began to decline, indicating its utilization by the bacterium to resume exponential growth following a stressed stationary phase. By the sixth day, all intermediate products had been fully utilized by the bacterium (Figure 4). At 300 ppm concentration of anthracene, the intermediate concentration was reduced on the 2nd day while achieving the exponential phase in the degradation rate plot (Figure 1). The highest concentrations of by-products were produced on the 5th day at a concentration of 300 ppm anthracene, compared to other values (Figure 5). On the 5th day in 400 ppm anthracene concentration, the degradation rate started to increase while increasing the intermediate production as well, after 4 d of stressed stationary phase. In 500 and 600 ppm of anthracene concentration plots, there were slight variations in the intermediate concentrations, but along with the degradation rates, the produced intermediates were reduced after 5 d.

At high anthracene concentrations, *B. velezensis* viability was substantially reduced, which can be attributed to the compound's inherent toxicity and hydrophobicity. Anthracene, as a PAH, could also become adsorbed onto the bacterial cell membrane due to its lipophilicity, disrupting membrane fluidity, and integrity. This leads to increased membrane permeability, cellular component leakage, and ultimately cell death (Hąc-Wydro, Połeć, and Broniatowski 2019).

Phytotoxicity assays confirmed that this by-product was not toxic to the phyllosphere in low concentrations, like 100 and 200 ppm. But as for the high concentrations of anthracene, they can be very toxic to the phyllosphere as well. The heights of the Mung seeds were gradually decreased when the anthracene concentration increased (Figure 6). When a line graph was plotted with the average height of the mung seeds in front of different concentrations, it displayed that the length of the Mung seeds gradually decreased when the anthracene concentration became high. When there was no spiked anthracene, the average length from shoot to root of the Mung seeds was 28.4 cm, and when the

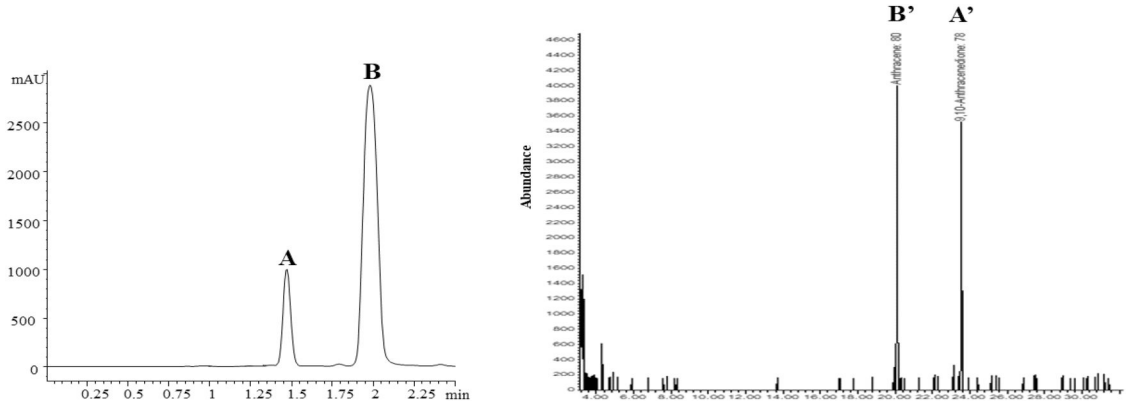

Figure 3. HPLC peaks with the retention times for the remaining anthracene and the by-product produced. {remaining anthracene retention time 1.979 min (peak B) and by-product retention time 1.471 min (peak a)}. GC-MS peaks for anthracene (peak B') and by-product (peak A').

