



HOKKAIDO UNIVERSITY

Title	Functional Specialization and Phenotypic Plasticity: A Mathematical Modeling Approach to Microbial Growth Dynamics
Author(s)	熊倉, 大騎
Degree Grantor	北海道大学
Degree Name	博士(生命科学)
Dissertation Number	甲第16276号
Issue Date	2025-03-25
DOI	https://doi.org/10.14943/doctoral.k16276
Doc URL	https://hdl.handle.net/2115/95430
Type	doctoral thesis
File Information	Daiki_Kumakura.pdf



博士学位論文

Functional Specialization and Phenotypic Plasticity:
A Mathematical Modeling Approach to Microbial Growth Dynamics

(機能分業と表現型可塑性：微生物の生存戦略に基づく増殖モデル研究)

熊倉 大騎

北海道大学大学院生命科学院

2025年3月

Summary in Japanese

本研究は、微生物の表現型可塑性と生存戦略を理解するために、機能分業システムを考慮した増殖モデルを提案し、微生物の増殖ダイナミクスを包括的に理解することを目的としている。微生物の増殖ダイナミクスは、培養時間や環境条件に応じて大きく変化する。

Monod (1949) による経験的な方程式は、微生物の増殖速度と制限基質との関係を説明する古典理論だが、現実の複雑な増殖ダイナミクスを完全に捉えることはできない。そこで、本研究では微生物が形成する亜集団の機能分業を考慮に入れた新たな数理モデルを構築し、培養過程における微生物の挙動をより精密に解析することを目指した。

従来の微生物培養モデルは、増殖期、定常期、死滅期の三つの期間に大別されるが、これらは微生物の増殖を十分に説明できるものではない。特に定常期は、微生物が完全に増殖を停止する期間とみなされているが、実際にはわずかな増殖や死滅が見られ、平衡状態には至っていない。また、既存のモデルは主に増殖期に焦点を当てており、培養後期における微生物の挙動はほとんど考慮されていない経緯がある。こうした状況を改善するため、本研究では、基質から得たエネルギーの分配を考慮した微生物の増殖モデルを提案し、亜集団形成とその役割についても分析を行った。

本研究では、制限基質と代謝産物を含む微生物の増殖について、新たな数理モデルを提案する。このモデルでは、微生物が基質を消費して得たエネルギーを、増殖を担う集団と代謝産物を分解する集団に分け、各亜集団の役割に基づく機能分業を仮定して増殖ダイナミクスを計算した。さらに、実際の培養データを用いてパラメータ推定を行い、代謝物の蓄積が微生物の増殖に与える影響を定量的に評価した。

数値解析の結果、微生物の増殖は6つの段階に分類できることが判明した。増殖期は3段階、定常期は2段階、死滅期は1段階として表され、特に死滅期後には代謝物が蓄積することで微生物の増殖能力が失われることが確認された。これにより、微生物はまず基質

を急速に消費して密度を高め、その後に環境を浄化するという二段階の戦略を採用している可能性が示唆された。パラメータ依存性の探索から、微生物が基質を消費して急速に増殖する戦略と、ゆっくりと増殖しながら環境を浄化する戦略を、状況に応じて使い分けている可能性が明らかになった。さらに、代謝物の蓄積に対する感受性の違いから、微生物は耐性を持つタイプ、中程度に敏感なタイプ、非常に敏感なタイプの3つに分類されることが確認された。データ分析から、これまで曖昧だった各期間を正確に定義することができたほか、微生物が培養環境中を浄化しながらゆっくりと増殖することが示され、微生物の増殖ダイナミクスにおける表現型可塑性と亜集団形成の重要性が明らかになった。

本研究により、微生物の増殖を阻害する要因や培養条件の変化に対する適応戦略を理解するための新たな視点を提供することができた。本研究の成果は、今後の微生物生態学および生物工学における応用に大いに貢献することが期待される。

Summary

This study aims to propose a growth model that considers the functional division system to understand the phenotypic plasticity and survival strategies of microorganisms and comprehensively understand the growth dynamics of microorganisms. The growth dynamics of microorganisms vary significantly depending on cultivation time and environmental conditions. The empirical equation by Monod (1949) is a classical theory that explains the relationship between microbial growth rate and limiting substrates, but it cannot fully capture the complex growth dynamics observed. Therefore, this study seeks to construct a new mathematical model that considers the functional division of microbial subpopulations and aims to analyze the behavior of microorganisms during the cultivation process.

Conventional microbial culture models are generally divided into three phases: the growth phase, the stationary phase, and the death phase. However, these phases do not sufficiently explain microbial growth dynamics. In particular, the stationary phase is often regarded as a period where microbial growth completely ceases, but slight growth and death exist, and equilibrium is not achieved. Additionally, existing models have primarily focused on the growth phase, with little consideration given to microbial behavior in the later stages of cultivation. To address these issues, this study proposes a microbial growth model that considers the allocation of energy obtained from substrates and analyzes the formation of subpopulations and their roles.

In this study, we propose a new mathematical model for microbial growth that includes limiting substrates and metabolic products. In this model, the energy obtained by microorganisms from consuming substrates is divided between a population responsible for growth and a population responsible for decomposing metabolic products. The growth dynamics were calculated under the assumption of functional division based on the roles of each subpopulation. Furthermore, parameter

estimation was conducted using actual culture data, and the impact of metabolite accumulation on microbial growth was quantitatively evaluated.

Numerical analysis revealed that microbial growth can be classified into six stages: three in the growth phase, two in the stationary phase, and one in the death phase. Notably, it was confirmed that after the death phase, the accumulation of metabolic products leads to the loss of microbial growth capacity. This suggests that microorganisms may adopt a two-stage strategy, first rapidly consuming the substrate to increase their density and then purifying the environment afterward. Exploration of parameter dependencies revealed that microorganisms might switch between a strategy of rapid growth by consuming substrates and a strategy of slow growth while purifying the environment, depending on the situation. Furthermore, based on differences in sensitivity to metabolite accumulation, microorganisms were classified into three types: resistant, moderately sensitive, and highly sensitive. Data analysis allowed for the precise definition of previously ambiguous stages and demonstrated that microorganisms grow slowly while purifying the cultivation environment. This highlighted the importance of phenotypic plasticity and subpopulation formation in the growth dynamics of microorganisms.

This study has provided a new perspective on understanding the factors that inhibit microbial growth and the adaptive strategies used in response to changes in cultivation conditions. The findings are expected to significantly contribute to future applications in microbial ecology and biotechnology.

Publication

- Kumakura, D., Yamaguchi, R., Hara, A., & Nakaoka, S. (2023). Disentangling the growth curve of microbial culture. *Journal of Theoretical Biology*, 111597.

<https://doi.org/10.1016/j.jtbi.2023.111597>.

Table of Contents

1. General Introduction	1
2. Introduction	5
3. Material and Methods	9
3.1. Model	9
3.2. Numerical analysis	14
3.2.1. Phase definition	14
3.2.2. Balancing the subpopulation	15
3.2.3. Resistance to the metabolite accumulation environment	16
3.3. Data analysis	17
4. Results	18
4.1. Stability analysis	18
4.2. Defining microbial growth phase	21
4.3. Parameter dependence	28
4.3.1. Single parameter dependence	28
4.3.2. Balance between the microbial growth and detoxification of byproducts	29
4.3.3. Resistance to environmental metabolite accumulation	30
4.4. Experimentally measured data analysis	33

5. Discussion	36
5.1. Motivation of the modeling	36
5.2. The summary of the analysis	37
5.2.1. Stability analysis	37
5.2.2. Defining the microbial growth phase	39
5.3. The summary of the results	41
5.3.1. Single parameter dependence	41
5.3.2. Balance between the microbial growth and detoxification of byproducts	42
5.3.3. Resistance to environmental metabolite accumulation	43
5.3.4. Data analysis	44
5.4. Biological implications of the results	45
5.5. Limitation of the current analysis and future works	47
6. General Discussion	49
7. References	53
8. Appendix	62
8.1. Nontrivial equilibrium points	62
8.2. Local stability analysis	64
8.3. Comprehensive parameter test	68
8.4. Nondimensionalization	72

8.4.1 Nondimensionalized model	72
8.4.2. Nontrivial equilibrium points	74
8.4.3. Local stability analysis	75
8.4.4. Comparison population dynamics with based model	78
8.4.5. Comprehensive parameter test	81
9. Acknowledgement	84

1. General Introduction

Microorganisms, particularly bacteria, proliferate predominantly through binary fission, a form of asexual reproduction that can lead to exponential population growth under favorable conditions. The factors determining growth rates are diverse, encompassing the availability of nutrients, temperature, pH, oxygen concentration, and various other physical and chemical parameters, all of which interact with the genetic background to influence microbial population dynamics. These fundamental growth phenomena have been formally described using mathematical models, including the logistic growth model (Verhulst, 1838; 1845) and the Monod equation (Monod, 1949). For decades, these models have served foundational frameworks for microbiology, environmental engineering, and bioprocess design. For example, the logistic growth model incorporates an environmental carrying capacity, capturing the transition from exponential growth in the early stages to a steady-state as resources become limited, or waste products are accumulating. In contrast, the Monod equation describes the relationship between substrate concentration and specific growth rate in a manner analogous to enzyme kinetics.

However, real-world microbial ecosystems exhibit far more complex behaviors than can be explained by these simplified models. Bacteria do not merely grow; they employ diverse strategic behaviors to survive and adapt under varying environmental conditions. For instance, they produce and release primary and secondary metabolites, engage in signaling and resource competition with other microorganisms, acquire new functional capabilities through genetic mutations and horizontal gene transfer, form persistent cells, and construct collective structures such as biofilms encased in extracellular matrices. These survival strategies often operate concurrently, allowing bacterial communities to thrive in dynamic and frequently hostile environments (Sharrar et al., 2020; Lenski

& Travisano, 1994; Frost et al., 2005; Lewis, 2010; Flemming & Wingender, 2010).

These strategies are activated in response to diverse external stimuli, ranging from nutrient availability and chemical stressors to physical pressures and exposure to host immune systems or antibiotics. Such flexibility enables bacterial populations to adapt flexibly to environmental changes. A key underlying concept in this adaptability is *phenotypic plasticity*. Phenotypic plasticity refers to the ability of cells with the same genetic makeup to alter their gene expression patterns in response to environmental conditions or internal states, thereby exhibiting distinct functional characteristics (phenotypes) (Elowitz et al., 2002; Ackermann, 2015). This plasticity allows bacteria to dynamically regulate processes such as metabolic pathway redistribution, induction of resistance genes, and the toggling of toxin production on or off, all of which contribute to enhancing their survival potential even in hostile environments.

In recent years, advances in single-cell analysis techniques, such as single-cell RNA sequencing (scRNA-seq), have illuminated cellular heterogeneity that was previously difficult to discern through bulk analyses (Kuchina et al., 2021; Brennan & Rosenthal, 2021; Homberger et al., 2022). scRNA-seq has overcome technical challenges specific to bacteria, such as the low abundance of polyadenylated transcripts and the presence of cell walls, enabling the high-resolution quantification of gene expression variability within genetically identical populations (Imdahl & Saliba, 2020; McNulty et al., 2023; Korshoj & Kielian, 2024; Yan et al., 2024). Consequently, it has become clear that cells sharing the same genotype can exhibit distinct phenotypes in response to environmental factors, cell density, and signaling molecules. These phenotypically distinct subpopulations often engage in division of labor, whereby specialized functional roles within the population enhance collective adaptability to environmental challenges (Homberger et al., 2022; Kuchina et al., 2021; Yan et al., 2024).

The formation of subpopulations and their functional specialization are closely linked to key processes in bacterial pathogenicity, including the internal structure and dynamics of biofilms, the production of public goods, toxin synthesis, and the acquisition of antibiotic resistance (Griffin et al., 2004; Flemming et al., 2023; Shokeen et al., 2021; McNulty et al., 2023; Hockenberry et al., 2021; Davis, 2020; Boles & Horswill, 2008; Garrett et al., 2019; Lyu et al., 2021). In this context, phenotypic plasticity and the resulting heterogeneity serve as evolutionary and physiological strategies enabling bacterial populations to respond effectively to diverse environmental stresses.

Nevertheless, capturing the variety of strategies and subpopulation dynamics supported by phenotypic plasticity remains challenging with existing average-based growth models. Traditional models often treat populations as homogeneous, neglecting their internal structure such as cellular heterogeneity and subpopulation formation. As a result, these models struggle to quantitatively explain more complex phenomena, including the mechanisms underlying growth deceleration during the late stages of cultivation, the self-intoxication effects caused by the accumulation of metabolic byproducts, and the roles played by specific functional subpopulations in shaping overall growth dynamics.

This study aims to bridge this gap. Specifically, it seeks to: (1) quantify and analyze phenotypic diversity and subpopulation formation at the single-cell level, (2) propose a novel mechanistic microbial growth model that incorporates substrate consumption alongside product accumulation, and (3) elucidate the quantitative impact of phenotypic plasticity on the population-level growth dynamics and survival strategies through alignment with experimental data. By integrating the internal structure and division of labor within bacterial populations into the modeling framework, this research strives to more accurately reproduce and predict microbial responses to environmental changes and adaptive evolution.

This approach is expected to not only deepen the fundamental understanding of microbiology but also pave the way for applications in biotechnology, environmental engineering, and medicine. Potential applications include improving resource utilization efficiency in industrial fermentation processes, managing microbial communities in bioreactors, and developing strategies to control antibiotic-resistant bacteria or combat emerging infectious diseases. By introducing the novel perspectives of phenotypic plasticity and cellular heterogeneity to the traditionally averaged understanding of microbial growth dynamics, this study aims to advance both our understanding of microbial ecosystems and the development of practical technologies.

2. Introduction

The bacterial growth rate and population density change with culture time. Monod (Monod, 1949) explained the relationship between the growth rate of bacteria and the concentration of the limiting substrate using a simple mathematical model. Based on Monod's equation, the maximum growth rate and equilibrium population density of a bacterial species can be determined (Liu, 2007). From the empirical perspective of Monod's equation, microbes have been found to grow based on a limited nutrient source (Monod, 1949). Later, a simple model that considered metabolites was effectively applied in the field of bioengineering (Garnier and Gaillet, 2015, Kumar Dutta et al., 1996, Birol et al., 1998).

Traditionally, a typical growth curve is divided into six phases: 1) lag, 2) acceleration, 3) exponential, 4) deceleration, 5) stationary, and 6) death (Monod, 1949). However, in many cases, cultures do not exhibit the typical growth pattern. This happens because the growth phases of microorganisms differ substantially, depending on their active conditions prior to culture initiation. The stationary phase is also not as simple as defined and is a period of slight growth or death rather than a complete equilibrium condition. Furthermore, most experimental studies are only interested in certain growth aspects; thus, all six phases have not been fully explored (Alvarez-Ramirez et al., 2019).

In predictive microbiology, validation focuses on only a few growth phases (Gil et al., 2014). A classical model was developed based on Monod's equation and is mainly focused on growth and stationary phases (Strigul et al., 2009). The model represents the process from the start of culture through lag time, rapid growth, and, finally, the stationary phase. In addition, Monod's equation proposes a model of product dynamics that depends on substrate consumption (Alvarez-

Ramirez et al., 2019). Growth inhibition factors (e.g., accumulation of toxic chemicals) may also affect the growth potential of microorganisms (Xu, 2020). Studies focusing on the growth potential in the late stage of culture have been overlooked because it had been interested in the exponential growth of microorganisms. Monod's equation can generate seemingly excellent population dynamics, but its versatility has been unexplored because it is an empirical equation (Liu, 2007). If we explain the long-term culture of microorganisms in a mechanical and simple form, we need to consider the growth of microorganisms, explicitly including the dynamics of substrates and products simultaneously. The explicit mathematical modeling of microbial growth and product kinetics in the late stage of culture can likely lead to a good estimation of the whole culture dynamics.

Microorganisms use the energy obtained from the substrate for various functions, such as cell division, secondary metabolism, flagellar locomotion, bioluminescence, and biofilm formation (Brodl et al., 2018, Pratt and Kolter, 1998, O'Toole et al., 2000, Tyc et al., 2017). Recently, single-cell RNA-seq analysis has shown that microorganisms perform various functions in the culture medium, even under the same culture conditions and time (Kuchina et al., 2021, Imdahl et al., 2020). Microorganism, even within the same species, exhibit different functions due to phenotypic plasticity and form distinct subgroups. Microorganisms use their acquired energy at the subpopulation level to perform a variety of roles. Mathematical models of the division of labor in microorganisms have been developed to mainly focus on competitive (or cooperative) relationships with public goods (Lynn and De Leenheer, 2019, Liu et al., 2021, Cooper et al., 2022). These provide long-term evolutionary conditions for the microbial division of labor between two species or strains. However, microbial growth dynamics for the formation of subpopulations at a short-term timescale (i.e., not evolutionary time scale but ecological one that affects population dynamics) of culture are yet to be analyzed. By analyzing how microorganisms divide and utilize the energy

acquired through substrate consumption for growth, the dynamics of microbial growth can be calculated by considering factors such as overcrowding, substrate depletion, and metabolite accumulation.

