https://doi.org/10.31861/biosystems2023.02.138
UDK 606:502]:[628.4.043:582.282.23+579.861+579.871

PECULIARITIES OF BISPHENOL A UTILISATION BY ENZYMES RHODOTORULA MINUTA, SACCHAROMYCES CEREVISIAE, CORYNEBACTERIUM GLUTAMICUM, MICROCOCCUS LUTEUS

M.V. VORONKA, L. M. VASINA

Yuriy Fedkovych Chernivtsi National University,
Ukraine, 58012, Chernivtsi, Kotsiubynsky 2 Str.
e-mail: l.vasina@chnu.edu.ua

One of the most serious problems today is environmental pollution by plastic and its derivatives. It is known that bisphenol A (BPA) is a widespread compound used in the production of various polymeric materials. When it gets into water, soil, air, food and beverages, it causes numerous toxic effects in the body. Bisphenol A disrupts the functioning of the endocrine, reproductive, immune, nervous, and respiratory systems (Kosior E., Crescenzi I., 2020). When ingested by the animal body through the diet, BPA can be absorbed by cells of the gastrointestinal tract and liver, where it undergoes biotransformation to form bisphenol A-glucuronide, and less commonly bisphenol A-sulfate (Durovcova I. et al., 2022). Little is known about the impact of BPA on the development of microorganisms; the possibility of converting the polutant by enzymes of a number of gram-negative bacteria and microscopic fungi has been reported (Ingale S. et al., 2021).

Today, effective and cost-effective strategies are being actively sought to remove xenobiotics from the environment and prevent their entry into the human body through trophic chains. Biodegradation is one of the most advanced technologies available today. Due to the efficient extracellular enzyme systems of ligninolytic and non-ligninolytic microscopic fungi and bacteria, BPA can be biotransformed to form non-toxic products (Daassi D. et al., 2016).

We have studied the possibility of biodegradation of bisphenol A by Rhodotorula minuta, Saccharomyces cerevisiae, Corynebacterium glutamicum, Micrococcus luteus. It was found that the cultivation of microorganisms on media containing bisphenol concentrations exceeding its MPC in the environment was characterised by a decrease in the accumulation of their biomass and colony formation. The introduction of 0.3, 0.4, 0.5, 3, 4, 5 mg/l of bisphenol A into the culture medium led to an increase in laccase, Mn-peroxidase and lignin peroxidase activities, most of all in R. minuta. The ability to utilise the polutant in the environment by the studied microorganisms is different. Studies indicating the indirect ability to convert bisphenol A by these gram-positive bacteria and fungi showed that the enzymatic systems of R. minuta utilised, on average, 13 % of the introduced xenobiotic, and M. luteus - about 6 % of BPA.

Keywords: bisphenol A, xenobiotic, oxidoreductases, biodegradation.

Introduction. The high level of anthropogenic impact on the environment causes environmental problems, the solution of which is one of the main tasks of modern society (Welden N. A., 2020). The amount of plastic polymers containing bisphenol A (BPA) is constantly increasing in the environment. Bisphenol A is an organic synthetic compound belonging to the group of diphenylmethane derivatives. BPA-based plastics are transparent and durable, and can be moulded into a variety of consumer products, such as water bottles, sports equipment, and computer accessories (Kosior E., Crescenzi I., 2020).

Generally, food-grade plastic materials consisting of polycarbonate and epoxy resins are the main source of bisphenol A in the human body. It is both a carcinogenic and a general toxic factor, affecting the functioning of the endocrine, nervous, cardiovascular, excretory, immune, and reproductive systems. BPA has a negative impact on the development and reproduction of animals, with some species being particularly sensitive, such as invertebrates, amphibians and fish (Rubin A. M., Seebacher F., 2022). In addition, BPA causes changes in the biochemical and microbial balance of soil, and disrupts plant growth and development (Tarafdar A. et al., 2022).

