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MODELING OF BIOSURFACTANT SYNTHESIS USING *BACILLUS ssp.*

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Bacteria belonging to the genus *Bacillus* produce biogenic surface-active compounds which are a sustainable alternative to synthetic ones. The amount of the target products depends significantly on the conditions of microorganism cultivation, especially the components of the medium. In this study, we utilized *Bacillus spp.* strain that can grow in a glycerol-containing medium. We used the Monod model to determine the growth kinetics of the bacteria depending on the concentration of nutrients in the medium. Our findings indicated that intensive growth of *Bacillus spp.* bacteria is observed during cultivation in the medium with a glycerol concentration of 30–40 g/L. The surface-active substances from *Bacillus spp.* was characterized by a sufficient level of foaming and foam stability. The highest value of foam stability was observed on the 7th day of cultivation.
Key words: biosurfactants; Monod model; foaming; foam stability.

Introduction

Surfactants are chemical compounds that utilized extensively in many different industries. This is because surfactants have a unique property that allows them to lower the surface tension or interfacial tension at the liquid/liquid surface, or other interfaces [1].

The value of the worldwide surfactant market climbed to USD 41.22 billion in 2021 from 39.42 billion USD in 2020. This market has a huge potential that might have a big impact on fossil fuel reserves; it is predicted to grow to 57.81 billion USD by 2028 (Fortune Business Insights, 2021) [2]. However, it is important to note that synthetic surfactants, byproducts of the oil refining process, can be harmful to human health and the environment. These surfactant molecules have a detrimental effect on soil microbiota at high concentrations, which interferes with biogeochemical processes [3].

For this reason, scientists are actively searching for alternative methods of producing environmentally friendly surfactants. Therefore, the use of biogenic surfactants is a promising direction. These compounds have physical and chemical properties that are similar to synthetic surfactants, but they are synthesized in the cells of living organisms [4].

Biosurfactants are classified by their microbial origin, molecular weight, and mode of action. Some of the most common microorganisms that produce biosurfactants are *Pseudomonas, Bacillus, Candida, Rhodococcus*, and *Corynebacterium* [5].

Microbial surfactants are versatile agents characterized by low toxicity and high activity under various conditions. They are widely used in the bioremediation of environmental pollutants in agriculture to improve the degradation of insecticides and pesticides, thereby improving soil fertility [6, 7].

Numerous investigations have demonstrated the potential of biosurfactants as a biologically active substance with antimicrobial, antibacterial and antiviral properties [8]. Moreover, they can exhibit these activities both separately or in combination with other substances, for example, methyl and ethyl esters of thiosulfonic acids [9].

It is known that bacteria of the genus *Bacillus* predominantly produce lipopeptides. They consist of a peptide fragment bound to a fatty acid. Representatives of the genus *Bacillus* synthesize three main groups of lipopeptides: surfactin, iturin, and fengicin, which differ in position, length, and isomers of fatty acids included in their composition. The most famous lipopeptide is surfactin, which is a heptapeptide conjugated to a β-hydroxy fatty acid with a chain length of 12 to 16 carbonates, forming a cyclic lactone ring structure [10]. *Bacillus* lipopeptides also include kurstakin, maltacin, polymyxin, bamylocin, and licheniformin [11].

Currently, biosurfactants are beneficial in a variety of applications due to their diverse characteristics. However, there are some limitations of their use. The main disadvantage is the high cost of production. The yield of surface-active substances of microbial origin is quite low, which significantly affects the cost of production. One of the ways to increase the biomass yield of microbial cells and target products is to optimize the conditions for cultivating microorganisms, especially the selection of media components.

The optimal composition of the medium can be selected experimentally or using mathematical modeling methods. The use of kinetic modeling in biotechnology is important due to the dynamics and complex structure of biological systems. This approach allows us to study systems as a holistic structure that changes over time and is important for understanding their behavior and interaction with the environment.

Therefore, the **goal of this work** is to model the synthesis of a surfactant's complex by microbial synthesis of *Bacillus spp.* on a medium with glycerol as the main source of carbon.