We propose a mechanistic microbial growth model, including substrates and products in the simplest model. The current study focused on cell division and secondary metabolism to model this type of dynamic behavior in bacteria. The microorganisms in the medium are divided into two populations: one that uses the energy obtained by consuming the limiting substrate for its growth and the other that decomposes the products obtained by the metabolism of the limiting substrate. This model investigated how microbial growth in the medium changed with the degree of microbial overcrowding, substrate consumption, and product accumulation.

In addition to understanding the dynamics of microbial growth, the aim of the model was to analyze the experimental growth curve data so that we could investigate the quantitative effect of microbial growth caused by the accumulation of metabolites. Autointoxication, an undesirable effect of accumulated metabolites on microbial growth, was taken into account in the previous mathematical analysis in the context of degradation of dioxin (Hojo et al., 2006). We here aim to build the model describing a general concept of autointoxication effects on the microbial growth. We use previously published data of the aerobic denitrifying bacterium *Pseudomonas balearica* RAD-17, which possesses nitrate reductase and can be used for denitrification (Ruan et al., 2020). *P. balearica* RAD-17 has efficient aerobic nitrogen removal capacity and is attracting attention as a bioaugmentation use (Ruan et al., 2020). In a nitrate medium, *P. balearica* can survive by metabolizing nitrate ions (NO_3^-), the only substrate. *P. balearica* uses nitrate as a substrate to produce nitrite ions (NO_2^-), a precursor to nitric oxide (NO), a toxic substance (McNulty et al., 2023). NO_2^- is then rapidly decomposed via NO to N_2O to become N_2 , which is safe for bacteria

(Ruan et al., 2020, Chaudhari et al., 2017). Through analysis of experimentally measured data with our current model, the detailed growth dynamics of *P. balearica* were revealed. It was also found that *P. balearica* rapidly degraded products during the stationary phase, which was not anticipated in the previous study. Thus, through the analysis of experimentally measured data, this model can improve our understanding of the detailed growth dynamics of certain microorganisms, particularly those influenced by secondary metabolites.

3. Material and Methods

3.1. Model

In this model, it is assumed that the same species can exhibit different functions and form different subpopulations due to the phenotypic plasticity of microorganisms. This has been suggested in single-cell RNA-seq studies of microorganisms (Kuchina et al., 2021, Imdahl et al., 2020). In this study, we considered the growth dynamics of microorganisms by focusing on the altruistic division of the functions system. Microorganisms use part of the energy obtained for asexual reproduction (microbial growth subpopulation) and part for the decomposition of metabolites produced in the process of obtaining energy (detoxification of byproducts subpopulation). To examine the altruistic division of the functions system (Fig. 1), we model a population-resource-metabolite dynamics as shown in Eqs. (1):

$$\frac{dN}{dt} = \mu a_1 R N p \left(1 - \frac{N}{K(M)}\right) - dN \quad (1-1)$$

$$\frac{dR}{dt} = \lambda - a_1 R N p - b_1 R N (1 - p) \quad (1-2)$$

$$\frac{dM}{dt} = a_2 R N p + b_2 R N (1 - p) - c M N (1 - p) \quad (1-3)$$

$$K(M) = K_0 + (K - K_0)e^{-fM} \quad (1-4)$$

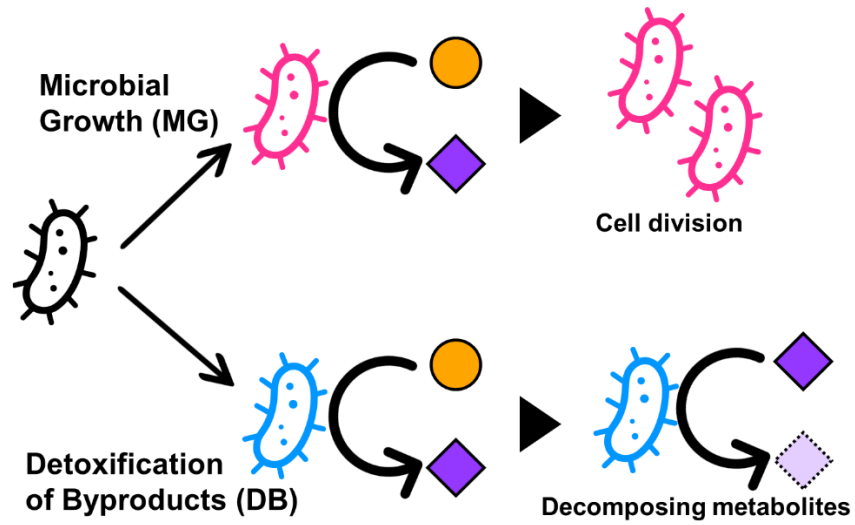


Fig. 1. Scheme of the model.

In this model, it is assumed that the same species can exhibit different functions and form different subpopulations due to the phenotypic plasticity of microorganisms. This has been suggested in single-cell RNA-seq studies of microorganisms (Kuchina et al., 2021, Imdahl et al., 2020). In this study, we considered the growth dynamics of microorganisms by focusing on the altruistic division of the functions system. Microorganisms use part of the energy obtained for asexual reproduction (microbial growth subpopulation) and part for the decomposition of metabolites produced in the process of obtaining energy (detoxification of byproducts subpopulation).

Each variable represents N (cell/ml) as the total cell density, R (ml) as the substrate concentration, and M (ml) as the metabolite concentration. The notations of parameters and values used in this study are listed in Table 1. Bacteria growth according to the growth rate for consuming the substrate. Their growth rate is defined as μ . In this study, we assume the model as $\mu = 1$ for simplicity. The focal microbe N plays two roles: consuming resources and growing, as well as decomposing metabolite M . Hereafter, the former is referred to as the microbial growth (MG) subpopulation and the latter the detoxification of byproducts (DB) subpopulation (different parameters: b_1 and b_2). The metabolite M is presumed to be a substance that adversely affects microbial growth function. For example, nitrite and hydrogen peroxide can be mentioned (Li et al., 2016, Ertmann and Gekara, 2019). The accumulation of these substances leads to oxidative stress, which inhibits bacterial growth and is considered a factor in growth inhibition. $p = \frac{R}{R+M+h}$ represents the fraction of substrates and metabolites (h is the constant value of zero escape). For simplicity, the division of the subpopulations here depends merely on the fraction to which the microorganisms are exposed. Microbial overcrowding $K(M)$, substrate depletion $-a_1RNp - b_1RN(1-p)$, and metabolite accumulation $a_2RNp + b_2RN(1-p)$ were considered as inhibitors of microbial growth, and these factors were incorporated into the model. The change in total cell density depends on growth rate based on the substrate consumption rate of the MG subpopulation, death rate d , and carrying capacity, depending on the metabolite concentration. The environment's carrying capacity $K(M)$ decreases from the carrying capacity of the optimal medium (medium when the carrying capacity is the maximum) with the increase in metabolites (Eq. 1–4). K_0 and K are the carrying capacities of the minimum and optimal media, respectively. f is the rate of decrease with metabolite accumulation. Substrates enter at a constant rate λ and are consumed at different rates by the MG subpopulation (a_1) and EI subpopulation (b_1) (Eq. 1–2). Different production rates were also

considered for the MG subpopulation (a_2) and EI subpopulation (b_2) for metabolite dynamics (Eq. 1–3). The EI subpopulation degraded at a constant rate (c). This model describes the situation of a chemostat when λ is nonzero and growth occurs by simply consuming the substrate in the medium when λ is zero.

Table 1. Parameter sets used in this study.

Parameter	Symbol	Value
Growth constant (cell/mL)	μ	1
Substrate consumption rate microbial growth subpopulation (1/(cell·min))	a_1	[0.00025, 0.004]
Metabolite production rate microbial growth subpopulation (1/(cell·min))	a_2	0.001
Substrate consumption rate environment improvement subpopulation (1/(cell·min))	b_1	[0.0005, 0.002]
Metabolite production rate environment improvement subpopulation (1/(cell·min))	b_2	0.001
Metabolite degradation rate (1/(cell·min))	c	0.01
Death rate (1/min)	d	0.01
Metabolite accumulation rate (1/mL)	f	[0.01, 100]
Constant value of zero escape	h	0.00001
Substrate influx rate (mL/min)	λ	[0, 1]
Optimum carrying capacity (cell)	K	[1.5, 3]
Minimum carrying capacity (cell)	K_0	1.5

3.2. Numerical analysis

3.2.1. Phase definition

Each phase of the microbial growth dynamics was defined to understand the dynamics. Defining the phase is required to determine the boundaries, and the feature values are calculated. The dynamics of total cell density, substrate amount, and metabolite amount were calculated by varying the substrate influx rate. For the calculation, the R package “deSolve” (Soetaert et al., 2010) was used, and the dynamics were visualized using “ggplot2” (Wickham, 2016). Other parameters used in the calculations are listed in Table 1. The following six dynamics-related features were calculated to detect the growth curve characteristics from the model: (i) maximum and (ii) minimum values of the change in the growth rate; (iii) maximum, (iv) zero, and (v) minimum values of the growth rate; and (vi) corresponding times. These six values were used to understand the detailed phase boundaries of the microbial growth dynamics.

3.2.2. Balancing the subpopulation

To investigate whether the division of the labor system affects the microbial growth potential, the change in the frequency of MG/DB strategies within the whole microorganism population was examined. Specifically, the relationship between the reaction ratio (RR ; the ratio of substrate consumption rate to metabolic production rate in each subpopulation (the ratio of MG subpopulations: $RR_{MG} = \frac{a_1}{a_2}$, the ratio of DB subpopulation: $RR_{EI} = \frac{b_1}{b_2}$) and growth strength (GS ; the ratio of RR of the microbial growth subpopulation to RR of the detoxification of byproducts subpopulation ($GS = \frac{RR_{MG}}{RR_{EI}}$) between the MG and EI subpopulations was numerically tested. The maximum total cell density ($\max(N)$) and length of the growth phase (time ($\max(\frac{dN}{dt})$)) were calculated when the GS and RR were varied. The length of the growth phase is the period until the growth rate reaches a maximum in the microbial dynamics of the model. The results were visualized as a heatmap using the R package "lattice" (Sarker, 2008), as shown in Fig. 4 The parameters used in this model are listed in table 1.

3.2.3. Resistance to the metabolite accumulation environment

In this study, the model was constructed by focusing on the dynamics of the decrease in environmental carrying capacity (Eq. 1–4). This modeling concept corresponds to the conditions under which microorganisms are sensitive to metabolites. Accordingly, two parameters were defined: the metabolite accumulation rate (f ; the rate of deterioration of carrying capacity due to metabolite accumulation) and the optimal carrying capacity (OC (K); the environment's carrying capacity on the optimal medium (when the carrying capacity is the maximum) of microorganisms), and the relationship between the metabolite accumulation rate and the OC was examined. The maximum total cell density ($\max(N)$) and length of the growth phase (time ($\max(\frac{dN}{dt})$)) were calculated when the metabolite accumulation rate (f) and OC were varied. The results were visualized as a heatmap using the R package “lattice” (Sarkar, 2008), as shown in Fig. 4. The parameters used in this model are listed in Table 1.

3.3. Data analysis

The products can serve as energy substrates for other microbes, but in the case of NO_2^- , we assumed that the adverse effect due to the toxicity of NO_2^- outweigh the energy benefits. To test the applicability of the model, experimentally measured data analysis was conducted. The aerobic denitrifying bacterium *P. balearica* RAD-17 was used, which possesses nitrate reductase and can be used for denitrification (Ruan et al., 2020). In this study, we used the time-series growth data of Ruan et al., 2020, which was extracted using WebPlotDigitizer v.4.6 (Ruan et al., 2020, Ankit, 2022). From the time-series data of cell density (OD600), substrate (NO_3^-), and metabolites (NO_2^-) shown in Ruan et al., 2020 (Ruan et al., 2020); the parameters were estimated using our model. The R package “deBInfer” (Boersch-Supan et al., 2017) was used for parameter estimation, and the dynamics were visualized using “ggplot2” (Wickham, 2016). Based on the estimated parameters, whether *P. balearica* RAD-17 focused on microbial growth or detoxification of byproducts in the division of the labor system was examined.

4. Results

4.1. Stability analysis

Initially, Eqs. (1) was examined under the influx condition (i.e., $\lambda > 0$). The total cell density (N), substrate concentration (R), and metabolite concentration (M) were constant. The equilibrium points were as follows (Section 6.1):

$$N^* = \frac{a_1 \lambda K}{a_1 \lambda + dK \left(a_1 + b_1 \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right)} \quad (2-1)$$

$$R^* = \frac{\left(1 + \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right) \left\{ a_1 \lambda + dK \left(a_1 + b_1 \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right) \right\}}{a_1 K \left(a_1 + b_1 \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right)} \quad (2-2)$$

$$M^* = \frac{\left(\frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right) \left(1 + \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right) \left\{ a_1 \lambda + dK \left(a_1 + b_1 \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right) \right\}}{a_1 K \left(a_1 + b_1 \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right)} \quad (2-3)$$

When there was an influx of the substrate, the cell density of the microorganism did not reach zero.

The substrate and metabolite concentrations did not reach zero but continued to take on a constant

value. Further, we considered the medium under the no-influx condition (i.e., $\lambda = 0$). The solution could be obtained by substituting $\lambda = 0$ for the equilibrium points up to Eqs. (2-1) - (2-3) as follows:

$$N^* = 0 \quad (3-1)$$

$$R^* = \frac{d}{a_1} \left(1 + \frac{b_2 + \sqrt{b_2^2 + 4a_2c}}{2c} \right) \quad (3-2)$$

$$M^* = \frac{d}{a_1} \left(1 + \frac{b_2 + \sqrt{b_2^2 + 4a_2c}}{2c} \right) \left(\frac{b_2 + \sqrt{b_2^2 + 4a_2c}}{2c} \right) \quad (3-3)$$

When there was no influx of the substrate, the cell density of the microorganism becomes zero. The substrate and metabolite concentrations did not reach zero but continued to take on a constant value.

Furthermore, the local stability around the equilibrium was evaluated using the eigenvalues of the Jacobian. Assuming a constant carrying capacity and $h = 0$, three eigenvalues Λ_1 (real solution), Λ_2 , and Λ_3 (complex solution) were obtained. The cubic equation for Λ is given as follows:

$$y_1\Lambda^3 + y_2\Lambda^2 + y_3\Lambda + y_4 = 0 \quad (4-1)$$

$$y_1 = -K(R + M)^3 < 0 \quad (4-2)$$

$$y_2 = -(R + M)\psi < 0 \quad (4-3)$$

$$y_4 = -\frac{y_{41}y_{42}y_{43}y_{44}}{16Ka_1c^3(2a_1c + b_1b_2 + b_1(4a_2c + b_2^2)^{0.5})^4} < 0 \quad (4-4)$$

where $\psi > 0$ and $y_{41}, y_{42}, y_{43}, y_{44} > 0$ hold (see Section 6.2 for details). Relation between roots and coefficients of cubic equation is $\Lambda_1\Lambda_2\Lambda_3 = -\frac{y_4}{y_1} < 0$ (Eqs. 4-2, 4-4). Since Λ_2 and Λ_3 are

complex solutions, they have a common real part, so $\Lambda_2\Lambda_3 > 0$. Thus, $\Lambda_1 < 0$.

For Equ. (4-1), let us assume that there are z positive real roots. According to Descartes's Rule of Signs, if $y_3 < 0$, there are no positive real roots, which means there are three negative real roots. On the other hand, if $y_3 > 0$, there can be either two or zero positive real roots, which implies there are either one (Λ_1) or three negative real roots. In summary, for the complex conjugate roots $\Lambda_2, \Lambda_3 = v \pm wi$, substituting the parameters used in Figure 2 into the conjugate complex solution yields $v < 0$. Hence, the equilibrium points under the parameters we used is stable spiral.

Furthermore, for Eqs. (1), the non-dimensionalized model is represented by Eqs. (9) in Section 6.4.1. Through the non-dimensionalization of parameters, the number of parameters in the model can be reduced as five. The equilibrium points of this model, similar to the original one, is determined as a single point, and when there is no inflow of substrate, the cell fraction can reach zero. Local stability analysis yields result similar to the original model, where the stable states vary depending on the permissible parameters (details in Section 6.4.2-6.4.3).

4.2. Defining microbial growth phase

Based on the results of the numerical solution, the microbial growth dynamics could be defined in six phases (Fig. 2). In particular, the growth phase was defined as the time from $\min(\frac{d^2N}{dt^2})$ to the point at which the bacterial growth rate was slowest, the stationary phase was defined as the time from $\min(\frac{dN}{dt})$ to the point at which the bacterial growth rate was the lowest, and the death phase was defined as the time after the stationary phase. The growth phase was defined in three stages. The early growth phase could be defined as the time from the start of culture to $\max(\frac{d^2N}{dt^2})$. Similarly, the middle growth phase corresponded to the time from $\max(\frac{d^2N}{dt^2})$ to $\max(\frac{dN}{dt})$, and the late growth phase corresponded to the time from $\max(\frac{dN}{dt})$ to $\min(\frac{d^2N}{dt^2})$. Further, the stationary phase could be divided into two phases. A positive stationary phase could be defined as the time from $\min(\frac{d^2N}{dt^2})$ to $\frac{dN}{dt} = 0$, and a negative stationary phase could also be defined as the time from $\frac{dN}{dt} = 0$ to $\min(\frac{dN}{dt})$. Finally, the death phase was represented as the time after $\min(\frac{dN}{dt})$.