BPA has antibacterial properties against both gram-positive and gram-negative bacteria (Ma Y. et al., 2019). Although this can be seen as a positive effect in certain applications, it can also be negative, as it disrupts the balance of microbial consortia, e.g. in the human and animal gut, autochthonous microflora of water, soil, and plant organs. Bisphenol results in disruption of the metabolic activity of microorganisms, which in turn determines the intensity of organic matter breakdown, nutrient cycling, etc.

It is possible to reduce the content of hazardous xenobiotics in the environment by using biodegradation technologies. Biodegradation is a natural process of breaking down organic matter by the enzymes of living organisms such as bacteria, fungi and other microorganisms (Sarma H. et al., 2019). Usually, xenobiotics of organic nature are used by them as sources of energy and carbon. These organisms break down substances into smaller components that can be reintegrated into the natural environment. Biodegradation technologies are used to purify water and soil, preserve agricultural products, and wood. One of the advantages of biodegradation technologies is their environmental friendliness, as they usually do not produce secondary, including toxic, waste generated by other methods of treatment and recovery.

To date, several mechanisms of BPA utilisation by microorganisms are known, including hydrolysis, reduction, oxidation, and conjugation, which are carried out by cellular and extracellular enzymes.

Our attention was drawn to microorganisms that are distributed in various ecological niches, capable of metabolising different sources of carbon and nitrogen and rapidly increasing biomass, and are undemanding to cultivation conditions, such as *Corynebacterium glutamicum, Micrococcus luteus, Rhodotorula minuta* and *Saccharomyces cerevisiae*.

Therefore, the aim of the study was to investigate the possibility of biodegradation of bisphenol A by the enzymes *Corynebacterium glutamicum, Micrococcus luteus, Rhodotorula minuta, Saccharomyces cerevisiae*. At the same time, to analyse the peculiarities of microbial development on media containing bisphenol A, the concentration of which exceeds the maximum permissible standards, to determine the laccase, Mn-peroxidase, lignin peroxidase activities of microorganisms and to evaluate the efficiency of bisphenol A biodegradation by microbial enzymes.

Materials and methods. Experimental studies were carried out on pure cultures of *C. glutamicum, M. luteus, R. minuta*, and *S. cerevisiae* (kindly provided by the staff of the D.K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine).

To obtain the inoculum, yeast was cultured in Sabouraud's liquid medium for 48 h at 28 °C, and bacteria in nutrient broth for 24 h at 30 °C. The main fermentation was carried out in 250 ml Erlenmeyer flasks in liquid media under similar cultivation conditions for 72 h for yeast and 48 h for bacteria. The inoculum content was 10%.

The effect of bisphenol A on the accumulation of microbial biomass was assessed in liquid medium with the addition of bisphenol A at concentrations of 0.03, 0.04, 0.05, 0.3, 0.4, 0.5, 3, 4, 5 mg/l during the main fermentation. The biomass was separated by centrifugation at 3500g for 10 min. The biomass accumulation was determined by measuring the dry weight (dried at 60 °C to a constant weight).

The number of colony-forming units of microorganisms was assessed on agarified media in Petri dishes by counting colonies.

Determination of laccase, Mn-peroxidase, ligninperoxidase activities and evaluation of the efficiency of bisphenol A utilisation by microorganisms was carried out by the spectrophotometric method (Ingale S. et al., 2021). The microorganisms were cultivated in a mineral-salt medium containing the studied concentrations of bisphenol A. The results of the experimental data were processed statistically using Microsoft Excel software. At the same time, the results were reliable at the level of reliability $p \leq 0.05$ according to the Student's criterion.

Results and discussion. Bisphenol A is a poorly soluble anthropogenic xenobiotic. The solubility of bisphenol A is a critical aspect that affects its environmental fate, transport and potential risks to living organisms and the environment. Its solubility depends on various factors such as temperature, pH and the presence of other dissolved substances. Taking into account the data from the literature (Moussavi G. et al., 2019), acetone was used as a solvent for experimental studies.