Materials and research methods

The experimental part of the work was carried out at the laboratory of CARPATOL LLC.

The object of the study was the Monod model to predict the bacterial cell growth.

The *Bacillus spp.* strain from the culture collection of CARPATOL LLC are cultivated in 750 cm^3 Erlenmeyer flasks containing 150 cm^3 of nutrient medium of the following composition, dm^3: $NaNO_3$ – 4; KH_2PO_4 – 1.2; $K_2HPO_4 \cdot 3H_2O$ – 2.0; $MgSO_4 \cdot 7H_2O$ – 0.5; glycerol – 40; sodium citrate – 5.0; distilled water – up to 1 dm^3.

The inoculum was a 48-h culture grown on a rolling pin at 14 rpm at 28 °C. The inoculum was taken in an amount of 10 % of the volume of the culture medium, and the cell titer was 5×108 CFU/cm^3.

Biomass was separated from the culture liquid by centrifugation at 6000 rpm for 20 min. Biosurfactants were isolated from the culture supernatant by precipitation with 10 % HCl solution to pH 3. The precipitate was kept at 4 °C for 12 h, separated by centrifugation (8000 rpm, 20 min) and dried to a constant weight. The percentage of biosynthesis product was expressed as dry residue.

The foaming ability of the biosurfactant solution was determined according to DSTU 3789-98 [12]. 100 ml of the supernatant was shaken in a graduated container for 1 min. Next, the height of the foam column was measured. Foam stability is defined as the time during which the formed foam is completely destroyed.

The Monod equation is a mathematical model used to describe the growth kinetics of microorganisms, in particular bacteria, under conditions of limited resources. This equation helps to predict how fast microorganisms will grow depending on the concentration of nutrients in the medium.

The basic equation of the Monod model was expressed as follows:

$$\mu = \mu_{max} \frac{S}{K_s + S}, \qquad (1)$$

μ – is the specific growth rate of microorganisms; μ_{max} – maximum possible specific growth rate under the most favorable conditions; S is the concentration of the limiting substrate; K_s is the concentration of the limiting substrate at which the specific growth rate is half of the maximum.

The modeling was performed using the Python programming language. The results were visualized using the Matplotlib library.

Results and discussion

One of the key advantages of kinetic modeling is the ability to predict the reactions of systems to changes in internal or external conditions. This approach allows solving practical problems, such as process optimization or the development of new technologies, in particular in biotechnology, medicine, agriculture, and other areas.

Kinetic modeling helps to reveal the mechanisms of action of biological systems, which is key to understanding the basic principles of their functioning. The development and construction of mathematical models and their analysis helps us to identify and explain internal relationships in biological processes.

To model the microbiological processes of biosurfactant production, we used the concentration of the key source of carbon in the culture medium –

glycerol (Fig. 1). The initial parameters μ_{max} and K_s for the kinetic model were taken for *Bacillus subtilis* [11].

It is known that the Monod kinetic model, shown in Fig. 1, is widely used in biological processes due to its versatility. For this study, the values of the specific microbial growth rate μ_{max} and the saturation constant K_s were taken to be 19.99 days^{-1} and 9.9995 g/L, respectively [11].

According to our experimental and modeled data (Fig. 1), the maximum growth rate of *Bacillus spp.* cells depends on the concentration of glycerol in the medium. Thus, within the glycerol concentration of 30–40 g/l in the culture medium, intensive growth of microorganisms is observed, while further increase in the glycerol concentration leads to insignificant bacterial growth. Further cell growth is insignificant, which is apparently regulated by cell maintenance and cell death.

In the exponential growth phase, cells grow intensively that is accompanied by an increase in cell mass. We can to count the number of cells at time *t*:

$$X = X_0 e^{\mu t}, \tag{2}$$

where: X is the concentration of cells at time t; X_0 is the initial cell concentration; μ is the specific growth rate of microorganisms.

Fig. 2 shows the dynamics of bacterial cell formation depending on the cultivation time and glycerol content in the medium. According to the model results, the maximum cell mass can theoretically be achieved on day 10 of cultivation.

