Study on Dynamic Model Construction and Analysis Method of Bacteria-algae Co-culture

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Abstract: To investigate the dynamic changes in growth and product synthesis of a mixed culture of Rhodotorula glutinis and Chlorella vulgaris, this study utilized fermentation data to analyze the rules and preliminary results of the microbial-algal symbiotic system. Employing Matlab, the author fitted a mathematical model proposed by predecessors, seeking parameters to establish a dynamic model for microbial fermentation. This model not only characterized parameter significance but also shed light on interactions, energy transfer mechanisms, and natural regulation balance during symbiotic fermentation. Results revealed that, under the influence of algae's photosynthetic carbon fixation, bacteria exhibited secondary growth, coupled with lipid synthesis and cell growth. The mixed culture model demonstrated a significant increase of 25.9% in biomass and 42.1% in oil content (reaching 30.694 g/L and 14.54 g/L, respectively). These findings suggest the model's potential as a theoretical guide for optimizing mixed culture fermentation processes.

Keywords： Bacteria and Algae Symbiosis; Mathematical Model to Reference; Dynamics; Fermentation Control Optimization

1. Introduction

Rhodotorula glutinis, a yeast strain renowned for its high oil production and pigment synthesis—particularly beta-carotene—stands as a versatile organism. This facultative aerobic yeast exhibits substantial oxygen consumption and carbon dioxide emission during its growth. In the fermentation process, the dissolved oxygen rate decreases proportionally with biomass accumulation. In conditions of oxygen insufficiency, the yeast undergoes anaerobic respiration, resulting in the production of volatile organic acids. This cascade effect leads to a rapid decline in the solution's pH, consequently inhibiting yeast growth.[1]

Similarly, Chlorella vulgaris, another strain with high oil and pigment production capabilities, contributes significantly to this symbiotic relationship. When utilizing CO2 for photoautotrophic growth, Chlorella vulgaris consumes both CO2 and small molecular organic acids.[2][3]This dual activity leads to an elevation in solution pH and the liberation of a substantial amount of oxygen. Consequently, the co-culture of Rhodotorula glutinis and Chlorella vulgaris demonstrates synergistic effects in gas exchange, substance exchange, and pH regulation.

Despite numerous reports on the mixed culture of bacterial strains, the current challenge lies in selecting optimal fermentation control measures. The key task is to maintain the respective population advantages of Rhodotorula glutinis and Chlorella vulgaris, facilitating a harmonious and stable co-culture of these two distinct species. This difficulty underscores the importance of identifying effective fermentation control means to unlock the full potential of their symbiotic relationship.

2. The Research Question

This study addresses the aforementioned challenges by focusing on the investigation and establishment of a stable mixed culture model. The primary aim is to construct growth and product synthesis kinetic models for the two microorganisms within the mixed culture system. This endeavor seeks to offer theoretical guidance for the selection of mixed culture modes, process optimization, and the subsequent pilot scale-up. The ultimate goal is to enhance the understanding of the symbiotic relationship between Rhodotorula glutinis and Chlorella vulgaris, facilitating practical applications and advancements in the field of mixed culture fermentation.

3. Methodology/Study

3.1 Mixed Culture

Following the storage on the slope, Chlorella sp. and Rhodotorula glutinosa were inoculated into 250 ml flasks (containing 150 ml of medium). The activation culture commenced in a constant temperature shaker and lasted for 72 hours under controlled conditions (temperature: 30 ° C, rotation speed: 180 rpm, light intensity: 3000 lux).

