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IMPACT OF BISPHENOL A IN POWDER FORM ON THE DEVELOPMENT OF *CORYNEBACTERIUM GLUTAMICUM* AND *MICROCOCCUS LUTEUS*

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Bisphenol A (BPA) is an important monomer in the production of polycarbonate plastic and its derivatives. The daily and widespread use of BPA-containing products has led to its wide distribution as a contaminant and xenobiotic in water, soil, and the atmosphere. Its impact is associated with disruptions in the endocrine, nervous, immune, and reproductive systems. Currently, methods for effective removal of BPA from the environment are actively being researched, including through enzymatic activity of microorganisms. Literature provides numerous data on the effects of dissolved xenobiotics on microbial viability, but there is a lack of information on the effects of solid powdered BPA. This study investigated the impact of granular BPA at concentrations significantly exceeding those found in soils on the growth and lignin peroxidase activity of Corynebacterium glutamicum and Micrococcus luteus.

It has been established that the pollutant in powdered form is capable of inhibiting the growth of both studied prokaryotic species within just 24 hours of cultivation. The diameter of the lysis zones ranged between 0.4-0.7 cm for M. luteus and 0.5-0.9 cm for C. glutamicum, depending on the dose of the pollutant applied. For C. glutamicum, a prolonged destructive impact of the compound was noted, evidenced by an increase in lysis diameter up to 168 hours into the experiment. In contrast, no definitive pattern was observed for M. luteus – maximum growth inhibition was observed at 48 hours, with no significant differences noted thereafter.

It has been observed that the introduction of powdered BPA in all studied concentrations, particularly at 7.5 mg/mL in liquid nutrient media, promotes the growth of microorganisms and increases the content of total protein and the activity of lignin peroxidase. These results are likely explained by the action of bisphenol A on microorganisms as a stress factor. Under these conditions, it is probable that protective mechanisms of bacteria, including those that aid in the utilization of bisphenol A, begin to be synthesized and activated..

Keywords: bisphenol A (BPA), BPA exposure, chemical pollutants, microbial development, bioremediation.

Introduction. Bisphenol A (BPA) is one of the earliest synthetic compounds, widely used as a precursor for epoxy resins and present in many consumer products. Alongside its use, there is a growing body of evidence regarding the adverse effects of BPA on various life processes, manifesting at different levels of organization - from cellular to biogeocenotic. Notably, bisphenol A is known to act as an endocrine disruptor, affecting hormonal balance (Nomiri, 2019), adversely affecting the reproductive system by reducing fertility, and exhibiting neurodegenerative effects that impair cognitive function. Furthermore, BPA is associated with metabolic diseases such as diabetes and obesity, and it can affect the functions of both the innate and adaptive immune systems (Molina-López, 2023). At the cellular level, BPA disrupts redox homeostasis, causes mitochondrial dysfunction, and induces apoptosis (Lee, 2018). The compound can accumulate in mammalian tissues, particularly in adipose tissue, due to its lipophilic properties (Besaratinia, 2023), and it can also accumulate in aquatic organisms (Sirasanagandla, 2022). In plants, BPA has been shown to inhibit photosynthesis (Kim, 2018).

Environmental contamination with bisphenol A is becoming an increasingly acute problem. It has been established that approximately 56 μg/l BPA can enter the body from water sources, from soil the range is between 1 to 150 μg/kg, and from air, individuals can inhale between 2 to 208 ng/m³ BPA (Manzoor, 2022). Based on new scientific data, EFSA experts have set a tolerable daily intake of bisphenol A at 0.2 ng/kg body weight per day, replacing the previous temporary value of 4 μg/kg body weight per day.

Reduction of BPA concentration in ecosystems can be achieved through the ability of certain microorganisms to transform it into less toxic substances. There are many microorganisms capable of degrading bisphenol A. Certain prokaryotes can use BPA as their sole source of carbon and energy. These include both gram-negative bacteria such as *Sphingomonas sp., Pseudomonas sp., Achromobacter sp., Novosphingobium sp., Nitrosomonas sp., Serratia sp., Bordetella sp., Alcaligenes sp., Pandoraea sp., Klebsiella sp*., and *Cupriavidus sp.,* as well as gram-positive bacteria like *Streptomyces sp. and Bacillus sp*. (Yu K., 2019). The mechanism of BPA degradation by

microorganisms may involve adsorption onto bacterial cell surfaces through electrostatic forces and/or enzymatic action (Endo, 2007). An important role in the transformation of BPA is played by lignin peroxidase (1.11.1.14) - a heme-containing enzyme, an H_2O_2-dependent oxidoreductase capable of cleaving a wide range of substrates with phenolic and non-phenolic structures (Singh, 2021).

The half-life of BPA varies from 4.5 to 4.7 days depending on the environment in which the compound is found (Santos, 2023). In water and soil, its half-life is approximately 5 days, while in air, it is less than one day.

However, BPA exhibits a toxic effect on the viability of the prokaryotes themselves, inhibiting their growth, development, and metabolic activity (Matsumura, 2015).