The expediency of choosing the studied concentrations is based on the MPC of bisphenol A microplastics in the media. The polutant is characterised by its local prevalence in open areas in amounts equal to or exceeding the MPC (Molina-Lopez A. M. et al., 2023). It can quickly enter fresh and marine water bodies. Destructive effects occur not only due to direct exposure to lethal/sublethal concentrations of the xenobiotic, but also to its decay products at optimal concentrations (Welden N. A., 2020).

At the first stage of our research, we studied the effect of bisphenol A in the culture medium on biomass accumulation and colony formation intensity after 48-72 hours of cultivation.

Significant changes in biomass were observed at all the indicated concentrations of bisphenol A, which were equal to or 10 and 100 times higher than the maximum permissible levels established for water (Fig. 1).

When bisphenol A was added to the microbial cultivation medium at a concentration equal to the maximum permissible dose, a 65-75% decrease in biomass accumulation was recorded. In particular, the biomass of *C. glutamicum* was on average 38 %, *M. luteus* - 33 %, *R. minuta* - 32 %, *S. cerevisiae* - 26 %.

It should be noted, in general, the higher resistance of the studied prokaryotes compared to microscopic fungi. Comparison of the effects of the studied BPA concentrations revealed a higher survival rate of prokaryotes, which was 1.3-1.4 times higher than that of eukaryotes.

Under the influence of bisphenol at concentrations of 0.3, 0.4, 0.5 mg/l, the decrease in biomass was even more critical and amounted to 17 % of the control values for *C. glutamicum*, 16 % for *M. luteus*, 15 % for *R. minuta*, and 13 % for *S. cerevisiae*.

The fact of different values in the accumulation of biomass by microorganisms under the influence of the highest studied concentrations is noteworthy. Lower eukaryotes were more resistant. The amount of biomass of *R. minuta* was, on average, 8 % of the control values, S. cerevisiae - 6 %.