Without substrate influx, the amount of substrate monotonically decreased and the amount of metabolites increased as the microorganisms grew (Fig. 3 upper right). When microbial growth became stagnant (around $t = 1000$ in Fig. 3), the decrease in substrate amount stopped. In contrast, the amount of metabolites increased with the population growth of microorganisms at the beginning but decreased after the negative stationary phase and reached equilibrium after $t = 600$.

When substrate influx occurred, metabolite dynamics could be divided into two patterns according to substrate consumption kinetics (Fig. 4). When there was little substrate influx, the substrate concentration decreased and then stopped decreasing. The cell density reached equilibrium

at a certain value. As in the case of no substrate, metabolites increased as the number of microorganisms increased in the beginning; however, after the death phase, the amount of metabolites decreased and stopped after $t = 600$ (Fig. 4(A)). In contrast, the substrate concentration increased and then plateaued when the substrate influx was high (Fig. 4(B)). The cell density reached equilibrium at a higher value than at a low substrate influx. Initially, the amount of metabolites increased with an increase in the amount of microorganisms but then plateaued.

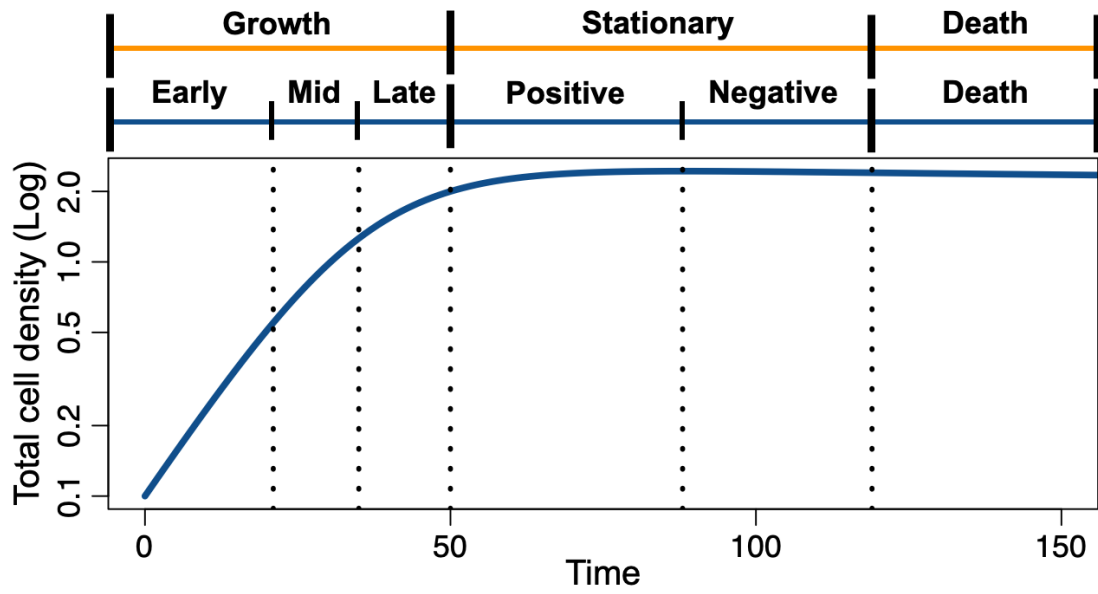


Fig. 2. Schematic illustration of phase definition derived by the current model.

The six phases were distinguished by the model constructed in this study, and the name of each phase is shown. Bacteria were divided into several stages according to the first or second derivative of the total cell density at the incubation time of the maximum or minimum (see Section 3.2 of the main text for the details of our definitions).

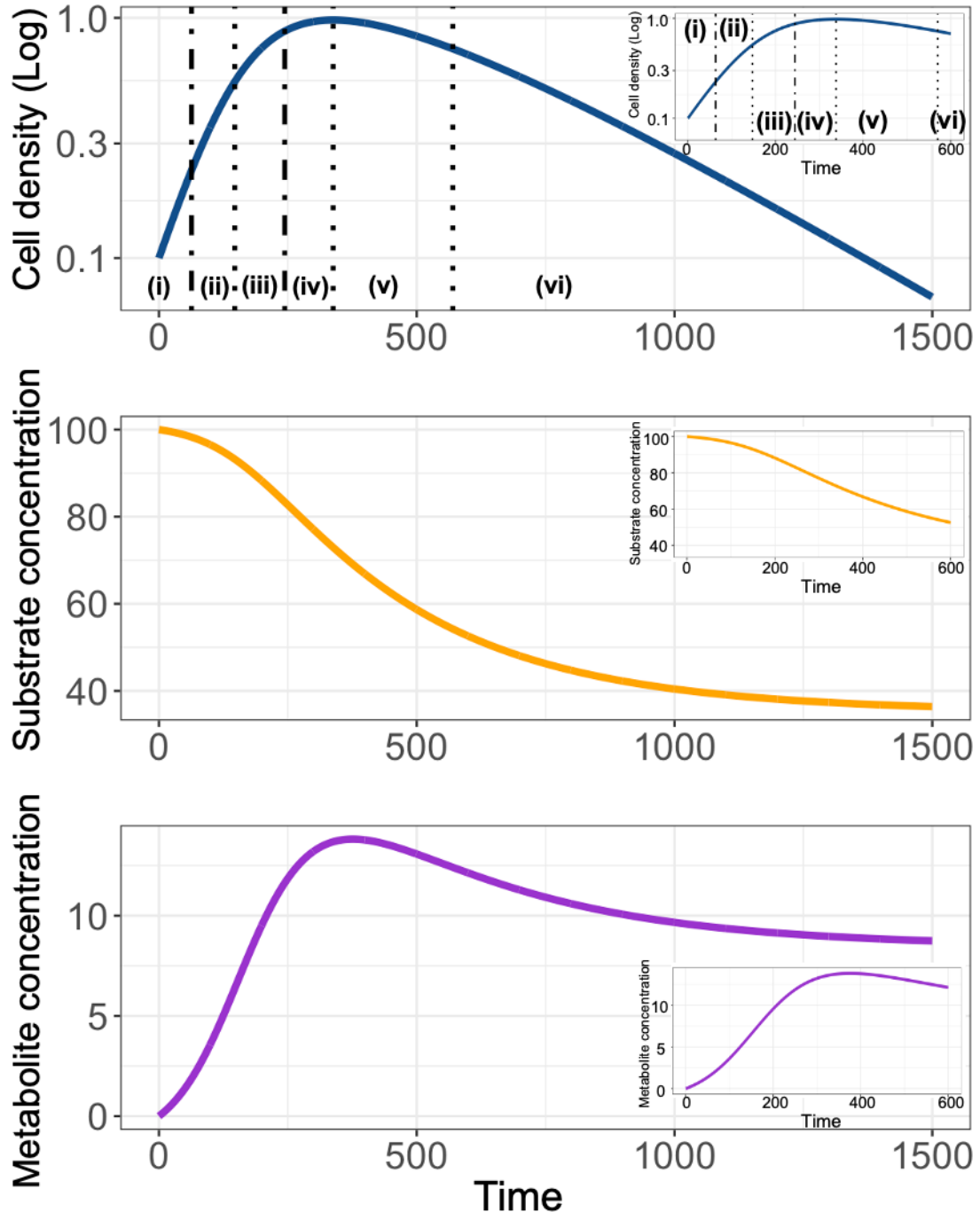


Fig. 3. Numerical solution for no-influx substrate.

Microbial growth dynamics (blue), substrate dynamics (orange), and metabolite dynamics (purple)

are shown in the parameter set ($N_0 = 0.1, R_0 = 100, M_0 = 0, \mu = 1, a_1 = 0.00025, a_2 =$

$0.001, b_1 = 0.0015, b_2 = 0.001, c = 0.01, d = 0.01, f = 0.01, h = 0.00001, \lambda = 0, K_0 = 1.5, K =$
 3). The small window in the upper right of each dynamic shows a detail view of growth phase (from time 0 to 600). The dot-dashed line indicates the time point of the maximum or minimum of the first derivative of the total cell density in the microbial growth dynamics, and the dotted line indicates the time point of the maximum, minimum, or zero of the second derivative of the total cell density. In the absence of substrate transfer, microorganisms grow rapidly at the beginning of the culture, but the growth rate decreases with microbial overcrowding and rapid accumulation of metabolites, and, eventually, they die slowly. After the death phase, the amount of substrate consumed by the microorganisms decreases, and the metabolites degrade, eventually reaching a plateau.

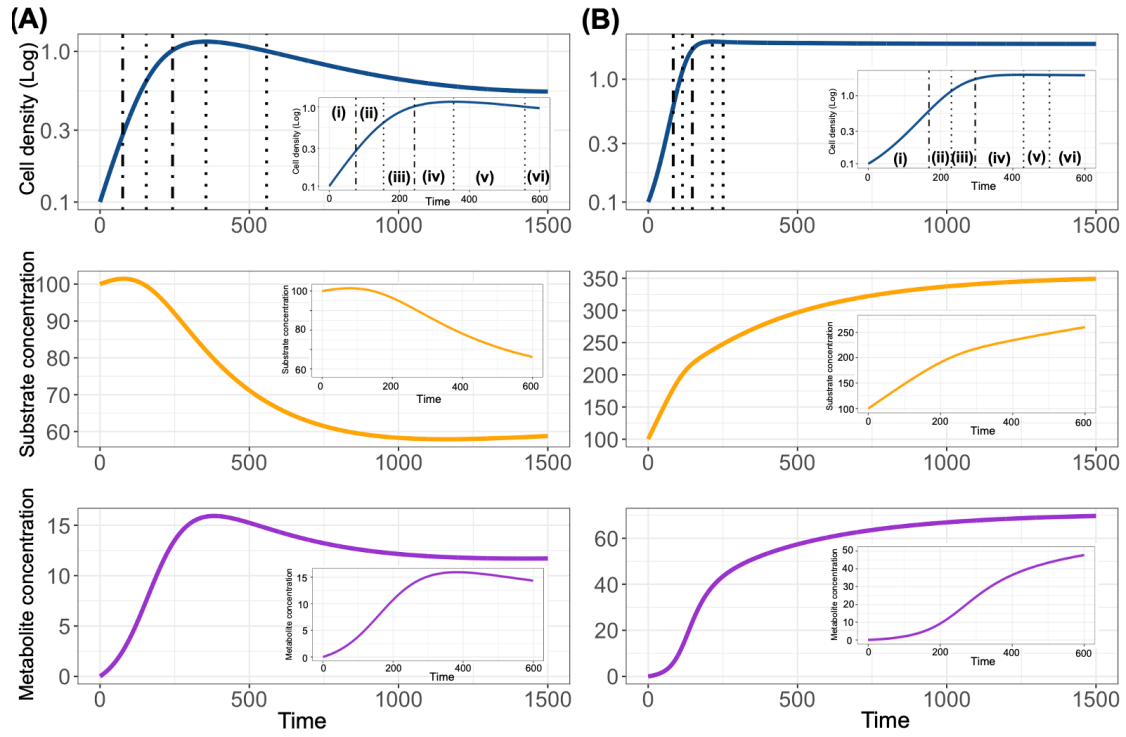


Fig. 4. Numerical solution for the influx substrate.

Microbial growth dynamics (blue), substrate dynamics (orange), and metabolite dynamics (purple)

are shown in the parameter sets ($N_0 = 0.1, R_0 = 100, M_0 = 0, \mu = 1, a_1 = 0.00025, a_2 =$

$0.001, b_1 = 0.0015, b_2 = 0.001, c = 0.01, d = 0.01, f = 0.01, h = 0.00001, K_0 = 1.5, K = 3$).

The small window in the upper right of each dynamic shows the initial state of the culture. The dot-dashed line indicates the time point of the maximum or minimum of the first derivative of the total cell density in the microbial growth dynamics, and the dotted line indicates the time point of the maximum, minimum, or zero of the second derivative of the total cell density.

(A) Numerical solution with $\lambda = 0.05$. Because the substrate influx is low, the population dynamics of the cell density is similar to the dynamics shown in Fig. 3. Because there was a small substrate influx, the substrate concentration started to increase. As the number of cells increases, the substrate is rapidly consumed in large quantities. Metabolites were degraded after the stationary phase, as

shown in Fig. 3.

(B) Numerical solution for $\lambda = 1$. Because the substrate influx is high, the cell density saturates at a constant high value, unlike Fig. 3. Because the substrate influx is more remarkable than that in (A), the amount of substrate continues to increase because the influx of more substrate than the microorganism consumes is constantly occurring. Subsequently, as the microorganisms saturate, the substrate takes a constant value (substrate influx and consumption are balanced).

4.3. Parameter dependence

4.3.1. Single parameter dependence

For the parameters in Equation (1), the changes in maximum cell density and proliferation period for each parameter are shown in Fig. 7 and Section 6.3. The substrate consumption rate (a_1) and death rate (d) directly involved in growth decreased the length of the growth period with an increase in maximum cell density. The increase in metabolic production rates (a_2 and b_2) led to an increase in maximum cell density and a shorter growth period. Similarly, the change in substrate consumption rate (b_1) for the DB subpopulation followed a similar condition as the metabolic production rates. The changes in maximum cell density and growth period associated with the variation in the decomposition rate (c) were different from the other parameters. As the decomposition rate increased, the maximum cell density increased, and the growth period followed an S-shaped curve (details in Section 6.3).

The non-dimensionalized form of Eqs. (1) is presented as Eqs. (9) in Section 6.4.1. The changes in the maximum cell fraction and proliferation period for each of these parameters are illustrated in Fig. 9 and Section 6.4.5. The parameters α , β , and γ exhibited similar variations. With an increase in the maximum cell fraction, the growth period decreased. The parameter η showed smaller changes in the maximum cell fraction ($\frac{R}{R+M}$) compared to the other parameters, and there was no significant impact on the growth period. Regarding the parameter ξ , an increase in the maximum cell fraction led to a longer growth period (details in Section 6.4.5).

4.3.2. Balance between the microbial growth and detoxification of byproducts

The relationship between the GS and RR_{MG} was shown in Fig. 5A. As the GS and RR_{MG} values increased, the maximum cell density increased, and the growth phase shortened.

4.3.3. Resistance to environmental metabolite accumulation

The relationship between the f and OC was shown in Fig. 5B. When f was small ($\text{Log}_{10}f < 0$), the effect of metabolite accumulation on the environmental carrying capacity $K(M)$ was very small. Thus, for each condition in which OC was increased, the maximum cell density increased, increasing the growth phase. When f was at the intermediate level ($\text{Log}_{10}f \simeq 0$), the carrying capacity $K(M)$ was moderately affected by metabolite accumulation. The maximum cell density remained almost unchanged around $K_0 = 1.5$. Therefore, as OC increased, $K(M)$ increased and microorganisms grew faster, but the growth phase was shortened because metabolite accumulation prevented the growth only up to $K_0 = 1.5$. When f was large ($\text{Log}_{10}f > 0$), even if a small amount of metabolite M was released, the term $(K - K_0)e^{-fM}$ in Equ. (1-4) becomes almost zero and the parameter K had little effect on $K(M)$. As a result, the environmental carrying capacity $K(M)$ was always almost K_0 , and the growth phase was constantly very short.

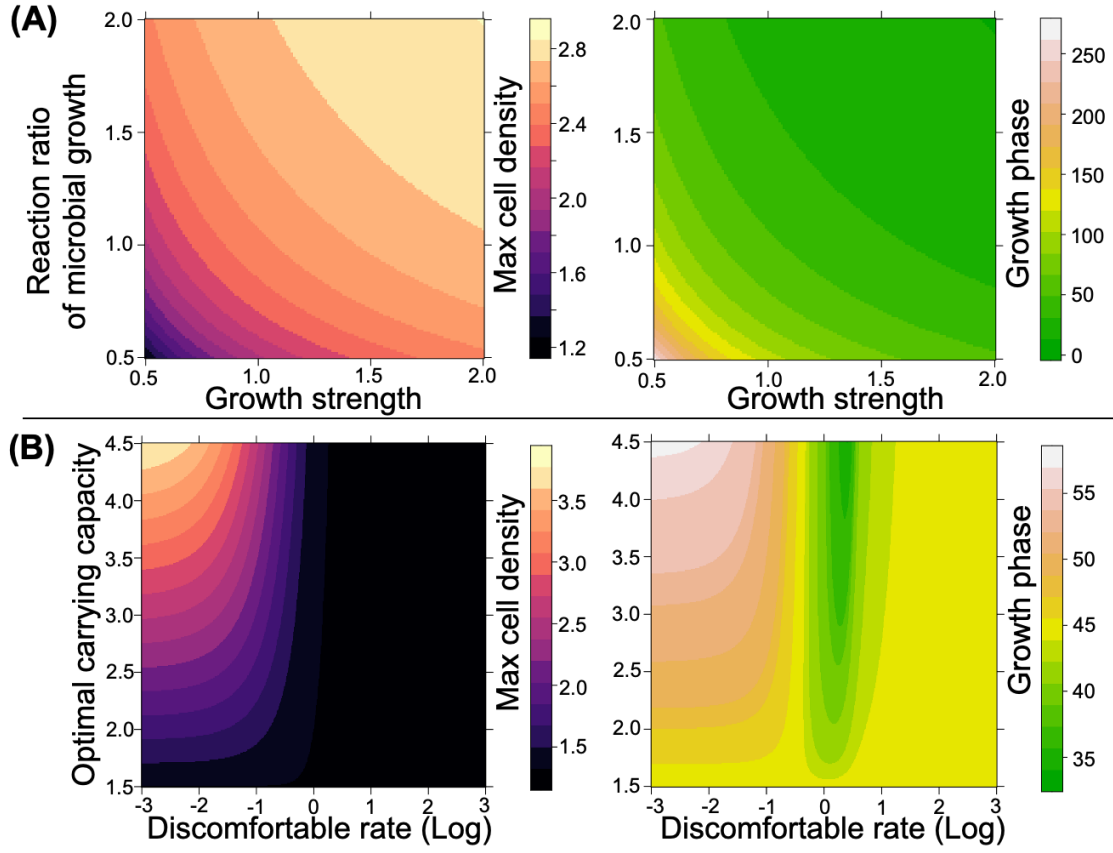


Fig. 5. Parameter dependence of microbial dynamics.