We were particularly interested in the widely distributed species *Corynebacterium glutamicum* and *Micrococcus luteus*. *C. glutamicum* is a classic research subject used in the industrial production of amino acids (L-glutamate and L-lysine) and has been found in soil. *Micrococcus luteus*, a gram-positive coccus from the family *Micrococcaceae*, is widespread in soil, air, and water. *M. luteus* is known for its potential to biodegrade various organic compounds, making it valuable for environmental cleanup of toxic substances (Kuyukina, 2016).

Materials and Methods. The experimental study was conducted using pure cultures of *C. glutamicum* and *M. luteus*. To obtain working bacterial cultures, the bacteria were grown in liquid nutrient broth for 24 hours at 37°C.

The effect of bisphenol A on the growth and development of prokaryotes was studied on an agar medium in Petri dishes. BPA polymer particles were spread on the surface of the nutrient medium at concentrations of 2.5, 5, and 7.5 mg/mL. Microorganism cultivation was conducted in a thermostat for 24 hours at 37°C. The colony lysis diameter was measured from 24 to 168 hours after the start of the experiment.

To determine the enzymatic activity, microorganisms were cultivated in 250 ml Erlenmeyer flasks. The volume of the liquid nutrient medium was 50 ml, the concentration of bisphenol A was 2.5, 5 and 7.5 mg/ml. The volume of the inoculum was 10% of the total volume of the culture medium. Cultivation conditions were identical to those previously described.

The biomass was separated from the culture liquid by centrifugation for 15 minutes at 3500 rpm. Subsequent manipulations were carried out using the supernatant fluid.

To determine lignin peroxidase activity, methylene blue was used as a substrate. The reaction mixture consisted of 2.2 ml enzyme extract, 0.1 ml methylene blue (1.2 mM), 0.6 ml sodium acetate buffer (0.5 M, pH = 4), and 0.1 ml H2O2 (2.7 mM) (Ingale, 2021). Under these conditions, the amount of enzyme required to oxidize 1 micromole of methylene blue per minute was defined as one unit of enzyme activity (U). Spectrophotometry was performed at a wavelength of 664 nm.

Total protein content was determined using the Lowry method (Lowry, 1951).

The research results were statistically analyzed using Microsoft Excel software.

Results and Discussion. In the initial stage of observations, the study examined the impact of powdered BPA on the growth and development of microorganisms. Experimental data indicated that the introduction of this xenobiotic form into the nutrient medium inhibited the growth of both bacterial species, regardless of concentration. Absence of colony growth was observed as early as 24 hours of cultivation. The diameter of lysis ranged from 0.4-0.7 cm for *M. luteus* and 0.5-0.9 cm for *C. glutamicum*, depending on the dose of the pollutant introduced (Fig. 1). Maximum inhibition of *M. luteus* growth due to BPA was recorded after 48 hours. *C. glutamicum* exhibited prolonged detrimental effects, with enhanced inhibition of colony formation observed even after 168 hours of observation.

Bisphenol A enters the environment due to the breakdown of polymer materials. Research on the influence of bisphenol A in its ethanol/methanol-soluble form suggests that certain bacteria have the ability to metabolize bisphenol A (Jia, 2020). Nonetheless, the compound also negatively affects other prokaryotes, inhibiting their growth and development. The likely cause of this is bisphenol A's ability to disrupt the structure and function of mitochondria, as well as enhance reactive oxygen species (ROS) production and oxidative stress (Kobroob, 2018). According to research (Hac-Wydro, 2019), bisphenol A can interact with lipid components of Gram-positive bacteria, which may also be a significant factor in terms of compound cytotoxicity. We observed a similar effect when introducing the polymeric form of the pollutant into agarized medium.

To determine the total protein content and lignin peroxidase activity facilitating bisphenol A utilization, liquid culture media were utilized. According to research findings, the presence of BPA in the culture liquid resulted in increased levels of both total protein and lignin peroxidase activity. The most significant changes were observed at the highest tested concentration of 7.5 mg/ml, particularly with more pronounced alterations during

the cultivation of *C. glutamicum*. In this case, the total protein content increased by 1.6 times, while lignin peroxidase activity increased by more than 3 times.

These results are likely explained by the action of bisphenol A on microorganisms as a stress factor. Under such conditions, it is probable that bacteria initiate the synthesis and activation of defensive mechanisms, including certain exogenous enzymes (Tian, 2022), which contribute to the utilization of bisphenol A.

Additionally, as mentioned earlier, BPA can interact with the cell wall or membranes, causing damage and compromising their integrity. This can lead to increased membrane permeability and activation of compensatory mechanisms, including enhanced synthesis of membrane proteins, which also contributes to the increase in total protein content within cells.

The increased activity of lignin peroxidase in the presence of BPA is indicative of a stress response in bacteria aimed at neutralizing the toxic effects of BPA and protecting cells from oxidative damage.