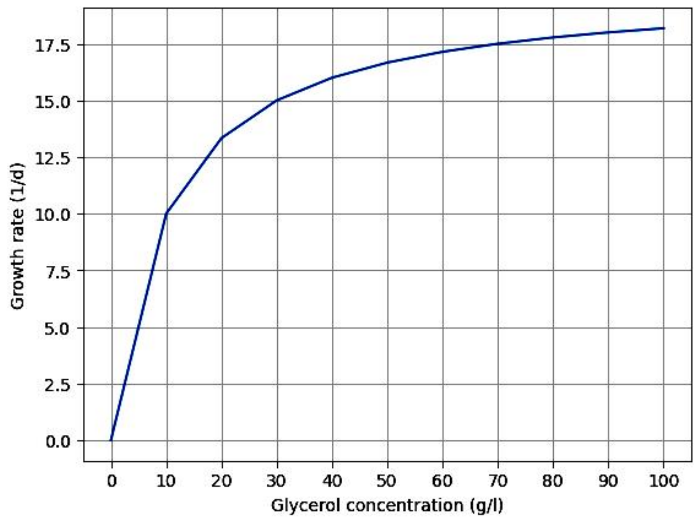

Fig. 1. Kinetic Monod model of the Bacillus spp. growth as a function of glycerol concentration in the culture medium

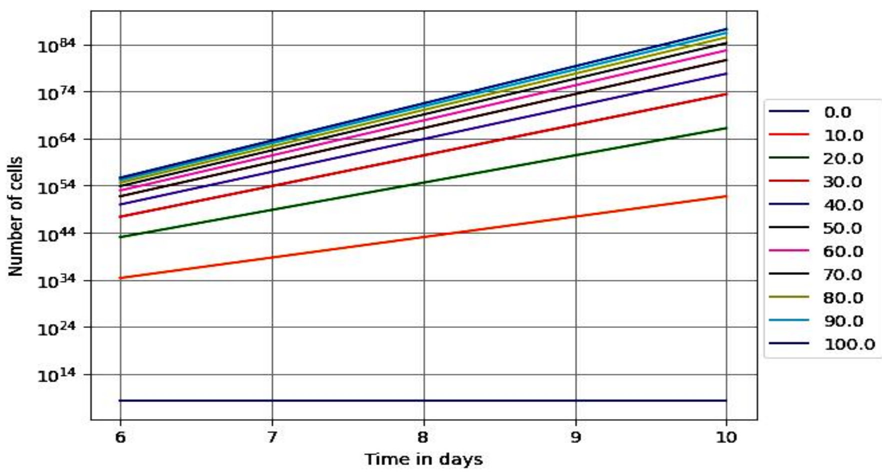

Fig. 2. The number of microbial cells of Bacillus spp. depending on the duration of cultivation and the glycerol concentration in the medium

Currently, there is sufficient data in the literature on the use of the Monod model to determine kinetic parameters, in particular, biomass growth rate and substrate utilization. For example, Ayub Md Som et al. (2021) used the Mono model to describe the processes of microbial wastewater treatment [12].

Malik A. Dawi et al. (2022) used the Monod equation to model the growth of microbial biomass with a limited supply of nutrients in the medium. They successfully used this methodology to reconstruct two studies of induced degradation of organic compounds in porous media by microorganisms: observations of the kinetics of *Pseudomonas putida* F1 during cultivation on benzene, toluene, and phenol in batch reactors and studies of carbon tetrachloride biodegradation by the denitrifying bacterium *Pseudomonas Stutzeri* KC [13].

The next step was to determine the ability of the *Bacillus spp.* to synthesize biosurfactants on nutrient media with a nitrogen source ($NaNO_3$) and with the addition of sodium citrate (4.0 g/l) or potassium citrate to the medium (Fig. 3).

Our results have shown that culture of *Bacillus spp.*, which are growing on the above-mentioned media, produces biosurfactants and their content ranges from 1.05 to 1.47 %. It is important to note that the maximum concentration of biogenic surfactants is reached on the 8th day of cultivation.