(A) The balance between microbial growth and detoxification of byproducts. The reaction ratio (RR) is the ratio of substrate consumption rate to metabolic production rate in each subpopulation (the ratio of MG subpopulations: $RR_{MG} = \frac{a_1}{a_2}$, the ratio of DB subpopulation: $RR_{DB} = \frac{b_1}{b_2}$). This reaction ratio is focused on the RR_{MG} . The growth strength (GS) is the ratio of RR of the microbial growth subpopulation to RR of the detoxification of byproducts subpopulation ($GS = \frac{RR_{MG}}{RR_{DB}}$). The parameter set is $N_0 = 0.1, R_0 = 100, M_0 = 0, \mu = 1, a_1 = [0.00025, 0.004], a_2 = 0.001, b_1 = [0.00050, 0.002], b_2 = 0.001, c = 0.01, d = 0.01, f = 0.01, h = 0.00001, \lambda = 0, K_0 = 1.5, K = 3$. The figure on the left shows the maximum cell density of the microorganism, and the figure on the

right shows the length of the growth phase of the microorganism (start of culture–late growth phase).

With an increase in RR_{MG} and GS values, the maximum cell density increases, and the growth phase shortens.

(B) Resistance to environmental metabolite accumulation. The metabolite accumulation rate (f) is the rate of change of the carrying capacity with a metabolite (parameter of f in Equ. (1-4)). The optimal carrying capacity (OC) is the carrying capacity of the environment on the optimal medium (parameter of K in Equ. (1-4)). The parameter set is $N_0 = 0.1, R_0 = 100, M_0 = 0, \mu = 1, a_1 = 0.001, a_2 = 0.001, b_1 = 0.001, b_2 = 0.001, c = 0.01, d = 0.01, f = [0.01, 100], h = 0.00001, \lambda = 0, K_0 = 1.5, K = [1.5, 3]$. The figure on the left shows the maximum cell density of the microorganism, and the figure on the right shows the length of the growth phase of the microorganism (start of the culture–late growth phase).

When f is low ($\text{Log}_{10}f < 0$), the maximum cell density increases and the growth phase lengthens with increasing OC . When the f is medium ($\text{Log}_{10}f = 0$), the maximum cell density does not change with increasing OC , however the growth phase shortens. When the f is medium ($\text{Log}_{10}f > 0$), the maximum cell density and the growth phase do not change with increasing OC .

4.4. Experimentally measured data analysis

The estimated parameters were used to reconstruct the dynamics of cell density, the substrate, and the metabolites (Fig. 6). Because there was no influx of NO_3^- as a substrate, $\lambda = 0$ was set when estimating the parameters. $h = 0.0001$ was used to avoid zero in the denominator of the population fraction (p). The growth phase of *P. balearica* was 15 h, the early growth phase was 9 h, the middle growth phase was 2 h, and the late growth phase was 4 h. The stationary phase was 25 h, the positive stationary phase was 1 h, and the negative stationary phase was 24 h. The death phase occurred 40 h after the start of incubation.

From the time-series data, it was observed that *P. balearica* almost completely degraded its metabolite, nitrite ions (NO_2^-), approximately 20 h after the start of incubation. It was also found that NO_2^- decomposition occurred in the negative stationary phase. Furthermore, after the substrate NO_3^- was depleted, *P. balearica* abundance slowly decreased.

The RR ($RR_{MG} = \frac{a_1}{a_2}$) of the MG subpopulation and RR ($RR_{DB} = \frac{b_1}{b_2}$) of the detoxification of byproducts (DB) subpopulation were 0.34 and 1.84, respectively. The GS ($\frac{RR_{MG}}{RR_{DB}}$) was 0.18.

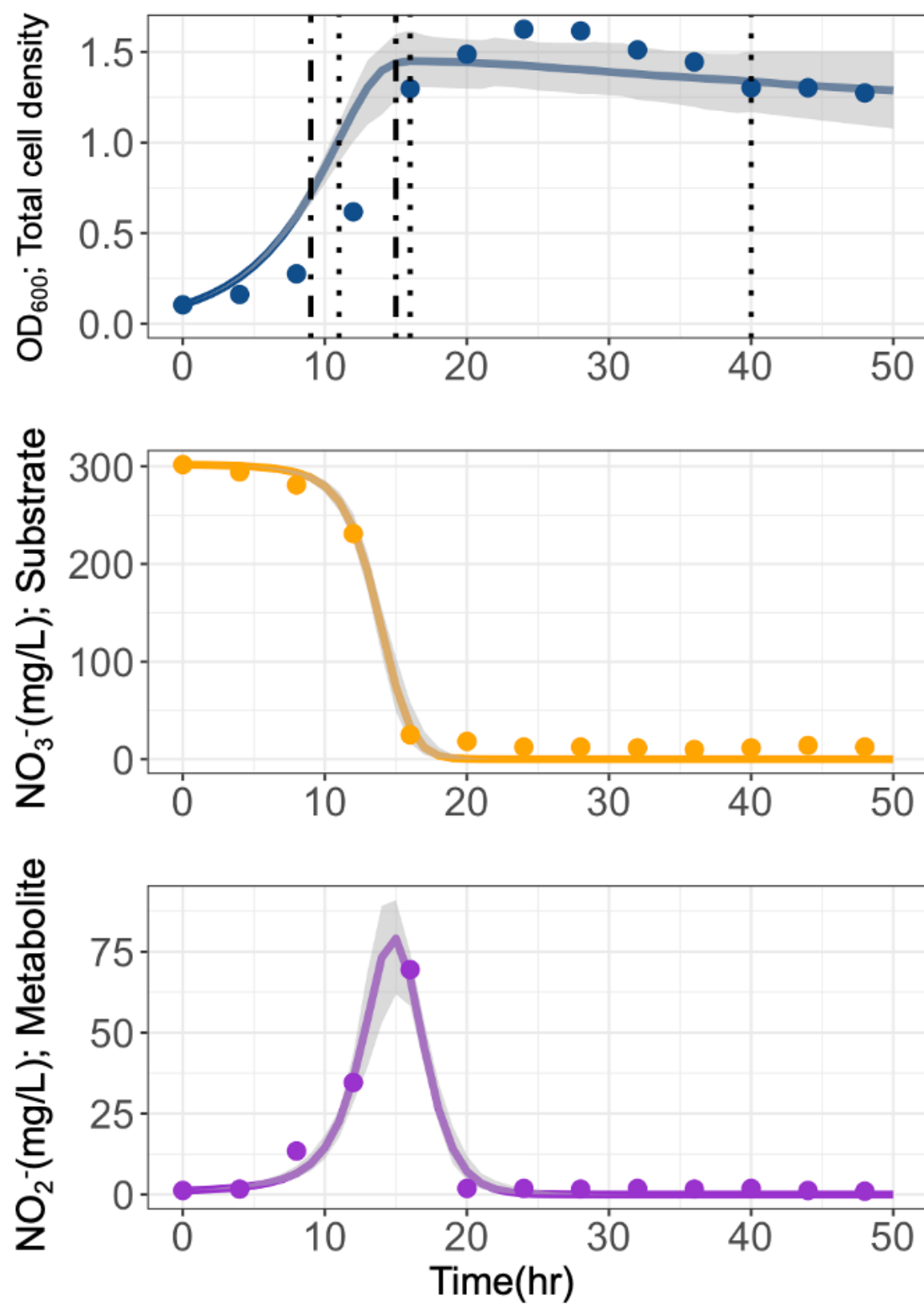


Fig. 6. Reconstructing the dynamics for the estimated parameters.

The scatter plot represents the experimentally measured data and the line plot represents the dynamics of the current model based on the estimated parameters. The gray ribbon plot indicates 95% confidence intervals. The top is the growth dynamics of *Pseudomonas balearica* RAD-17 (OD600), the middle is the limiting substrate (nitrate) dynamics (mg/L), and the bottom is the metabolite (nitrite) dynamics (mg/L). The dot-dashed line indicates the time point of the maximum or minimum of the first derivative of the total cell density in the microbial growth dynamics, and the dotted line indicates the time point of the maximum, minimum, or zero of the second derivative of the total cell density. The estimated dynamics show that the growth phase is 15 h (early 9 h; middle 2 h; late 4 h), the stationary phase is 25 h (positive 1 h; negative 24 h), and the death phase is 40 h from the start of incubation.

5. Discussion

5.1. Motivation of the modeling

In this study, our focus was on the altruistic division of the functions system of microorganisms to model growth dynamics. We assumed that microorganisms allocate the energy obtained for asexual reproduction and part of the decomposition of the metabolites produced. The results provided an understanding of the details of the growth and stationary phases, which could not be explicitly modeled previously. It was also assumed that microorganisms adopt two strategies: one that rapidly consumes substrates and grows and another that slowly grows while cleaning up within the growth environment. According to the parameter sensitivity analysis, there were two types of microorganisms with respect to the sensitivity to environmental change: those with some tolerance to metabolite accumulation and those with sensitivity. Through data analysis, the model was able to estimate not only cell density but also the dynamics of substrates and metabolites.

5.2. The summary of the analysis

5.2.1. Stability analysis

The results of the local stability analysis showed that the microorganisms converged to an equilibrium point in this model. In the case of substrate influx, the microorganisms converged to a non-zero value (Equ. 2-1). The parameters included the substrate influx (λ), the degradation rate of metabolites (c), substrate consumption rate (b_1), and metabolite production rate (b_2) of the DB subpopulation. This suggested that the DB subpopulation may play a significant role in determining the cell density of microorganisms. In Eqs (2-2) and (2-3), the equilibrium points of the substrates and metabolites and the microorganisms' environmental carrying capacity ($K(M)$) were included. This suggested that the substrate and metabolites in the medium contribute to the size of the microbial community that mass acts with them.

In the case of substrate no-influx, the cell density of the microorganisms was eventually zero (Equ. 3-1). It has been suggested that microorganisms die when their limiting nutrient source (substrate) is depleted. In Eqs (3-2) and (3-3), the equilibrium points of the substrates and metabolites, the environmental carrying capacity ($K(M)$) was not included, and the parameter $\frac{d}{a_1}$ remained. Under conditions without substrate supplied, the microbial community did not contribute to the substrate or metabolites in the medium. The MG subpopulation was determined by the ratio of the microbial death rate to the substrate consumption rate. This suggested that substrate and metabolite concentrations were determined according to microbial characteristics, such as the ratio of the specific growth rate to the death rate of microorganisms that mass-act with substrates and metabolites. When $\frac{d}{a_1}$ was high (i.e., when the death rate was high), the substrate used was not optimal for the microorganism. Therefore, the microorganisms would not utilize the substrate as

much, and R^* was expected to be high in the final state. However, when $\frac{d}{a_1}$ was low (i.e., when the death rate was low), the substrate is valuable because it is relatively more necessary or significant for their growth or metabolism. Therefore, microorganisms were expected to use more substrate, and R^* would be low in the final state.

5.2.2. Defining the microbial growth phase

The dynamics of the system could be analytically divided into six phases (Fig. 2). During the early growth phase, the first derivative of the total cell density increased monotonically. The second derivative of total cell density also increased monotonically, so the microorganisms adapted to the environment and gradually increased their growth potential by consuming substrates after metabolic preparation. The middle growth phase was the interval until the first derivative of the total cell density reached its maximum value. In the late growth phase, the first derivative of the total cell density decreased monotonically, and the second derivative of the total cell density decreased monotonically to a minimum. The microorganism numbers grew slowly. In the positive stationary phase, the first derivative of the total cell density approached zero but is positive, which was the interval where the growth rate $\left(a_1 RNp \left(1 - \frac{N}{K(M)}\right)\right) \geq$ the death rate (dN) . In the negative stationary phase, the growth rate was negative from zero and was the interval until the first derivative of the total cell density reached a minimum. In the death phase, the first derivative of total cell density is always negative. The number of microorganisms continued to decrease monotonically as microorganisms died slowly. This six-phase classification is more detailed than existing microbial growth models (Huang, 2013, Vanimpe et al., 2005).

Without substrate influx, the microorganisms multiplied at the beginning of the culture, but the growth rate decreased with the overcrowding of the microorganisms and rapid accumulation of metabolites, and, finally, they died slowly (Fig. 3). Because there was a sufficient amount of substrate in the parameter set used in this numerical simulation, the factors involved in microbial growth were considered to be microbial density and metabolite accumulation. After the death phase, microorganisms lose their growth potential by accumulating metabolites. We found that

microorganisms did not necessarily utilize all substrates in sufficient amounts. Furthermore, microorganisms produced metabolites rather than degraded them during the growth phase. After the death phase, the consumption of substrates and degradation of metabolites slow down and eventually reach a plateau.

We calculated the dynamics, assuming that the growth rate depends on substrate consumption, metabolite accumulation, and cell overcrowding. After the death phase, the number of microorganisms monotonically decreased. However, it was not possible to address in detail the initial growth stage of microorganisms used in the culture media and the metabolic state of the microorganisms after the death phase from the present model. The metabolism that takes place for the growth of the microorganisms requires preparation, and once the microorganism loses its proliferative potential, preparation needs to occur before it can grow again. The preparation time may correspond to the time required to express metabolic enzymes, which corresponds to the so-called lag phase (Buchanan and Cygnarowicz, 1990, Prats et al., 2008). Understanding the mechanisms that allow microorganisms to quickly acclimate to their environment will allow us to culture them under optimal conditions (Rolfe et al., 2012).

With substrate influx, the higher the amount of substrate transferred, the higher the maximum cell density of the microorganisms (Fig. 4). This was because the growth phase is characterized by rapid growth, owing to the constant supply of sufficient substrate. In addition, when the substrate influx was small, metabolites were degraded after the death phase, similar to the condition of no substrate influx. It has been suggested that microorganisms first increase cell density by rapidly consuming substrates and then optimizing the environment.

5.3. The summary of the results

5.3.1. Single parameter dependence

Parameters a_1 and d directly influence the net growth of microorganism. Therefore, an increase in net growth results in rapid attainment of high maximum cell density. Metabolic production rates a_2 and b_2 have a direct impact on the amount of metabolites produced, leading to a negative influence on the fraction of net growth. The substrate consumption rate b_1 for the DB subpopulation reduces the relative substrate utilization of the MG subpopulation. The decomposition rate c directly affects the amount of metabolites, thus exerting a positive influence on the fraction $(\frac{R}{R+M})$ of net growth (details in Section 6.3).

Furthermore, the analysis using non-dimensionalized parameters represents the variations in each parameter with respect to a_1 . Parameters α , β , and γ indicate the changes in b_1 , a_2 , and b_2 relative to a_1 . As these parameters increase, the relative decrease in a_1 negatively affects net growth. Parameter η represents the changes in the decomposition rate c with respect to a_1 . An increase in η results in a relative decrease in the fraction of metabolites. Additionally, an increase in ξ positively influences net growth, but its impact is relatively smaller compared to other parameters (details in Section 6.4.5).

5.3.2. Balance between the microbial growth and detoxification of byproducts

The RR was the ratio of the substrate consumption rate to the metabolite production rate in each subpopulation. The higher the RR , the more substrates were consumed, and metabolites were acquired. The GS was the RR ratio of the DB subpopulation to the MG subpopulation. A high GS implied that the metabolic reaction is more active in the MG subpopulation than in the DB subpopulation. An active metabolic response in the MG subpopulation may indicate a high microbial growth potential.

The higher the RR (reaction ratio), the faster the substrate consumption rate. Therefore, the maximum cell density and the length of the proliferation period until reaching the final state vary. Consequently, regarding $p \left(= \frac{R}{R+M+h} \right)$, when the RR is high, indicating a high substrate consumption rate, there is a temporary possibility of obtaining a high value for p . Ultimately, p can reach a certain value as it converges to the equilibrium points shown in Eqs. (2) and (3).