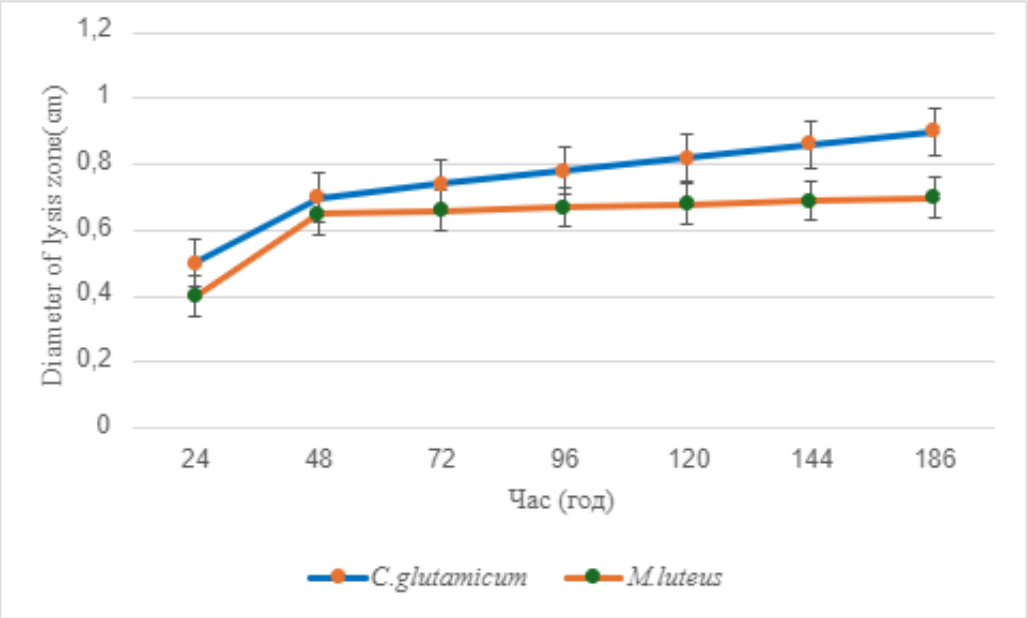

Fig. 1. Effect of powdered BPA (5 mg/ml) on colony formation activity of *M. luteus* and *C. glutamicum*.

Conclusions. Therefore, the addition of granular Bisphenol A induces an inhibitory effect on prokaryotic development, resulting in the lysis of *C. glutamicum* and *M. luteus* colonies within 24 hours of cultivation. In the cultural medium, there is an elevation in total protein content and the activity of the pollutant-utilizing enzyme lignin peroxidase, suggesting the potential application of these studied cultures in bioremediation technologies for environments contaminated with bisphenol A.

Interests disclosure. *The authors declare no conflict of interest.*

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ВПЛИВ ПОРОШКОПОДІБНОГО БІСФЕНОЛУ А НА РОЗВИТОК *CORYNEBACTERIUM GLUTAMICUM* ТА *MICROCOCCUS LUTEUS*

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Бісфенол А (BPA) є важливим мономером у виробництві полікарбонатного пластику та його похідних. Щоденне та повсюдне використання BPA-продуктів призвело до широкого розповсюдження BPA у воді, ґрунті та атмосфері, що визначає його як полютанта та ксенобіотика. Вплив хімічної речовини пов'язаний з порушенням роботи ендокринної, нервової, імунної та репродуктивної систем. Сьогодні активно досліджуються методи ефективного видалення бісфенолу А з навколишнього середовища, в тому числі за рахунок ферментативної активності мікроорганізмів. У літературі зустрічаються чисельні дані щодо впливу

розчиненого ксенобіотику на життєдіяльність мікроорганізмів, проте відсутні дані про ефекти, що спричиняє бісфенол А у твердій порошковій формі. У роботі досліджували впливу порошкоподібного бісфенолу А у концентраціях, що значно перевищують виявлений вміст у грунтах, на розвиток та лігніннероксидазну активність Corynebacterium glutamicum та Micrococcus luteus.

Встановлено, що полютант в порошковій формі здатен інгібувати розвиток обох досліджуваних видів прокаріот уже через 24 год культивування. Діаметр лізису колоній варіював в межах 0,4-0,7 см для M. luteus та 0,5-0,9 см для C. glutamicum, залежно від внесеної дози полютанта. Для C. glutamicum зафіксовано пролонгований деструктивний вплив сполуки, вважаючи на зростання діаметру лізису колоній аж до 168 год експерименту. Для M. luteus, максимальний гальмівний ефект встановлений через 48 год культивування, надалі достовірних відмінностей показника не візначали.

Відзначено, що при внесенні порошкової форми БФА в усіх досліджуваних концентраціях, найбільшою мірою у концентрації 7,5 мг/мл в рідке поживне середовище, при культивуванні мікроорганізмів зростають і вміст загального протеїну і активність лігніннероксидази. Ймовірно, що такі результати можна пояснити дією бісфенолу А на мікроорганізми як стресового чинника. За таких умов, ймовірно, починають синтезуватися та активізуватися захисні механізми бактерій, в тому числі й ті, що сприяють утилізації бісфенолу А.

Ключові слова: бісфенол А (BPA), вплив BPA, хімічні забруднювачі, розвиток мікроорганізмів, біоремедіація.

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