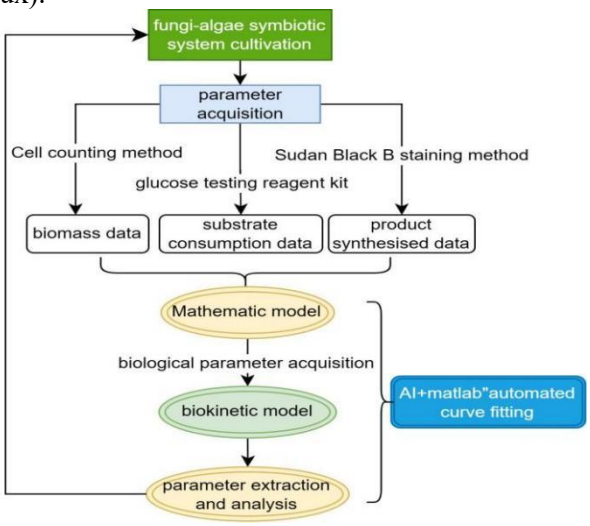

Figure 1. Technical Road Map

Subsequently, the activated seeds were inoculated with a 3% inoculum into the mixed culture. The culture conditions for the mixed culture were consistent with those during the activation phase. Sampling occurred at 24-hour intervals, spanning a total fermentation period of approximately 168 hours.

This cultivation and sampling protocol was designed to capture the dynamic changes in the mixed culture system and facilitate a comprehensive analysis of growth and product synthesis kinetics. The regular intervals of sampling provide a detailed understanding of the symbiotic relationship between Chlorella sp. and Rhodotorula glutinosa throughout the fermentation process.

3.2 Parametric Measurement

3.2.1 Biomass Measurements

By measuring the correlation between algal count and chlorophyll content, a standard curve was established to calculate the algal number by chlorophyll a content. The Cytometry samples were centrifuged at 4000rmin for 10 min and then dried in a constant temperature drying chamber to a constant weight, the absorbance at 750nm, 645nm, 663nm and 652nm was measured. The content of chlorophyll a was calculated according to formula (1) .[5]

$$chl_a = [11.64 * (OD_{663} - OD_{750}) - 2.16 * (OD_{645} - OD_{750}) + 0.10 * （OD_{630} - OD_{750}）] * V \quad (1)$$

The standard curve of biomass and biomass concentration of Rhodotorula glutinis was established by means of microscopic counting and further drying.

3.2.2 Glucose Concentration Test

At first, the standard curve was made by kit reaction, and then the sample was determined.

3.2.3 Oil Concentration Test

The oil content was assessed using the Sudan Black B staining method. Initially, a Standard Oil content solution was prepared using peanut oil. In the staining process, 0.1 g of Sudan Black B was dissolved in 100 ml of a 70% ethanol solution. Subsequently, 0.4 ml of the Sudan Black B staining solution was added to the test sample, while another tube (control tube) received 0.4 ml of 70% ethanol. The contents were thoroughly mixed and heated in a boiling water bath for 10 minutes.

As a result of this process, the product exhibited a blue color. The absorption value of the test sample was measured at 645 nm. This method, employing Sudan Black B staining, offers a reliable means to quantify the oil content, with the standard solution serving as a reference for accurate comparison and analysis.

3.3 Model Specification

M.H. Zwietering[4] reparameterized the Gompertz equation. The Gompertz equation is as follows:

$$c(X) = Ae^{\{-Ce^{(-b)t}\}} \quad (2)$$

By performing the second-order derivative of c(X) and setting the second-order derivative to 0, find the inflection $\frac{dc(X)_i}{dt_i}$ point. The inflection point ti corresponds to the maximum specific growth rate μm. Find the tangent equation y=kx+b through the inflection point. The tangent equation The intercept on the abscissa is defined as the delay time λ, and the derived equation is:

$$c(X) = Ae^{\left\{-e^{\left[\frac{\mu_m}{A}(\lambda - t)\right]}\right\}} \quad (3)$$

Among them, λ: the time of the delay phase, h; μm: the maximum specific growth rate, h-1; A: the number of cells in the stable phase, g/L.

At this time, this biologically meaningful model can provide guidance information for directional optimization during the bacterial growth process.