5.3.3. Resistance to environmental metabolite accumulation

As shown in Fig. 5B, there were three types of microbial growth depending on their ability to detect metabolite accumulation. When f was small ($\text{Log}_{10}f < 0$), the growth of microorganisms was not disturbed by quantitative changes in the metabolites and could grow until the optimal carrying capacity, as long as other conditions of their habitat allow. When f was about 1 ($\text{Log}_{10}f \simeq 0$), they were moderately sensitive to quantitative changes in metabolites and could grow only to a certain density even when the optimal carrying capacity in the absence of metabolites increased. When f was large ($\text{Log}_{10}f > 0$), they were very sensitive to the metabolites in the environment, and it switched growth dynamics when they detected metabolites even a little amount. In such conditions, OC no longer had large effect on the growth dynamics.

5.3.4. Data analysis

From the analysis based on our model, the periods of the growth, stationary, and death phases, which were previously ambiguous, were determined precisely. Since the negative stationary phase was found to be 16–40 h from the start of cultivation, it was possible to estimate when *P. balearica* had the highest metabolic performance from the start of culture. Thus, it was possible to estimate the state of *P. balearica* for efficient nitrate reduction.

According to the results of experimentally measured data analysis, the analysis of GS showed that the RR of *P. balearica* was higher in the DB subpopulation than in the MG subpopulation under the present conditions of the nitric acid medium. Because the GS is low, the energy utilized for cell division and metabolite degradation may differ among *P. balearica*. However, the metabolite here refers to NO_2^- , and no specific form of energy is assumed. The results of the parameter test suggested that *P. balearica* grows slowly during clean-up within the growth environment.

5.4. Biological implications of the results

As the RR and GS values of the microorganisms increased, the maximum cell density increased, and the growth phase shortened. As a_1 (the growth rate of the microorganisms) increased, RR_{MG} and GS increased, but the carrying capacity of the environment did not change. Thus, the higher the RR and GS values, the more microorganisms focused on MG rather than DB in the division system. The more substrates consumed, the more rapidly microorganisms could grow. The rapid multiplication of microorganisms may transiently increase their viability in the habitat.

In contrast, the lower the RR and GS values, the more the microorganisms focused on DB rather than MG in the division of the labor system, and the slower they grew. The slow growth of microorganisms may enhance their long-term survival in these habitats.

The growth curve of a microorganism was determined by a variety of factors. One of these factors was the negative effect of metabolite accumulation on microbial growth; autointoxication (Gauthier and Flatau, 1976). An example of a mathematical analysis that introduces the concept of autointoxication was the degradation of dioxin (Hojo et al., 2006). However, it has not been possible to quantitatively compare the effects of autotoxicity. Therefore, we investigated the impact of autotoxicity by examining f (metabolite accumulation rate) and OC (Optimal carrying capacity) in this model.

The maximum cell density of a microorganism and the length of its growth phase depended on the values of f and Optimal carrying capacity (OC ; K). Although both f and OC determined the level of environmental carrying capacity ($K(M)$), the two parameters represent different aspects of the carrying capacity. The parameter f corresponds to the sensitivity of microorganisms to the quantitative changes in metabolites, while OC represents the environmental

aspect other than the effect of metabolites. *OC* reflects the conditions which allow microorganisms to grow, such as nutrition and space in their habitat (Huete-Stauffer et al., 2015, Indekeu and Sznajd-Weron, 2003).

P. balearica was an aerobic denitrifying bacterium that utilizes NO_3^- and NO_2^- as electron acceptors (Ruan et al., 2020, Bridou et al., 2018, Semenova et al., 2020). The nitrate medium only had NO_3^- as an electron acceptor, which corresponded to the substrate of this model. Furthermore, NO_2^- produced in the nitrate reduction reaction corresponded to the metabolite of this model. Because *P. balearica* was capable of denitrification, nitrate reduction ($\text{NO}_3^- \rightarrow \text{NO}$), nitric oxide reduction ($\text{NO} \rightarrow \text{N}_2\text{O}$), and nitrous oxide reduction ($\text{N}_2\text{O} \rightarrow \text{N}_2$) occurred in multiple steps after the nitrate reduction reaction. Therefore, *P. balearica* grew on a nitrate medium eventually converted its toxic NO precursor, the metabolite NO_2^- to N_2 , which is harmless to bacteria (McNulty et al., 2023).

It has been suggested that *P. balearica* consumes a substrate (NO_3^-) for growth and degrades metabolites (NO_2^-). The growth of *P. balearica* slowed as the subpopulation of metabolite degradation increased. When the substrate was depleted, the growth rate changed from stagnant to negative (after 16 h), and *P. balearica* died. The rapid degradation of the metabolite NO_2^- suggested that *P. balearica* undergoes cell division and metabolite degradation during growth.

5.5. Limitation of the current analysis and future works

In this study, we developed a microbial growth model based on the assumption that microorganisms divide their metabolic energy into two subpopulations: an MG subpopulation (cell division) and an DB subpopulation (degradation of metabolites). With the development of single-cell analysis technology, it has become clear that there is heterogeneity in the expression state of microorganisms, even within the same medium and culture time (Kuchina et al., 2021, Imdahl et al., 2020, McNulty et al., 2023, Homberger et al., 2023). The heterogeneity of the expression profiles in the microbial population suggests that they do not grow uniformly in the environment (Ackermann, 2015).

Therefore, it is suggested that microorganisms do not use the energy obtained from metabolism only for specific functions but can be divided into functional clusters (Han et al., 2020). Furthermore, it is crucial for bacterial survival tactics such as bet-hedging, which allows for rapid adaptation to changing environments, or biofilm formation, where individual cells have distinct roles in a community, to be considered (Grimbergen et al., 2015, Morawska et al., 2022, Rumbaugh and Sauer, 2020). The model we developed enables us to understand the growth dynamics of microorganisms in detail by considering their overcrowding, degree of substrate consumption, and degree of metabolite accumulation.

Previous models, which estimate the dynamics by assuming that all the acquired energy is allocated to growth, only represent the average growth rate of the microorganisms. In the present study, we examined growth dynamics at the functional subpopulation level, focusing on growth and function (Monod, 1949, Huang, 2013, López et al., 2004). Therefore, it is possible to express the growth of bacterial species divided into multiple functional subpopulations based on the assumption that all energy is used for growth. Single-cell microbial analysis can clarify the functions of each cell (Imdahl et al., 2020). It will then be possible to divide the bacterial species into functional

subpopulations and measure cell density. In the future, microbial analysis at the single-cell level will provide detailed information on the heterogeneity of roles, even in a single microorganism population, and how they grow. Our model of microbial growth based on the altruistic division of the functions will help improve the understanding of the functionality of each individual microorganism that changes depending on the culture conditions.

6. General Discussion

This study implicates both theoretical and practical significance by proposing a paradigm shift in how we model microbial population growth. Historically, fundamental models such as the logistic growth model (Verhulst, 1838; 1845) and the Monod's equation (Monod, 1949) have been widely employed. These models successfully capture growth constraints through simple factors such as environmental carrying capacity and substrate concentration, treating the microbial population as a homogeneous entity exhibiting average behavior. However, microbial habitats are far more complex. They are influenced not only by fluctuating resources, waste product accumulation, and various stress factors, but also by intricate cell-to-cell interactions, the exchange of genetic material, and phenotypic plasticity that results in dynamically emerging and disappearing heterogeneous subpopulations (Ackermann, 2015; Frost et al., 2005; Lewis, 2010; Kuchina et al., 2021).

In this study, I explicitly incorporated such complexities into a new modeling framework, thereby establishing an approach capable of representing both the diversity within bacterial populations and their dynamic division of labor—factors that have been difficult to fully capture with conventional models. More specifically, I incorporated the formation of subpopulations—heterogeneous groups arising from phenotypic plasticity and gene expression within genetically identical cells—into the model and postulated specialized subsets of cells responsible for substrate uptake, metabolic byproduct degradation, and the control of growth rates. By doing so, I ventured beyond the limitations of simple average-based representations and advanced toward an understanding of microbial populations as “communities” with multifaceted internal structures. This perspective enables a more comprehensive explanation of the complex behaviors observed during the late and death phases of culture, and represents a significant paradigm shift from traditional,

oversimplified modeling approaches.

Of particular note is the treatment of autointoxication, which was previously regarded simply as an environmental factor (i.e., the accumulation of waste products). This study suggests that autointoxication can drive division of labor and functional differentiation within the population. Specifically, the emergence of subpopulations sensitive to high concentrations of accumulated metabolites, alongside other subpopulations specialized in their degradation and detoxification, provides an intrinsic mechanism by which the community mitigates unfavorable conditions. As a result, growth-specialized subpopulation can capitalize on short-term resource utilization, while the detoxification-oriented subpopulation ensures long-term viability and adaptability. Such a dynamic interplay thus enables the microbial community to remain responsive to environmental changes and stressors, going well beyond simplified interpretations from traditional models.

Recent studies have strongly suggested that such “functional differentiation” is an integral part of bet-hedging and strategic diversification within microbial populations (Grimbergen et al., 2015; Morawska et al., 2022). By conceptualizing the community as a dynamic network of subpopulations interacting through resource and metabolite exchange, the modeling framework presented here lays a quantitative foundation for understanding aspects often overlooked by conventional approaches such as the internal structural complexity of biofilms (Stewart & Franklin, 2008; Flemming & Wingender, 2010) and the production of public goods (Griffin et al., 2004). Furthermore, the proposed model offers a promising avenue for exploring the conditions under which persistent cells arise (Lewis, 2010), as well as examining how horizontal gene transfer (Frost et al., 2005; Johnston et al., 2014) facilitates the acquisition of new functions, all in relation to the subpopulation architecture. In this manner, it opens the door to a deeper, more nuanced understanding of the evolutionary and ecological processes shaping microbial communities.

Moreover, advances in single-cell analysis technologies, particularly scRNA-seq, will greatly enhance the empirical validation of this model. While bulk analyses historically overlooked cell-to-cell heterogeneity, single-cell omics techniques (Kuchina et al., 2021; Homberger et al., 2022) now enable high-resolution, time-sensitive quantification of these differences. By integrating such experimental data into the model, it becomes possible to pinpoint gene expression profiles and metabolic activity maps to evaluate the conditions, tipping points, and stability of distinct growth and labor-dividing strategies within subpopulations. Furthermore, by fitting model parameters to these single-cell datasets, hypothetical mechanisms of functional differentiation can be tested and substantiated through direct experimental evidence.

From an applied perspective, this model holds considerable potential. In industrial biotechnology, environmental bioprocessing, wastewater treatment, and agricultural soil improvement, a better understanding of complex microbial communities can be leveraged to adapt to varying environmental conditions, mitigate autointoxication effects, and optimize the production of valuable compounds or the degradation of harmful substances. For example, engineering cultivating conditions that promote the formation of specific subpopulations or employing mixed cultures of functionally specialized strains may lead to more efficient fermentation processes and enhanced environmental remediation. In the medical field, a deeper understanding of the diversity and resistance mechanisms exhibited by biofilm-forming microbes could, in turn, inform strategies aimed at suppressing phenotypic plasticity. Such insights would potentially improve infection control and strengthen therapeutic interventions against biofilm-associated pathogens.

Looking ahead, extending and refining the current model remains a key challenge. While this framework has primarily focused on phenotypic diversity and functional differentiation within a single species and strain, many natural and industrial environments involve multispecies

communities, necessitating the incorporation of interspecies interactions, syntrophy, and horizontal gene transfer (Morris et al., 2013; Thomas & Nielsen, 2005). Equally important is expanding the temporal and spatial scales of the model. Much remains unknown about how subpopulation dynamics unfold when environmental factors are heterogeneous and undergo dynamic changes. Continued progress in computational methods, integration with multiscale models spanning molecular to community levels, and the application of machine learning for large-scale data analysis are all promising directions for tackling more complex ecosystems.

In conclusion, this model represents a deliberate balancing act between biological realism and theoretical simplicity. Excessive complexity can hinder comprehension and analysis, while oversimplification risks failing to adequately explain actual community dynamics. The central contribution of this work is to demonstrate how moving beyond average-based models—and explicitly incorporating features such as phenotypic plasticity and the formation of subpopulations—can profoundly advance both our understanding of microbial ecology and the design of practical biotechnological applications. As single-cell analytical techniques and mathematical modeling methods continue to improve, new horizons will open for more strategically controlling and harnessing microbial communities in both natural and engineered environments.

Taken together, this study highlights the importance of internal diversity and functional specialization in shaping microbial population dynamics and provides a robust conceptual and methodological foundation for their quantitative and integrative understanding. In doing so, it offers an opportunity to reassess the evolutionary and functional significance of microbial communities from a comprehensive perspective that spans microbiology, ecology, biotechnology, and medicine.

7. References

- Ackermann, M. (2015) A functional perspective on phenotypic heterogeneity in microorganisms. *Nat Rev Microbiol* 13: 497–508.
- Alvarez-Ramirez J, Meraz M, Jaime Vernon-Carter E. (2019) A theoretical derivation of the monod equation with a kinetics sense. *Biochemical Engineering Journal* 150:107305.
- Ankit R. 2022. WebPlotDigitizer. <https://automeris.io/WebPlotDigitizer>
- Birol G, Doruker P, Kirdar B, İlgen Önsan Z, Ülgen K. (1998) Mathematical description of ethanol fermentation by immobilized *Saccharomyces cerevisiae*. *Process Biochemistry* 33:763–771.
- Boersch-Supan PH, Ryan SJ, Johnson LR. (2017) deBInfer: Bayesian inference for dynamical models of biological systems in R. *Methods in Ecology and Evolution* 8:511–518.
- Boles, B. R., & Horswill, A. R. (2008). Agr-mediated dispersal of *Staphylococcus aureus* biofilms. *PLoS Pathogens*, 4(4), e1000052.
- Brennan, M. A., & Rosenthal, A. Z. (2021). Single-Cell RNA Sequencing Elucidates the Structure and Organization of Microbial Communities. *Frontiers in Microbiology*, 12, 713128.
- Bridou, R., Rodriguez-Gonzalez, P., Stoichev, T., Amouroux, D., Monperrus, M., Navarro, P., et al. (2018) Methylation and dealkylation of tin compounds by sulfate- and nitrate-reducing bacteria. *Chemosphere* 208: 871–879.
- Brodl, E., Winkler, A., and Macheroux, P. (2018) Molecular Mechanisms of Bacterial Bioluminescence. *Comput Struct Biotechnol J* 16: 551–564.

Buchanan, R.L. and Cygnarowicz, M.L. (1990) A mathematical approach toward defining and calculating the duration of the lag phase. *Food Microbiol* 7: 237–240.

Chaudhari, S.S., Kim, M., Lei, S., Razvi, F., Alqarzaee, A.A., Hutfless, E.H., et al. (2017) Nitrite Derived from Endogenous Bacterial Nitric Oxide Synthase Activity Promotes Aerobic Respiration. *MBio* 8.:

Cooper GA, Liu M, Peña J, West SA. (2022) The evolution of mechanisms to produce phenotypic heterogeneity in microorganisms. *Nature Communications* 13:195.

Davis, K. M. (2020). For the greater (bacterial) good: Heterogeneous expression of energetically costly virulence factors. *Infection and Immunity*, 88(7).

Elowitz, M. B., Levine, A. J., Siggia, E. D., & Swain, P. S. (2002). Stochastic gene expression in a single cell. *Science (New York, N.Y.)*, 297(5584), 1183–1186.

Erttmann, S.F. and Gekara, N.O. (2019) Hydrogen peroxide release by bacteria suppresses inflammasome-dependent innate immunity. *Nat Commun* 10: 3493.

Flemming, H.-C., & Wingender, J. (2010). The biofilm matrix. *Nature Reviews. Microbiology*, 8(9), 623–633.

Flemming, H.-C., van Hullebusch, E. D., Neu, T. R., Nielsen, P. H., Seviour, T., Stoodley, P., Wingender, J., & Wurtz, S. (2023). The biofilm matrix: multitasking in a shared space. *Nature Reviews. Microbiology*, 21(2), 70–86.

Frost, L. S., Leplae, R., Summers, A. O., & Toussaint, A. (2005). Mobile genetic elements: the agents of open source evolution. *Nature Reviews. Microbiology*, 3(9), 722–732.

Garnier A, Gaillet B. (2015) Analytical solution of Luedeking-Piret equation for a batch

fermentation obeying Monod growth kinetics. *Biotechnology and Bioengineering* 112:2468–2474.

Garrett, E. M., Sekulovic, O., Wetzel, D., Jones, J. B., Edwards, A. N., Vargas-Cuevas, G., McBride, S. M., & Tamayo, R. (2019). Phase variation of a signal transduction system controls *Clostridioides difficile* colony morphology, motility, and virulence. *PLoS Biology*, 17(10), e3000379.

Gauthier, M.J. and Flatau, G.N. (1976) Antibacterial activity of marine violet-pigmented *Alteromonas* with special reference to the production of brominated compounds. *Can J Microbiol* 22: 1612–1619.

Griffin, A. S., West, S. A., & Buckling, A. (2004). Cooperation and competition in pathogenic bacteria. *Nature*, 430(7003), 1024–1027.

Gil MM, Miller FA, Silva CLM, Brandão TRS. (2014) Application of optimal experimental design concept to improve the estimation of model parameters in microbial thermal inactivation kinetics. *Journal of Food Engineering* 134:59–66.

Grimbergen, A.J., Siebring, J., Solopova, A., and Kuipers, O.P. (2015) Microbial bet-hedging: the power of being different. *Curr Opin Microbiol* 25: 67–72.