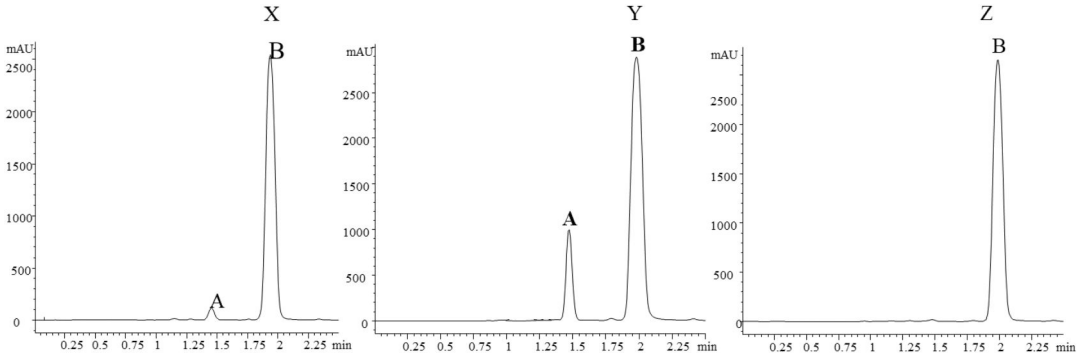

Figure 4. Remaining anthracene peak and intermediate formation on the 0th day (X), the third day (Y), and the sixth day (Z) by HPLC analysis in 200 ppm concentration of anthracene. {remaining anthracene retention time 1.979 min (peak B) and by-product retention time 1.471 min (peak A)}.

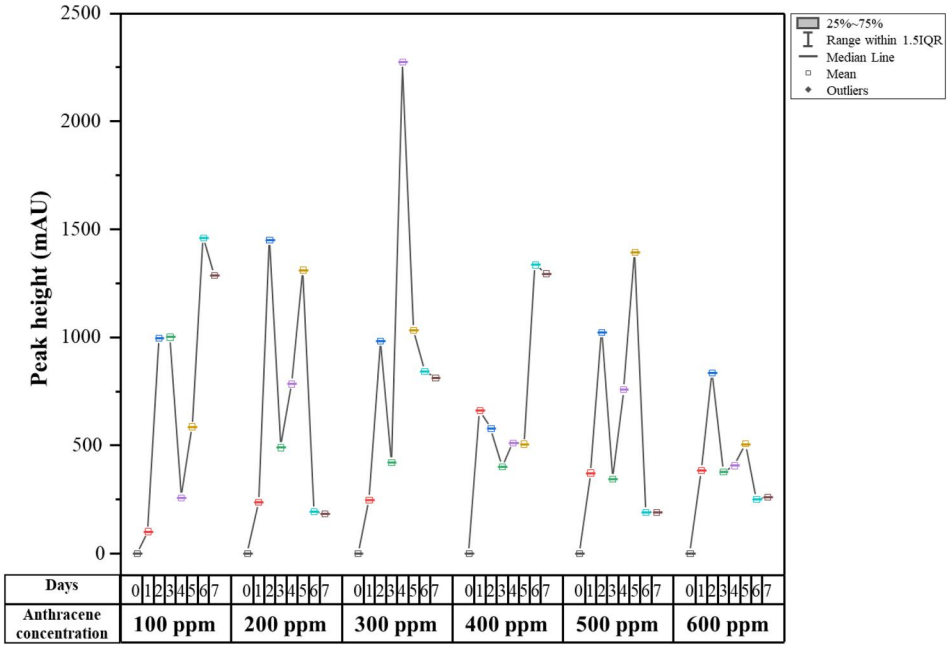

Figure 5. Intermediate concentrations (peak heights) of by-products produced by *B. velezensis* during incubation with various anthracene concentrations.