4. Conclusion

4.1 Growth Process

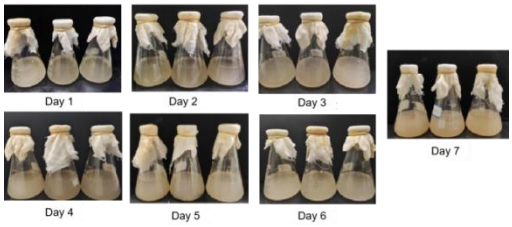

Figure 2. Growth Record

In the initial stages of the experiment, the bacterial biomass was relatively low. As the organisms proliferated, a gradual decline in glucose mass was observed, concomitant with an increase in the biomass of both bacteria and algae. Notably, a discernible positive correlation emerged between the augmentation of bacterial and algal biomass and the synthesis of products, particularly the quality of oil. The growing organisms provided the necessary energy and carbon sources for the synthesis process, leading to a gradual increase in the mass of lipids synthesized.

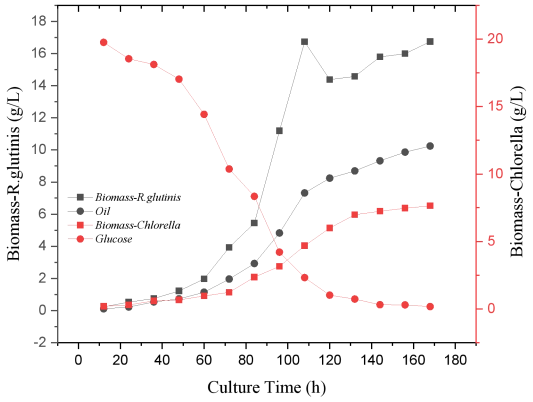

Figure 3. Data Integration

Interestingly, the augmentation in oil quality exhibited a lag behind the increase in bacterial and algal biomass. This delay suggests that lipid synthesis is a relatively slow process, requiring sufficient time to accumulate substantial products. This hysteresis effect might be attributed to the intricate metabolic pathways and synthetic mechanisms inherent to the organisms.[3]

As the experiment progressed, a sharp decline in glucose mass was observed, contrasting with a continued increase in oil mass. During this phase, the biomass of algae experienced a gradual increase. This phenomenon could be attributed to the sugars generated through algae photosynthetic carbon fixation, which likely sustained the growth and metabolism of bacteria to a certain extent. This mutual interaction between bacteria and algae potentially contributed to an enhanced efficiency in the synthesis of products, particularly the increasing oil mass.

4.2 Dynamic Model

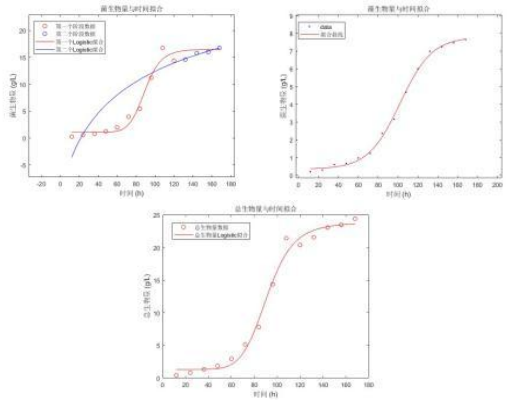

Figure 4. Results of Biomass Dynamics

Here, the following results are obtained through automatic analysis by matlab:

4.3 Bacterial Growth Kinetic Model

$$\begin{cases} X_{B1} = 248.64 - \frac{248.15}{1+(\frac{t}{200.54})^{4.29}} & 0 \le t \le 144 \\ X_{B2} = 18.26e^{(e^{0.02(t-43.90)})} & 144 < t \le 168 \end{cases} \quad (4)$$