Han Y, Zhang F. (2020) Heterogeneity coordinates bacterial multi-gene expression in single cells. *PloS Computational Biology* 16:e1007643.

Hockenberry, A. M., Micali, G., Takács, G., Weng, J., Hardt, W.-D., & Ackermann, M. (2021). Microbiota-derived metabolites inhibit *Salmonella* virulent subpopulation development by acting on single-cell behaviors. *Proceedings of the National Academy of Sciences of the United States of America*, 118(31), e2103027118.

Homberger, C., Barquist, L., & Vogel, J. (2022). Ushering in a new era of single-cell transcriptomics in bacteria. *microLife*, 3, uqac020.

Homberger, C., Hayward, R.J., Barquist, L., and Vogel, J. (2023) Improved Bacterial Single-Cell RNA-Seq through Automated MATQ-Seq and Cas9-Based Removal of rRNA Reads. *Mbio* e0355722.

Huang, L. (2013) Optimization of a new mathematical model for bacterial growth. *Food Control* 32: 283–288.

Huete-Stauffer, T.M., Arandia-Gorostidi, N., Díaz-Pérez, L., and Morán, X.A.G. (2015) Temperature dependences of growth rates and carrying capacities of marine bacteria depart from metabolic theoretical predictions. *FEMS Microbiol Ecol* 91.:

Imdahl F, Vafadarnejad E, Homberger C, Saliba A-E, Vogel J. (2020) Single-cell RNA-sequencing reports growth-condition-specific global transcriptomes of individual bacteria. *Nature Microbiology* 5:1202–1206.

Imdahl, F., & Saliba, A.-E. (2020). Advances and challenges in single-cell RNA-seq of microbial communities. *Current Opinion in Microbiology*, 57, 102–110.

Indekeu, J.O. and Sznajd-Weron, K. (2003) Hierarchical population model with a carrying capacity distribution for bacterial biofilms. *Phys Rev E Stat Nonlin Soft Matter Phys* 68: 061904.

Johnston, C., Martin, B., Fichant, G., Polard, P., & Claverys, J.-P. (2014). Bacterial transformation: distribution, shared mechanisms and divergent control. *Nature Reviews. Microbiology*, 12(3), 181–196.

Korshoj, L. E., & Kielian, T. (2024). Bacterial single-cell RNA sequencing captures

biofilm transcriptional heterogeneity and differential responses to immune pressure. *Nature Communications*, 15(1), 10184.

Kuchina A, Brettner LM, Paleologu L, Roco CM, Rosenberg AB, Carignano A, Kibler R, Hirano M, DePaolo RW, Seelig G. (2021) Microbial single-cell RNA sequencing by split-pool barcoding. *Science* 371.

Kumar Dutta S, Mukherjee A, Chakraborty P. (1996) Effect of product inhibition on lactic acid fermentation: Simulation and modeling. *Applied Microbiology and Biotechnology* 46:410–413.

Lenski, R. E., & Travisano, M. (1994). Dynamics of adaptation and diversification: a 10,000-generation experiment with bacterial populations. *Proceedings of the National Academy of Sciences of the United States of America*, 91(15), 6808–6814.

Lewis, K. (2010). Persister cells. *Annual Review of Microbiology*, 64(1), 357–372.

Li, G., Vilcherrez, D., Carvajal-Arroyo, J.M., Sierra-Alvarez, R., and Field, J.A. (2016) Exogenous nitrate attenuates nitrite toxicity to anaerobic ammonium oxidizing (anammox) bacteria. *Chemosphere* 144: 2360–2367.

Liu M, West SA, Coope GA. (2021) Relatedness and the evolution of mechanisms to divide labor in microorganisms. *Ecology and Evolution* 11:14475–14489.

Liu Y. (2007) Overview of some theoretical approaches for derivation of the Monod equation. *Applied Microbiology and Biotechnology* 73:1241–1250.

López, S., Prieto, M., Dijkstra, J., Dhanoa, M.S., and France, J. (2004) Statistical evaluation of mathematical models for microbial growth. *Int J Food Microbiol* 96: 289–300.

Lynn BK, De Leenheer P. (2019) Division of labor in bacterial populations. *Mathematical*

Biosciences 316:108257.

Lyu, Z., Yang, A., Villanueva, P., Singh, A., & Ling, J. (2021). Heterogeneous flagellar expression in single Salmonella cells promotes diversity in antibiotic tolerance. *mBio*, 12(5), e0237421.

McNulty, R., Sritharan, D., Pahng, S.H., Meisch, J.P., Liu, S., Brennan, M.A., et al. (2023) Probe-based bacterial single-cell RNA sequencing predicts toxin regulation. *Nat Microbiol*.

Miho Hojo, Yutaka Kimura, Yumio Yato, and Yoshikuni Urushiwaga (2006) On Mathematical Models of Microbial Degradation (Studies in Nonlinear and Convex Analysis). ISM Research Institute for Mathematical Analysis Kokyuroku 1484: 145-149.

Monod J. (1949) The growth of bacterial cultures. *Annual Review of Microbiology* 3:371–394.

Morawska, L.P., Hernandez-Valdes, J.A., and Kuipers, O.P. (2022) Diversity of bet-hedging strategies in microbial communities-Recent cases and insights. *WIREs Mech Dis* 14: e1544.

Morris, B. E. L., Henneberger, R., Huber, H., & Moissl-Eichinger, C. (2013). Microbial syntrophy: interaction for the common good. *FEMS Microbiology Reviews*, 37(3), 384–406.

O'Toole, G., Kaplan, H.B., and Kolter, R. (2000) Biofilm formation as microbial development. *Annu Rev Microbiol* 54: 49–79.

Prats, C., Giró, A., Ferrer, J., López, D., and Vives-Rego, J. (2008) Analysis and IbM simulation of the stages in bacterial lag phase: basis for an updated definition. *J Theor Biol* 252: 56–68.

Pratt, L.A. and Kolter, R. (1998) Genetic analysis of *Escherichia coli* biofilm formation:

roles of flagella, motility, chemotaxis and type I pili. *Mol Microbiol* 30: 285–293.

Rolfe, M.D., Rice, C.J., Lucchini, S., Pin, C., Thompson, A., Cameron, A.D.S., et al. (2012) Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. *J Bacteriol* 194: 686–701.

Ruan Y, Taherzadeh MJ, Kong D, Lu H, Zhao H, Xu X, Liu Y, Cai L. (2020) Nitrogen removal performance and metabolic pathways analysis of a novel aerobic denitrifying halotolerant *Pseudomonas balearica* strain RAD-17. *Microorganisms* 8.

Ruan Y, Taherzadeh MJ, Kong D, Lu H, Zhao H, Xu X, Liu Y, Cai L. (2020) Nitrogen removal performance and metabolic pathways analysis of a novel aerobic denitrifying halotolerant *Pseudomonas balearica* strain RAD-17. *Microorganisms* 8.

Rumbaugh, K.P. and Sauer, K. (2020) Biofilm dispersion. *Nat Rev Microbiol* 18: 571–586.

Sarkar D. (2008) *Lattice: Multivariate Data Visualization with R*. Springer, New York.

Semenova, E.M., Ershov, A.P., Sokolova, D.S., Tourova, T.P., and Nazina, T.N. (2020) Diversity and Biotechnological Potential of Nitrate-Reducing Bacteria from Heavy-Oil Reservoirs (Russia). *Microbiology* 89: 685–696.

Sharrar, A. M., Crits-Christoph, A., Méheust, R., Diamond, S., Starr, E. P., & Banfield, J. F. (2020). Bacterial secondary metabolite biosynthetic potential in soil varies with phylum, depth, and vegetation type. *mBio*, 11(3), e00416-20.

Shokeen, B., Dinis, M. D. B., Haghighi, F., Tran, N. C., & Lux, R. (2021). Omics and interspecies interaction. *Periodontology* 2000, 85(1), 101–111.

Soetaert K, Petzoldt T, Setzer RW. (2010) Solving differential equations in R: Package

deSolve. *Journal of Statistical Software* 33(9):1–25.

Stewart, P. S., & Franklin, M. J. (2008). Physiological heterogeneity in biofilms. *Nature Reviews. Microbiology*, 6(3), 199–210.

Strigul N, Dette H, Melas VB. (2009) A practical guide for optimal designs of experiments in the Monod model. *Environmental Modelling & Software* 24:1019–1026.

Thomas, C. M., & Nielsen, K. M. (2005). Mechanisms of, and barriers to, horizontal gene transfer between bacteria. *Nature Reviews. Microbiology*, 3(9), 711–721.

Tyc, O., Song, C., Dickschat, J.S., Vos, M., and Garbeva, P. (2017) The Ecological Role of Volatile and Soluble Secondary Metabolites Produced by Soil Bacteria. *Trends Microbiol* 25: 280–292.

Van Impe, J.F., Poschet, F., Geeraerd, A.H., and Vereecken, K.M. (2005) Towards a novel class of predictive microbial growth models. *Int J Food Microbiol* 100: 97–105.

P.-F. Verhulst. (1838) Notice sur la loi que la population suit dans son accroissement. *Correspondance mathématique et physique*. 10: 113–121.

P.-F. Verhulst. (1845) Recherches mathématiques sur la loi d'accroissement de la population. *Nouveaux Mémoires de l'Académie Royale des Sciences et Belles-Lettres de Bruxelles*. 18: 1–42.

Wickham H. (2016) *Ggplot2: Elegant Graphics for Data Analysis*. Springer-Verlag, New York.

Xu P. (2020) Analytical solution for a hybrid Logistic-Monod cell growth model in batch and continuous stirred tank reactor culture. *Biotechnology and Bioengineering* 117:873–878.

Yan, X., Liao, H., Wang, C., Huang, C., Zhang, W., Guo, C., & Pu, Y. (2024). An improved bacterial single-cell RNA-seq reveals biofilm heterogeneity. In eLife.

8. Appendix

8.1. Nontrivial equilibrium point

The model shown in Eqs. (1) is as follows:

$$\frac{dN}{dt} = \mu a_1 R N p \left(1 - \frac{N}{K(M)}\right) - dN \quad (1-1)$$

$$\frac{dR}{dt} = \lambda - a_1 R N p - b_1 R N (1 - p) \quad (1-2)$$

$$\frac{dM}{dt} = a_2 R N p + b_2 R N (1 - p) - c M N (1 - p) \quad (1-3)$$

$$K(M) = K_0 + (K - K_0)e^{-fM} \quad (1-4)$$

$p = \frac{R}{R+M+h}$ represents the fraction of amounts of substrate and metabolites (h is the constant value of zero escape). To simplify the subsequent analysis, let us assume that $K(M) = K$ (i.e., constant value) and $h = 0$. Using the assumption that metabolite is not negative value ($M \geq 0$), we can obtain the following set of equilibrium values.

$$N^* = \frac{a_1 \lambda K}{a_1 \lambda + dK \left(a_1 + b_1 \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right)} \quad (2-1)$$

$$R^* = \frac{\left(1 + \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right) \left\{ a_1 \lambda + dK \left(a_1 + b_1 \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right) \right\}}{a_1 K \left(a_1 + b_1 \frac{b_2 + \sqrt{b_2^2 + 4a_2 c}}{2c} \right)} \quad (2-2)$$

$$M^* = \frac{\left(\frac{b_2 + \sqrt{b_2^2 + 4a_2c}}{2c}\right) \left(1 + \frac{b_2 + \sqrt{b_2^2 + 4a_2c}}{2c}\right) \left\{a_1\lambda + dK \left(a_1 + b_1 \frac{b_2 + \sqrt{b_2^2 + 4a_2c}}{2c}\right)\right\}}{a_1K \left(a_1 + b_1 \frac{b_2 + \sqrt{b_2^2 + 4a_2c}}{2c}\right)} \quad (2-3)$$

8.2. Local stability analysis

The eigenvalues of the Jacobian of the dynamics assessed at the above-shown nontrivial equilibrium can be used to calculate the local stability around the equilibrium. In a similar manner, we assume that a constant value of carrying capacity and $h = 0$. The Jacobian matrix is as follows:

$$J = \begin{bmatrix} J_1 & J_2 & J_3 \\ J_4 & J_5 & J_6 \\ J_7 & J_8 & J_9 \end{bmatrix} \quad (5-1)$$

$$J_1 = \frac{R^2 a_1 \cdot \left(1 - \frac{N}{K}\right)}{M + R} - d - \frac{NR^2 a_1}{K(M + R)} \quad (5-2)$$

$$J_2 = N \left(-\frac{R^2 a_1 \cdot \left(1 - \frac{N}{K}\right)}{(M + R)^2} + \frac{2Ra_1 \cdot \left(1 - \frac{N}{K}\right)}{M + R} \right) \quad (5-3)$$

$$J_3 = -\frac{NR^2 a_1 \cdot \left(1 - \frac{N}{K}\right)}{(M + R)^2} \quad (5-4)$$

$$J_4 = -R \left(\frac{Mb_1}{M + R} + \frac{Ra_1}{M + R} \right) \quad (5-5)$$

$$J_5 = -NR \left(-\frac{Mb_1}{(M + R)^2} - \frac{Ra_1}{(M + R)^2} + \frac{a_1}{M + R} \right) - N \left(\frac{Mb_1}{M + R} + \frac{Ra_1}{M + R} \right) \quad (5-6)$$

$$J_6 = -NR \left(-\frac{Mb_1}{(M + R)^2} - \frac{Ra_1}{(M + R)^2} + \frac{b_1}{M + R} \right) \quad (5-7)$$

$$J_7 = -\frac{M^2 c}{M + R} + \frac{MRb_2}{M + R} + \frac{R^2 a_2}{M + R} \quad (5-8)$$

$$J_8 = N \left(\frac{M^2 c}{(M + R)^2} - \frac{MRb_2}{(M + R)^2} + \frac{Mb_2}{M + R} - \frac{R^2 a_2}{(M + R)^2} + \frac{2Ra_2}{M + R} \right) \quad (5-9)$$

$$J_9 = N \left(\frac{M^2 c}{(M + R)^2} - \frac{MRb_2}{(M + R)^2} - \frac{2Mc}{M + R} - \frac{R^2 a_2}{(M + R)^2} + \frac{Rb_2}{M + R} \right) \quad (5-10)$$

Let the three eigenvalues be Λ_1 (real solution), Λ_2 , and Λ_3 (complex solution). Then, the cubic

equation of Λ is as follows:

$$y_1\Lambda^3 + y_2\Lambda^2 + y_3\Lambda + y_4 = 0 \quad (6-1)$$

$$y_1 = -K(R + M)^3 < 0 \quad (6-2)$$

$$y_2 = -(R + M)\psi < 0 \quad (6-3)$$

$$y_4 = -\frac{y_{41}y_{42}y_{43}y_{44}}{16Ka_1c^3(2a_1c + b_1b_2 + b_1(4a_2c + b_2^2)^{0.5})^4} < 0 \quad (6-4)$$

$$y_{41} = \lambda^2(b_2 + 2c + (4a_2c + b_2^2)^{0.5})^3 > 0 \quad (6-5)$$

$$y_{42} = (2Ka_1cd + Kb_1b_2d + Kb_1d(4a_2c + b_2^2)^{0.5} + 2\lambda a_1c) > 0 \quad (6-6)$$

$$y_{43} = \left(\frac{4a_1c(4a_2c + b_2^2)^{0.5} + 4a_2b_1c + b_1b_2^2}{+2b_1b_2(4a_2c + b_2^2)^{0.5} + b_1(4a_2c + b_2^2)^{1.0}} \right) > 0 \quad (6-7)$$

$$y_{44} = \left(\frac{2Ka_1b_2cd + 4Ka_1c^2d + 2Ka_1cd(4a_2c + b_2^2)^{0.5} + Kb_1b_2^2d + 2Kb_1b_2cd + 2Kb_1b_2d(4a_2c + b_2^2)^{0.5} + 2Kb_1cd(4a_2c + b_2^2)^{0.5} + Kb_1d(4a_2c + b_2^2)^{1.0} + 2\lambda a_1b_2c + 4\lambda a_1c^2 + 2\lambda a_1c(4a_2c + b_2^2)^{0.5}}{ } \right) > 0 \quad (6-8)$$

Where $\psi(> 0)$ is as follows:

$$\begin{aligned}
& \lambda \left(2\lambda a_1 c + K \left(\frac{2a_1 c d + b_1 b_2 d}{+b_1 d \sqrt{4a_2 c + b_2^2}} \right) \right) \\
& \left(b_2 + 2c + \sqrt{4a_2 c + b_2^2} \right)^2 \\
& \left(\lambda \left(\frac{2a_1 b_2^2 c + 8a_1 b_2 c^2}{+8a_1 c^3 + 2a_1 c(4a_2 c + b_2^2)} \right) \right. \\
& \quad \left. + \sqrt{4a_2 c + b_2^2} (4a_1 b_2 c + 8a_1 c^2) \right) \\
& \quad \left(\begin{aligned} & 8a_1^2 b_2 c^2 + 8a_1^2 c^3 + 8a_1 a_2 c^3 \\ & + 6a_1 b_1 b_2^2 c + 4a_1 b_1 b_2 c^2 \\ & + 2a_1 b_2^2 c^2 + 4a_2 b_1 b_2 c^2 \\ & + b_1^2 b_2^3 + b_1 b_2^3 c \\ & + (b_1^2 + b_1 c) \sqrt{4a_2 c + b_2^2}^3 \end{aligned} \right) \\
& \quad + K^2 \left(\sqrt{4a_2 c + b_2^2} \left(\begin{aligned} & 8a_1^2 c^2 + 12a_1 b_1 b_2 c \\ & 4a_1 b_1 c^2 + 4a_1 b_2 c^2 \\ & 8a_1 c^3 + 4a_2 b_1 c^2 \\ & 3b_1^2 b_2^2 + 3b_1 b_2^2 c \\ & + 4b_1 b_2 c^2 \end{aligned} \right) \right. \\
& \quad \left. + (4a_2 c + b_2^2) \left(\begin{aligned} & 6a_1 b_1 c + 2a_1 c^2 \\ & + 3b_1^2 b_2 + 3b_1 b_2 c + 4b_1 c^2 \end{aligned} \right) \right) \\
& \quad + K \left(\begin{aligned} & 2a_1 b_2^2 c d + 8a_1 b_2 c^2 d + 8a_1 c^3 d \\ & + b_1 b_2^3 d + 4b_1 b_2^2 c d \\ & + 4b_1 b_2 c^2 d + b_1 d \sqrt{4a_2 c + b_2^2}^3 \end{aligned} \right) \\
& \quad + \sqrt{4a_2 c + b_2^2} \left(\begin{aligned} & 4a_1 b_2 c d + 8a_1 c^2 d \\ & + 3b_1 b_2^2 d + 8b_1 b_2 c d + 4b_1 c^2 d \end{aligned} \right) \\
& \quad \left. + (4a_2 c + b_2^2) (2a_1 c d + 3b_1 b_2 d + 4b_1 c d) \right) \\
\psi = & \frac{\left(\begin{aligned} & 8a_1 c^3 K^2 \left(2a_1 c + b_1 b_2 + b_1 \sqrt{4a_2 c + b_2^2} \right)^3 \end{aligned} \right)}{8a_1 c^3 K^2 \left(2a_1 c + b_1 b_2 + b_1 \sqrt{4a_2 c + b_2^2} \right)^3} \quad (7)
\end{aligned}$$

Relation between roots and coefficients of cubic equation is $\Lambda_1 \Lambda_2 \Lambda_3 = -\frac{y_4}{y_1} < 0$ (6-2, 6-4). Since

Λ_2 and Λ_3 are complex solutions, they have a common real part, so $\Lambda_2 \Lambda_3 > 0$. Thus, $\Lambda_1 < 0$.