It is well known that one of the main characteristics that determines the practical application of biogenic surfactants is their ability to foam. Biosurfactants are used in cosmetics and pharmaceuticals, in foam systems for extinguishing fires, and in the food industry. Another important characteristic of foam is its stability, which depends on the ability of the foaming agent to form stable interfacial films and a viscous continuous phase. Although almost all surfactants are capable of foaming, not all of them can form stable foams.

The results of the study of foaming of products based on *Bacillus spp*. obtained during cultivation on nutrient media with glycerol and citrate are shown in Fig. 4.

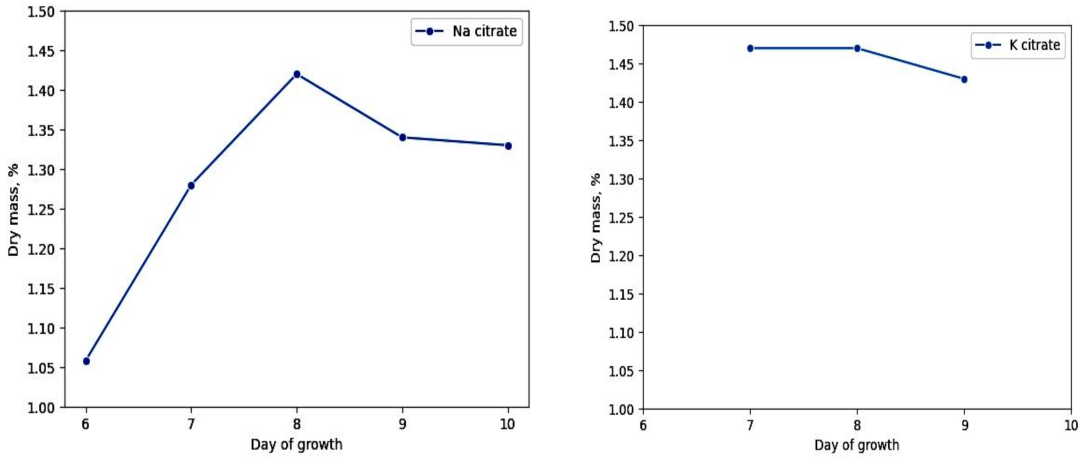

Fig. 3. Dependence of the content of synthesized biosurfactant on the duration of cultivation

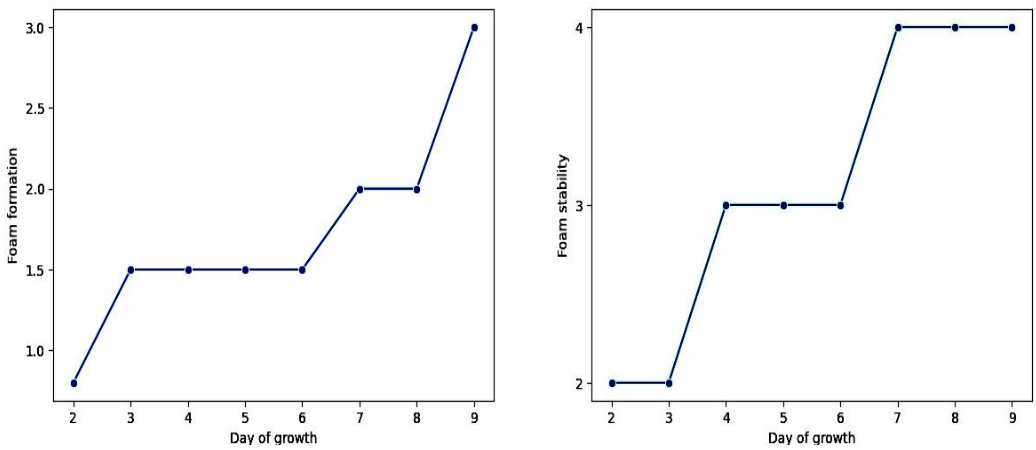

Fig. 4. Dependence of foaming and its stability on the duration of cultivation

The obtained results showed that both foaming and foam stability, as a characteristic of biosurfactants synthesized by *Bacillus spp.*, significantly depend on the duration of microorganisms cultivation. Thus, foaming increases on the 3rd day of cultivation and reaches its maximum on the 9th day, while the maximum value of foam stability was recorded on the 7th day. Thus, it can be argued that biogenic surfactants, obtained with B*acillus spp.* bacteria, provided a sufficient level of foaming and foam stability. It is known that these characteristics depend on the chemical structure of biosurfactants [1].