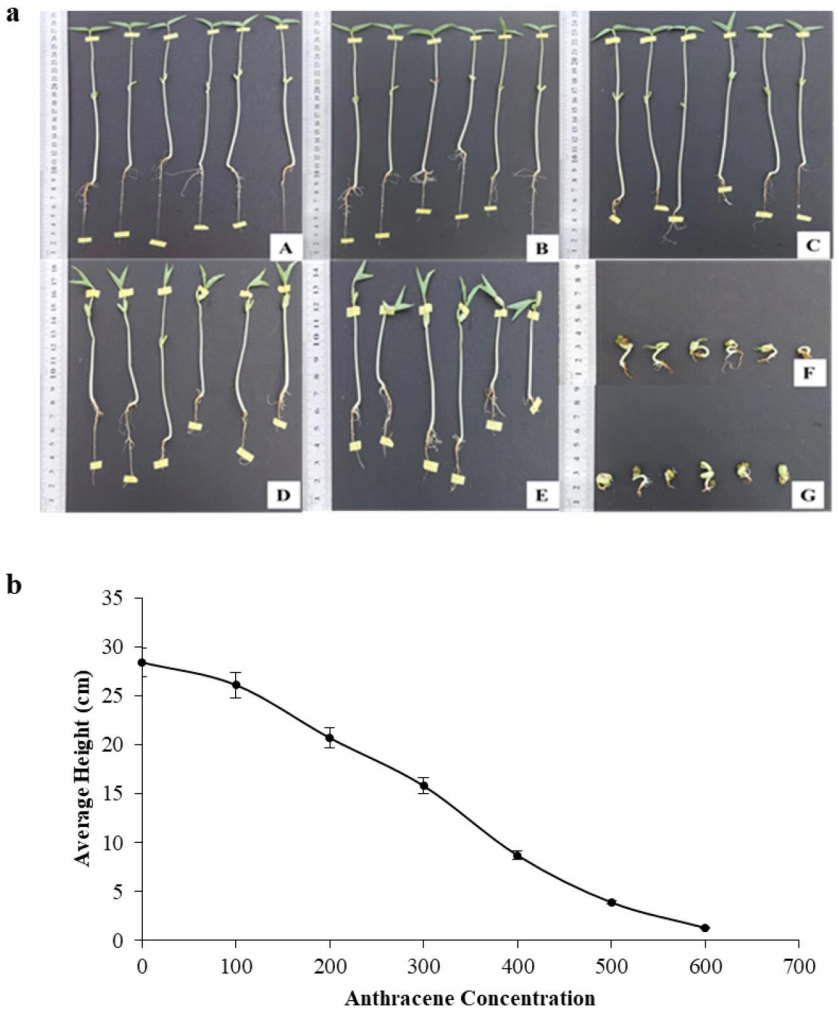

Figure 6. (a) Six Mung seeds which were sprayed with the filtered culture solutions from 3rd day; A: Negative control (sprayed with BBH broth), B: 100 ppm culture solution, C: 200 ppm culture solution, D: 300 ppm culture solution, E: 400 ppm culture solution, F: 500 ppm culture solution, and G: 600 ppm culture solution. (b) The line graph displays the average height of the six mung seeds in front of the different concentrations.

concentration increased by 100 ppm every time, the length decreased. At high anthracene concentrations of 500 ppm and 600 ppm, mung seeds began to germinate slightly, but their growth was inhibited, and they started to wither. At elevated anthracene concentrations, due to substrate inhibition, the ability of *B. velezensis* to degrade anthracene was reduced as discussed previously. As a result, a higher amount of unmetabolized anthracene remained in the supernatant, causing toxicity to mung beans by interfering with seed germination and early growth (Paul et al. 2025).

As future recommendations, to improve statistical reliability, finer anthracene concentration breaks between 100 ppm and 600 ppm, which will enhance precision and allow for a better understanding of the inhibition threshold.

Conclusion

Batch tests were conducted to examine the interaction of anthracene with individual components using pure cultures of *B. velezensis* under aerobic conditions. This bacterium was selected as an ideal microorganism to study the kinetics of anthracene degradation at different concentrations (100, 200, 300, 400, 500, and 600 ppm). The results revealed that bacterial survival decreased at higher concentrations of anthracene, leading to growth inhibition. Furthermore, GC-MS analysis identified the degradation by-product as 9,10-anthracenedione. At 100 ppm anthracene, *B. velezensis* further degraded this intermediate into carbon dioxide and water. Phytotoxicity assays confirmed that the intermediate was not harmful to the phyllosphere or the bacterium itself. Anthracene degradation by

the bacterium was rapid, resulting in significant biomass growth. This study deviated from the typical Michaelis – Menten kinetic model due to substrate inhibition caused by anthracene toxicity. Therefore, the Michaelis – Menten equation is not applicable in cases where substrate inhibition occurs. Statistical analysis showed that among the models tested, Wayman and Tseng's model provided the best fit for the data. However, two parameter values within this model were not statistically significant. To strengthen the analysis, it was deemed necessary to interpolate the seven data points in question. By performing this interpolation, a more comprehensive dataset can be obtained, enabling a more accurate determination of the optimal fit for Wayman and Tseng's model.

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Disclosure statement

The authors confirm that they have no conflicts of interest with respect to the work described in this manuscript.

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Data availability

Data will be made available on request.

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