For (6-1), let's assume that there are z positive real roots. According to Descartes's

Rule of Signs, if $y_3 < 0$, there are no positive real roots, which means there are three negative

real roots. On the other hand, if $y_3 > 0$, there can be either two or zero positive real roots, which implies there are either one (Λ_1) or three negative real roots. In summary, for the complex conjugate roots $\Lambda_2, \Lambda_3 = v \pm wi$:

- If $v > 0$, it corresponds to an unstable spiral.
- If $v < 0$, it corresponds to a stable spiral.
- If $v = 0$, it corresponds to a center.

8.3. Comprehensive parameter test

The maximum cell density and the proliferation period varied with the changes in parameters (Fig. 7).

For the MG subpopulation, a higher substrate consumption rate, a_1 , led to an increase in maximum cell density and a shorter growth period. When a_1 approached a value close to 1, the maximum cell density converged to the environmental carrying capacity, and the growth period reached a small value. Parameter a_1 directly influenced microbial growth. An increase in a_1 resulted in higher substrate utilization by the MG subpopulation and a higher microbial growth term. As a result, net growth increased, leading to the rapid attainment of a high maximum cell density. On the other hand, a higher metabolic production rate, a_2 , caused a decrease in maximum cell density and a shorter growth period. A higher a_2 increased the production of metabolic products, which negatively affected the environmental carrying capacity. This led to a decrease in fraction $(\frac{R}{R+M})$. These two factors resulted in a decrease in net growth and a reduction in maximum cell density. Furthermore, as the maximum cell density decreased, the time required to reach the maximum cell density became longer, thereby lengthening the growth period.

For the DB subpopulation, a higher substrate consumption rate, b_1 , resulted in a decrease in maximum cell density and a shorter growth period. A higher b_1 led to increased substrate utilization by the DB subpopulation, reducing the relative substrate utilization by the MG subpopulation. This relative decrease in substrate consumption, along with a decrease in fraction $(\frac{R}{R+M})$, caused a decrease in net growth and a reduction in maximum cell density. Additionally, as the maximum cell density decreased, the time required to reach the maximum cell density became longer, leading to a longer growth period. On the other hand, a higher metabolic production rate, b_2 ,

caused a decrease in maximum cell density. An increase in b_2 led to higher metabolic production, resulting in a relative decrease in fraction $(\frac{R}{R+M})$. This inhibited microbial growth and led to a decrease in maximum cell density. While the growth period initially increased with an increase in b_2 , it eventually decreased after reaching a certain threshold. Initially, the changes in b_2 did not have a significant impact on the maximum cell density. Therefore, although the fraction decreased, the maximum cell density remained relatively unchanged. The maximum cell density did not change, but the slight decrease in net growth resulted in a long time to reach the maximum growth rate, thereby extending the growth period. As the parameters increased to a certain extent, the decrease in maximum cell density became more pronounced. Consequently, the time required to reach the maximum cell density from the initial value decreased, leading to a shorter growth period.

An increase in the metabolic product degradation rate, c , caused an increase in maximum cell density. An increase in the degradation rate resulted in a decrease in the concentration of the metabolic product M , thereby improving the environmental carrying capacity and increasing the fraction $(\frac{R}{R+M})$. These two factors contributed to an increase in maximum cell density. The growth period initially decreased but later followed an S-shaped curve, increasing with a further increase in the degradation rate. Initially, an increase in the degradation rate led to an improvement in the environmental carrying capacity and an increase in the fraction, enabling the rapid attainment of maximum cell density. However, once the degradation rate reached a certain level, the increase in maximum cell density became significant, leading to a longer time to reach the maximum cell density. Beyond a certain point, the increase in the degradation rate caused a gradual decrease in the increase of maximum cell density, resulting in a shorter time to reach the maximum cell density. Therefore, to achieve a high maximum cell density and a longer growth period, the degradation rate needs to be within a certain range.

A higher death rate, d , resulted in the opposite outcome compared to a_1 . As the death rate increased, the maximum cell density decreased, and the growth period became longer. The death rate directly negatively impacted microbial growth. An increase in the death rate led to a higher rate of microbial decline. Consequently, the net growth decreased. In this case, the decrease in maximum cell density did not occur due to the environmental carrying capacity. Instead, it was a result of the decreased net growth, which led to a lower microbial growth rate in response to substrate consumption. As a result, the maximum cell density decreased. Moreover, due to the increase in the number of deaths, the time required to reach the maximum cell density increased, leading to a longer growth period.

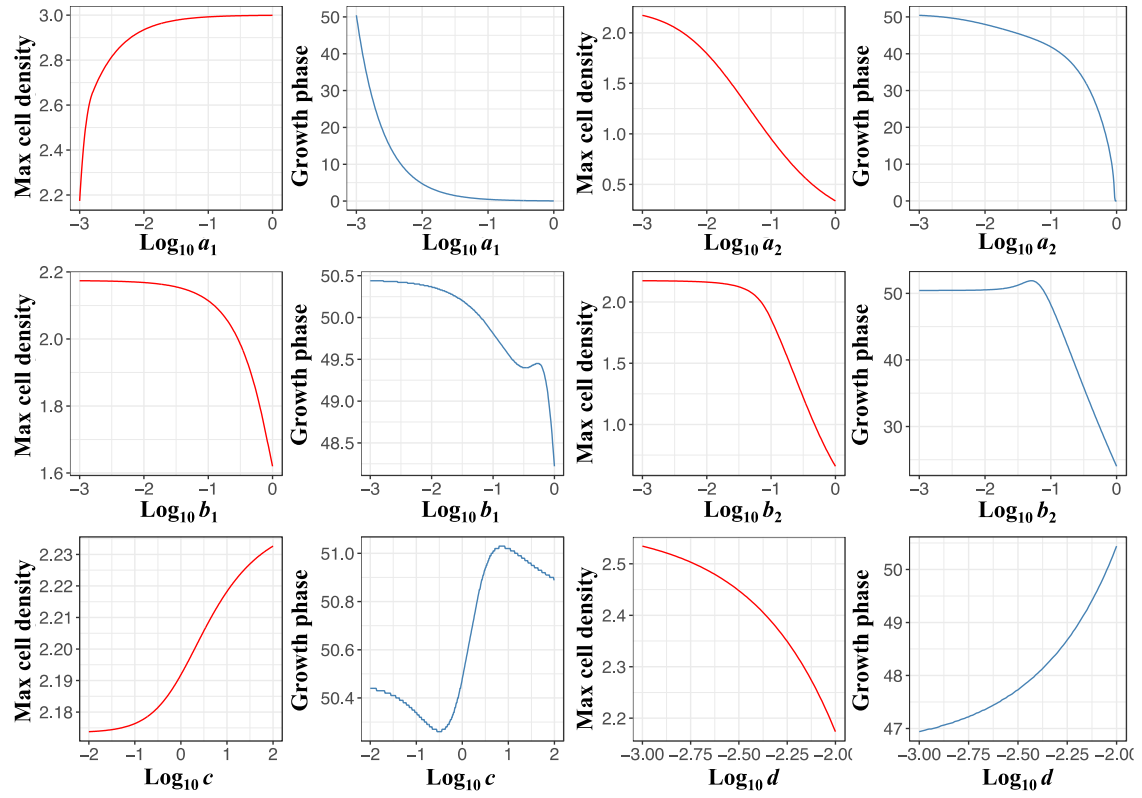


Fig. 7. Single parameter dependence of microbial dynamics.

Here is the plot of the maximum cell density and the growth phase when each parameter is varied individually. The red line represents the maximum cell density, and the blue line represents the growth phase. The parameter set excluding the parameter of interest is $N_0 = 0.1, R_0 = 100, M_0 = 0, \mu = 1, a_1 = 0.001, a_2 = 0.001, b_1 = 0.001, b_2 = 0.001, c = 0.01, d = 0.01, f = 0.01, h = 0, \lambda = 0, K_0 = 1.5, K = 3$.

8.4. Nondimensionalization

8.4.1 Nondimensionalized model

The model shown in Eqs. (1) is as follows:

$$\frac{dN}{dt} = \mu a_1 R N p \left(1 - \frac{N}{K(M)}\right) - dN \quad (1-1)$$

$$\frac{dR}{dt} = \lambda - a_1 R N p - b_1 R N (1 - p) \quad (1-2)$$

$$\frac{dM}{dt} = a_2 R N p + b_2 R N (1 - p) - c M N (1 - p) \quad (1-3)$$

$$K(M) = K_0 + (K - K_0)e^{-fM} \quad (1-4)$$

$p = \frac{R}{R+M+h}$ represents the fraction of amounts of substrate and metabolites (h is the constant value of zero escape). To simplify the subsequent analysis, let us assume that $K(M) = K$ (i.e., constant value) and $h = 0$. Using the assumption that metabolite is not negative value ($M \geq 0$), $p = \frac{R}{R+M+h}$ represents the fraction of amounts of substrate and metabolites (h is the constant value of zero escape). To simplify the subsequent analysis, let us assume that $K(M) = K$ (i.e., constant value) and $h = 0$. Using the assumption that metabolite is not negative value ($M \geq 0$), we can obtain the following set of equilibrium values. Define the non-dimensionalization parameters as follows:

$$\begin{aligned} n &= \frac{N}{K}, r = \frac{\mu}{K} R, m = \frac{\mu}{K} M, \tau = a_1 K t, \\ \alpha &= \frac{b_1}{a_1}, \beta = \frac{a_2}{a_1}, \gamma = \frac{b_2}{a_1}, \varepsilon = \frac{\lambda \mu}{a_1 K^2}, \eta = \frac{c}{a_1}, \xi = \frac{d}{a_1 K} \end{aligned} \quad (8)$$

Substitute these dimensionless parameters into the original model to obtain a dimensionless model.

$$\frac{dn}{d\tau} = r \frac{r}{r+m} n(1-n) - \xi n \quad (9-1)$$

$$\frac{dr}{d\tau} = \varepsilon - r \frac{r}{r+m} n - \alpha r \frac{m}{r+m} n \quad (9-2)$$

$$\frac{dm}{d\tau} = \beta r \frac{r}{r+m} n + \gamma r \frac{m}{r+m} n - \eta m \frac{m}{r+m} n \quad (9-3)$$

By making it dimensionless, we can reduce the number of parameters by three from the original model.

8.4.2. Nontrivial equilibrium point

For dimensionless models Eqs. (9), assume $n \geq 0$, $r \geq 0$, and $m \geq 0$. We can obtain the following set of equilibrium values.

$$n^* = \frac{\varepsilon}{1 + \alpha x} \left(\frac{1}{\frac{\varepsilon}{1 + \alpha x} + \xi} \right) \quad (10 - 1)$$

$$r^* = (1 + x) \left(\frac{\varepsilon}{1 + \alpha x} + \xi \right) \quad (10 - 2)$$

$$m^* = x(1 + x) \left(\frac{\varepsilon}{1 + \alpha x} + \xi \right) \quad (10 - 3)$$

Where $x = \frac{\gamma + \sqrt{\gamma^2 + 4\beta\eta}}{2\eta}$. When there was an influx proportion of the substrate, the microorganism proportion did not reach zero. The substrate and metabolite proportions did not reach zero but continued to take on a constant value. Further, we considered the medium under the no-influx condition (i.e., $\lambda = 0$; $\varepsilon = 0$). The solution could be obtained by substituting $\varepsilon = 0$ for the equilibrium points up to Eqs. (10) as follows:

$$n^* = 0 \quad (11 - 1)$$

$$r^* = (1 + x)\xi \quad (11 - 2)$$

$$m^* = x(1 + x)\xi \quad (11 - 3)$$

When there was no influx proportion of the substrate, the microorganism proportion becomes zero.

The substrate and metabolite proportions did not reach zero but continued to take on a constant value.

8.4.3. Local stability analysis

The eigenvalues of the Jacobian of the dynamics assessed at the above-shown nontrivial equilibrium can be used to calculate the local stability around the equilibrium (10-1) – (10-3). The Jacobian matrix is as follows:

$$J = \begin{bmatrix} J_1 & J_2 & J_3 \\ J_4 & J_5 & J_6 \\ J_7 & J_8 & J_9 \end{bmatrix} \quad (12-1)$$

$$J_1 = \frac{-nr^2 + r^2(1-n) - \xi(r+m)}{r+m} \quad (12-2)$$

$$J_2 = \frac{nr(-2m-r)(n-1)}{(r+m)^2} \quad (12-3)$$

$$J_3 = \frac{nr^2(n-1)}{(r+m)^2} \quad (12-4)$$

$$J_4 = \frac{r(-\alpha m - r)}{r+m} \quad (12-5)$$

$$J_5 = \frac{n\{r(\alpha m + r) - (r+m)(\alpha m + 2r)\}}{(r+m)^2} \quad (12-6)$$

$$J_6 = \frac{nr(-2m-r)(n-1)}{(r+m)^2} \quad (12-7)$$

$$J_7 = \frac{\beta r^2 - \eta m^2 + \gamma r m}{r+m} \quad (12-8)$$

$$J_8 = \frac{n\{-\beta r^2 + \eta m^2 - \gamma r m + (r+m)(2\beta r + \gamma m)\}}{(r+m)^2} \quad (12-9)$$

$$J_9 = \frac{n\{-\beta r^2 + \eta m^2 - \gamma r m + (r+m)(-2\eta m + \gamma r)\}}{(r+m)^2} \quad (12-10)$$

Let the three eigenvalues be λ_1 (real solution), λ_2 , and λ_3 (complex solution). Then, the cubic equation of λ is as follows:

$$y_1\Lambda^3 + y_2\Lambda^2 + y_3\Lambda + y_4 = 0 \quad (13-1)$$

$$y_1 = -(r+m)^3 < 0 \quad (13-2)$$

$$y_2 = -(r+m)\psi < 0 \quad (13-3)$$

$$y_4 = -\frac{y_{41}y_{42}y_{43}y_{44}}{16\eta^3(\alpha\gamma + \alpha(4\beta\eta + \gamma^2)^{0.5} + 2\eta)^4} < 0 \quad (13-4)$$

$$y_{41} = \epsilon^2(2\eta + \gamma + (4\beta\eta + \gamma^2)^{0.5})^3 > 0 \quad (13-5)$$

$$y_{42} = (\alpha\gamma\xi + \alpha\xi(4\beta\eta + \gamma^2)^{0.5} + 2\epsilon\eta + 2\eta\xi) > 0 \quad (13-6)$$

$$y_{43} = \left(\begin{array}{l} 4\alpha\beta\eta + \alpha\gamma^2 + 2\alpha\gamma(4\beta\eta + \gamma^2)^{0.5} \\ + \alpha(4\beta\eta + \gamma^2)^{1.0} + 4\eta(4\beta\eta + \gamma^2)^{0.5} \end{array} \right) > 0 \quad (13-7)$$

$$y_{44} = \left(\begin{array}{l} 2\alpha\eta\gamma\xi + 2\alpha\eta\xi(4\beta\eta + \gamma^2)^{0.5} + \alpha\gamma^2\xi \\ + 2\alpha\gamma\xi(4\beta\eta + \gamma^2)^{0.5} + \alpha\xi(4\beta\eta + \gamma^2)^{1.0} \\ + 4\epsilon\eta^2 + 2\epsilon\eta\gamma + 2\epsilon\eta(4\beta\eta + \gamma^2)^{0.5} \\ + 4\eta^2\xi + 2\eta\gamma\xi + 2\eta\xi(4\beta\eta + \gamma^2)^{0.5} \end{array} \right) > 0 \quad (13-8)$$