The relationships between the studied parameters are shown in Fig. 5. This is the Pearson correlation matrix, which indicates the degree of relationship between two variables and allows us to determine how much and in what direction one variable changes relative to another.

Pearson's correlation calculates a linear relationship between two variables and takes a value in the range from –1 to 1. A value close to 1 indicates a positive linear relationship: when one variable increases, the other variable increases accordingly. A value close to –1 indicates a negative linear relationship. A value close to 0 indicates that there is no linear relationship between the variables.

Based on the presented correlation matrix, we can conclude that all variables show a strong positive correlation.

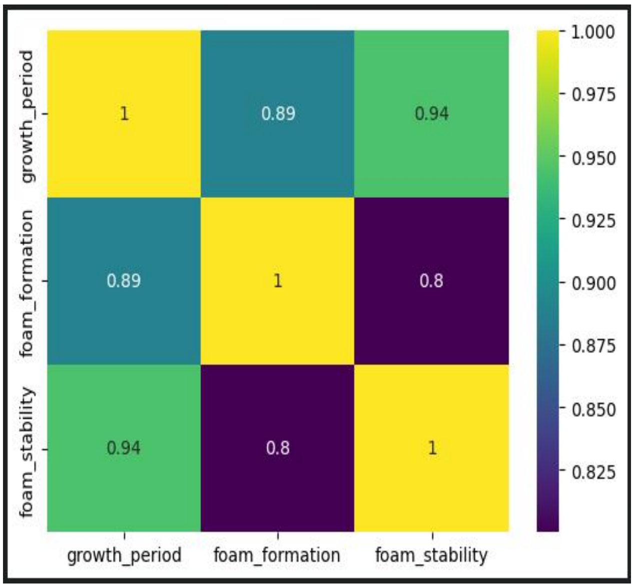

Fig. 5. The interrelationships of biosurfactant synthesis in the form of Pearson correlation

Conclusions

Kinetic Monod model of the *Bacillus spp.* growth on the glycerol contained culture medium was developed.

Our finding based on experimental and modeled data, have been shown that the growth rate of microorganisns depends on the glycerol concentration in the medium. The concentration of glycerol in the medium at the level of 30–60 g/L ensures optimal growth of *Bacillus spp.* cells.

Bacillus spp. culture, growing on media containing glycerol and NaNO3, as a source of carbon and nitrogen, produces biosurfactants which content ranges from 1.05 to 1.47 %. The highest concentration of biogenic surfactants is reached on the 8th day of cultivation.

The biosurfactants obtained by microbial synthesis of *Bacillus spp.* provided a sufficient level of foaming and foam stability. The peak of foaming was observed on the 9th day while the maximum value of the foam stability – on the 7th day.

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МОДЕЛЮВАННЯ СИНТЕЗУ БІОСУРФАКТАНТІВ З ВИКОРИСТАННЯМ *BACILLUS ssp.*

Бактерії роду *Bacillus* продукують біогенні поверхнево-активні сполуки, які є альтернативою синтетичним. Вихід цільових продуктів істотно залежить від умов культивування мікроорганізмів, особливо від компонентів живильного середовища. У цьому дослідженні ми використовували штам *Bacillus spp.*, здатний рости в середовищі, що містить гліцерин. Для визначення кінетики росту бактерій залежно від концентрації поживних речовин у середовищі ми використовували модель Моно. Результати показали, що інтенсивний ріст бактерій *Bacillus spp.* спостерігається у разі їх культивування у середовищі з концентрацією гліцеролу 30–40 г/л. Поверхнево-активні речовини, отримані за допомогою *Bacillus spp.*, характеризувалися достатнім рівнем піноутворення та піностабільності. Найвище значення піностійкості спостерігали на сьому добу культивування.

Ключові слова: біосурфактанти; модель Моно; піноутворення; стабільність піни.