RB12=0.991；RB22=0.940

Xmin：0.205 Xmax：17.413

μm： 0.723 λ：60.29

Algae growth kinetic model

$$X_Y = 8.15 - \frac{7.68}{1+(\frac{t}{103.62})^{6.04}} \quad (5)$$

RY2= 0.978

Xmin：0.149 Xmax：7.7966

μm：0.1159 λ：102.19

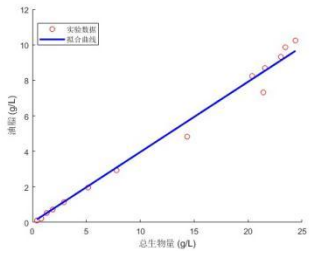

Figure 5. Product Synthesis Model Fitting Results

Product synthesis kinetic model[6]

$$p(t) = 0.403 * (23.77 - \frac{22.46}{1+(\frac{t}{91.26})^{7.91}}) - 0.147 \quad (6)$$

R2=0.978

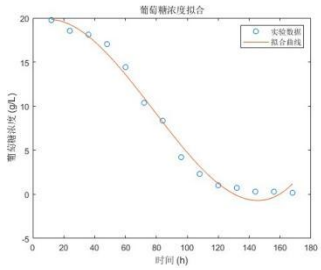

Figure 6. Matrix Depletion Model Fitting Results

Substrate consumption kinetic model[6]

$$S(t) = 24.801 - \frac{7.692}{200.45*3.29}(1+\frac{t}{200.45})^{-3.29} - 0.021(23.77 - \frac{22.46}{1+(\frac{t}{91.26})^{7.92}}) \quad (7)$$

R2=0.981

α(Bacterial growth coefficient)=0.031;

β(algae growth)= 0.021;

$\gamma(\text{product synthesis}) = 0.029;$

δ(respiratory consumption)= 0.011. (8)

4.3 Model Analysis and Application Results

Building upon the results outlined in the report, a comprehensive analysis of the rules governing bacterial growth, product synthesis, and substrate consumption was conducted, leading to an overarching correlation. The primary focus was on maximizing oil production while minimizing substrate utilization. The model report highlighted the coupling of lipid synthesis with biomass concentration, emphasizing the significance of algae growth in the symbiotic relationship between bacteria and algae. Optimal resource utilization and increased output were identified as potential benefits of prioritizing higher algae concentration.

Consequently, a strategic approach was adopted to curtail glucose concentration in the early stages, aiming to inhibit algae growth while ensuring the maintenance of bacterial biomass for higher output. Specific measures were implemented, with a 15% sugar concentration in the initial stages, followed by an additional 10% on the third day of culture. This protocol was sustained for seven days, during which biomass and oil quality were regularly monitored.

The optimized mixed culture model demonstrated a substantial improvement, with a 25.9% increase in biomass and a remarkable 42.1% increase in oil content, reaching 30.694 g/L and 14.54 g/L, respectively. These results underscore the efficacy of the proposed model in offering superior theoretical guidance for optimizing the mixed culture fermentation process. The success of this optimized approach validates its potential application for enhancing both biomass and oil content in symbiotic fermentation systems.

5. Prospect

Building on the foundation of existing research outcomes, the focus of my ongoing investigation will delve into the molecular mechanisms underlying the symbiotic relationship between bacteria and algae. A comprehensive exploration of the causes behind the observed secondary growth phenomenon will be undertaken, aiming for a deeper understanding of the intricate interactions within the symbiosis system. To accomplish this, I plan to integrate additional insights from molecular biology, incorporating more advanced mathematical and computational methodologies.

The forthcoming research endeavors will prioritize the enhancement of computer data processing and calculation capabilities, leveraging both mathematical knowledge and computer science expertise. This approach aims to refine and broaden the scope of biological analyses, enabling more professional and comprehensive insights. By adopting a multidisciplinary approach, I aim to extract richer conclusions and conduct in-depth analyses of the significance of each specific time point throughout the fermentation process.

The ultimate goal of this extended study is to contribute to the collective understanding of symbiotic fermentation systems, paving the way for advancements in both theoretical knowledge and practical applications. [7]Through the integration of molecular biology, mathematics, and computer science, this research seeks to unravel the complexities of bacterial and algal symbiosis, providing valuable insights for future biotechnological applications and innovations.

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