Where $\psi(> 0)$ is as follows:

$$\psi = \frac{\epsilon(2\eta + \gamma + \sqrt{4\beta\eta + \gamma^2})^2 \left(\begin{array}{l} \alpha\gamma\xi + \alpha\xi\sqrt{4\beta\eta + \gamma^2} \\ + 2\epsilon\eta + 2\eta\xi \end{array} \right) (\varphi_1 + \varphi_2 + \varphi_3 + \varphi_4)}{8\eta^3(2\eta + \alpha\gamma + \alpha\sqrt{4\beta\eta + \gamma^2})^3} \quad (14-1)$$

$$\begin{aligned} \varphi_1 = & \alpha^2\gamma^3 + 4\alpha\beta\eta^2\gamma + 4\alpha\eta^2\gamma\xi \\ & + 4\alpha\eta^2\gamma + \alpha\eta\gamma^3 + 4\alpha\eta\gamma^2\xi \\ & + 6\alpha\eta\gamma^2 + \alpha\gamma^3\xi + 8\beta\eta^3 \\ & + 8\epsilon\eta^3 + 8\epsilon\eta^2\gamma + 2\epsilon\eta\gamma^2 \\ & + 8\eta^3\xi + 8\eta^3 + 2\eta^2\gamma^2 \\ & + 8\eta^2\gamma\xi + 8\eta^2\gamma + 2\eta\gamma^2 \end{aligned} \quad (14-2)$$

$$\varphi_2 = (4\beta\eta + \gamma^2)^{0.5} \cdot \begin{pmatrix} 3\alpha^2\gamma^2 + 4\alpha\beta\eta^2 + 4\alpha\eta^2\gamma \\ +4\alpha\eta^2\xi + 4\alpha\eta^2 + 3\alpha\eta\gamma^2 \\ +8\alpha\eta\gamma\xi + 12\alpha\eta\gamma + 3\alpha\gamma^2\xi \\ +8\epsilon\eta^2 + 4\epsilon\eta\gamma + 8\eta^3 \\ +4\eta^2\gamma + 8\eta^2\xi + 8\eta^2 + 4\eta\gamma\xi \end{pmatrix} \quad (14-3)$$

$$\varphi_3 = (4\beta\eta + \gamma^2)^{1.0} \cdot \begin{pmatrix} 3\alpha^2\gamma + 4\alpha\eta^2 + 3\alpha\eta\gamma \\ +4\alpha\eta\xi + 6\alpha\eta + 3\alpha\gamma\xi \\ +2\epsilon\eta + 2\eta^2 + 2\eta\xi \end{pmatrix} \quad (14-4)$$

$$\varphi_4 = \alpha(4\beta\eta + \gamma^2)^{1.5}(\alpha + \eta + \xi) \quad (14-5)$$

Relation between roots and coefficients of cubic equation is $\Lambda_1\Lambda_2\Lambda_3 = -\frac{y_4}{y_1} < 0$ (13-2, 13-4).

Since Λ_2 and Λ_3 are complex solutions, they have a common real part, so $\Lambda_2\Lambda_3 > 0$. Thus, $\Lambda_1 < 0$.

For (13-1), let's assume that there are z positive real roots. According to Descartes's Rule of Signs, if $y_3 < 0$, there are no positive real roots, which means there are three negative real roots. On the other hand, if $y_3 > 0$, there can be either two or zero positive real roots, which implies there are either one (Λ_1) or three negative real roots. In summary, for the complex conjugate roots $\Lambda_2, \Lambda_3 = v \pm wi$:

- If $v > 0$, it corresponds to an unstable spiral.
- If $v < 0$, it corresponds to a stable spiral.
- If $v = 0$, it corresponds to a center.

8.4.4. Comparison population dynamics with based model

We performed population dynamics calculations using the parameter set identical to Fig. 3 of the main text but applied to a dimensionless model (Fig. 8). The phase was classified into six stages. Some slight discrepancies were observed in the boundary values of each phase compared to the original model. In the original model, the carrying capacity varied with metabolite concentration, but this is thought to be because the carrying capacity was kept constant for simplicity as the model was made dimensionless.

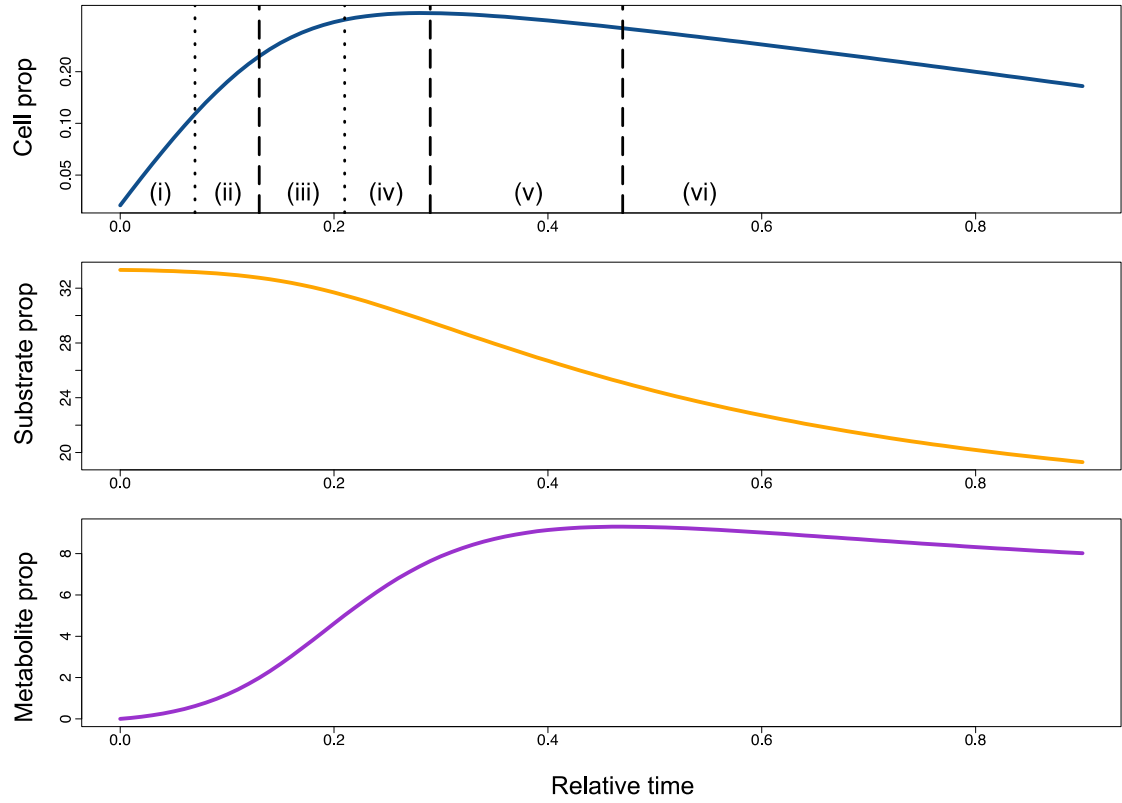


Fig. 8. Numerical solution for no-influx substrate on Eqs. 11.

Relative microbial growth (blue), relative substrate (orange), and relative metabolite dynamics (purple) are shown in the parameter set ($N_0 = 0.1, R_0 = 100, M_0 = 0, \mu = 1, a_1 = 0.00025, a_2 = 0.001, b_1 = 0.0015, b_2 = 0.001, c = 0.01, d = 0.01, h = 0.00001, \lambda = 0, K = 3$)).

We used the dot-dashed line to represent the relative time point where the first derivative of the total cell proportion in microbial growth dynamics reaches its maximum or minimum. Similarly, the dotted line indicates the relative time point where the second derivative of the total cell proportion reaches its maximum, minimum, or zero.

In the absence of substrate transfer, microorganisms exhibit rapid growth at the beginning of the culture. However, as microbial overcrowding and the accumulation of metabolites occur, the growth

rate gradually decreases, leading to a slow decline in the proportion. Following the death phase, the consumption of substrate by microorganisms decreases, and the metabolites start to degrade, eventually reaching a plateau.

8.4.5. Comprehensive parameter test

The maximum cell proportion and length of the growth phase were calculated when several single nondimensionalized parameters were changed (Fig. 9).

The maximum cell density and the proliferation period varied with the changes in parameters.

An increase in $\alpha = \frac{b_1}{a_1}$ resulted in a decrease in the maximum cell proportion and a shorter growth phase. The increase in α led to a relative increase in b_1 , making the DB subpopulation more capable of consuming substrate in the environment. Consequently, the relative substrate utilization by the MG subpopulation decreased, negatively impacting microbial growth.

An increase in $\beta = \frac{a_2}{a_1}$ and $\gamma = \frac{b_2}{a_1}$ resulted in a decrease in the maximum cell proportion.

The increases in β and γ caused a relative increase in the metabolic product production rate. The increased metabolic production rate led to a higher production of metabolites, resulting in a relative decrease in the fraction $(\frac{r}{r+m})$. As a result, the microbial growth rate was suppressed, leading to a decrease in the maximum cell proportion. The length of the growth phase became longer as β and γ increased, but beyond a certain value, the growth period became shorter. The changes in parameters initially did not have a significant impact on the maximum cell proportion. Therefore, although the fraction decreased, the maximum cell proportion remained almost unchanged. The maximum cell proportion remained unchanged, but the growth rate slightly decreased, causing the time to reach the maximum growth rate to increase, thereby prolonging the growth period. As the parameters increased to a certain extent, the decrease in the maximum cell proportion became more significant. Consequently, the time from the initial value to reach the maximum cell proportion decreased, leading to a shorter growth period.

An increase in $\eta = \frac{c}{a_1}$ resulted in an increase in the maximum cell proportion, while the growth period remained unchanged. The increase in η led to a relative increase in the degradation rate c . The increased degradation rate suppressed the production of metabolites, resulting in a relative increase in the fraction $(\frac{r}{r+m})$. Thus, it contributed to a slight increase in the maximum cell proportion. On the other hand, it did not affect the growth period. This was because the influence on the maximum cell proportion was too small to affect the length of the growth period.

An increase in $\xi = \frac{d}{a_1 K}$ resulted in a decrease in the maximum cell proportion and a longer growth period. The increase in ξ led to a relative increase in the death rate d . The death rate directly negatively affected microbial growth. The increase in the death rate led to an increase in the death rate of microorganisms. As a result, the net growth rate of microorganisms decreased. Therefore, with a lower net growth rate, the amount of microbial growth in relation to substrate consumption decreased, leading to a decrease in the maximum cell proportion. The increase in the number of deaths prolonged the time to reach the maximum cell proportion, resulting in a longer growth period.

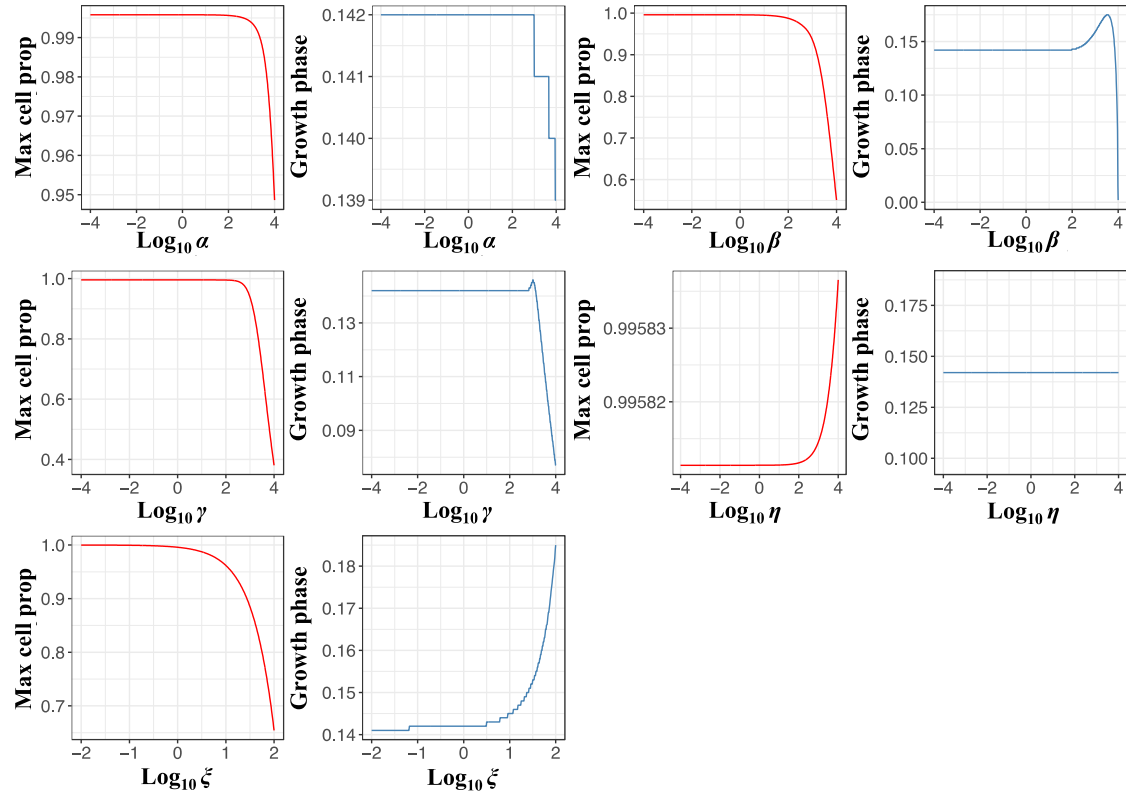


Fig. 9. Single nondimensionalized parameter dependence of microbial dynamics.

Here is the plot of the maximum cell proportion and the growth phase when each parameter is varied individually. The red line represents the maximum cell density, and the blue line represents the growth phase. The parameter set excluding the parameter of interest is $n_0 = \frac{0.1}{3}, r_0 = \frac{100}{3}, M_0 = 0, \alpha = 0.1, \beta = 0.1, \gamma = 0.1, \varepsilon = 0, \eta = 0.01, \xi = 0.1, h = 0, \tau = 1$.

9. Acknowledgement

I would like to express my deepest gratitude to Associate Professor Shinji Nakaoka of the Laboratory of Mathematical Biology, Faculty of Advanced Life Science, Hokkaido University, for his guidance over the past five years. In his laboratory, I had the opportunity to engage in mathematical biology, bioinformatics, and experimental work, gaining invaluable knowledge in these fields.

I am also sincerely thankful to Assistant Professor Ryo Yamaguchi of the same laboratory. Your instruction in the fundamentals of mathematical biology and modeling was crucial to my research. Additionally, I extend my heartfelt thanks to Assistant Professor Akane Hara of the Faculty of Pharmacy and Pharmaceutical Sciences, University of Toyama, for your insights into mathematical modeling, which greatly enhanced my research.

My deepest appreciation goes to the members of our laboratory. I want to especially thank Keiko Saito (Assistant) for her warm and comprehensive support throughout my research activities and student life. I am also grateful to Junya Sunagawa, with whom I collaborated as one of the early members of this laboratory, as we worked together to build it from the ground up.

I would also like to express my gratitude to Professor Toshiyuki Nakagaki (Laboratory of Physical Ethology) and Professor Hisashi Haga (Laboratory of Cell Dynamics) for their time and effort in reviewing this doctoral dissertation.

Furthermore, I am deeply grateful to Tetsuo Hatsuda (Program Director), Catherine Beauchemin (Deputy Program Director), Jeffrey Fawcett (Senior Research Scientist), and Hitomi Wada (Assistant) from the Interdisciplinary Theoretical and Mathematical Sciences Program (iTHEMS), RIKEN. The stimulating discussions and the conducive research environment at RIKEN

have shaped my academic journey and set my research path in motion.

I would like to acknowledge the financial support provided by the Ambitious Fellowship, EXEX Fellowship, and RIKEN Junior Research Associate Program, which made this research possible. Thank you for your generous support.

I am also grateful to my former horses, Sakura Superior and Nogaro. Reflecting on memories and looking at their photos helped me overcome the challenging moments during my research.

Lastly, I would like to express my heartfelt gratitude to my family. My grandfathers, Soukichi George Kumakura and Toshio Fukushima, despite living through turbulent times, held a deep admiration for academic pursuits. Your passion for knowledge and the guidance you imparted have greatly influenced who I am today. To my parents, Takamoto and Yuka, your unwavering support and dedication have been the foundation upon which I have been able to pursue my academic journey. Thank you.