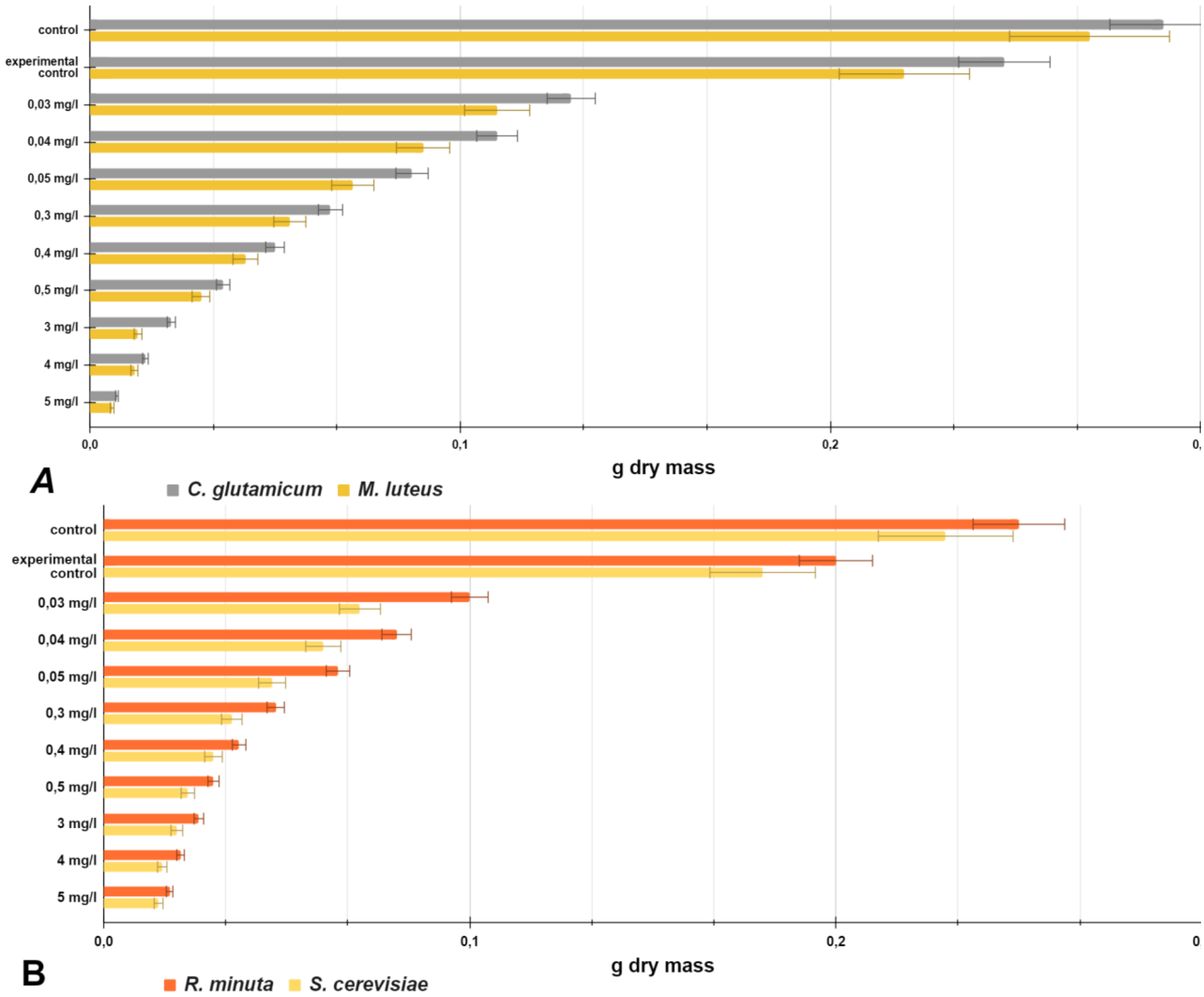

Fig. 1. Effect of bisphenol A on biomass accumulation of bacteria (A) and microscopic fungi (B)

Note: * - statistically significant difference compared to the control, p≤0.05

The use of solid nutrient media made it possible to determine the number of live cells of bacteria and microscopic fungi in the presence of bisphenol A. We noted the following patterns:

- the effect of bisphenol A caused a more significant toxic effect on the vital activity of lower eukaryotes (intensity of colony formation) compared to prokaryotes at concentrations of bisphenol A of 0.3, 0.4, 0.5 mg/l

-at concentrations of bisphenol A 100 times higher than the MPC (3, 4, 5 mg/l), a lower intensity of prokaryotic colony formation was recorded compared to microscopic fungi. For all the species studied, their number differs by 25-33%;

- The most resistant cultures to bisphenol A were *C. glutamicum* at bisphenol A concentrations 10 times higher than the MPC and *R. minuta* at bisphenol A concentrations 100 times higher than the MPC. This feature is especially evident at high concentrations, at which the number of live cells of *Rhodotorula* is at least twice as high as that of *Corynebacterium*.

Literature data (Ingale S. et al., 2021; Sarma H. et al., 2019; Eio E. J. et al., 2015) indicate that various types of bacteria, fungi, algae, which are autochthonous representatives of the microflora of soils, fresh and marine waters, are capable of degrading BPA. The ability of these microorganisms to degrade bisphenol A is due to enzymes responsible for the degradation or metabolism of bisphenol, such as laccase, Mn-peroxidase, lignin peroxidase, UDP-glucuronosyltransferase, sulfotransferase, polyphenol oxidase, cytochrome P450 and others (Moussavi G. et al. 2019). Among these oxidoreductases, the most interesting are laccase, Mn-dependent peroxidases, lignin peroxidases, the activity of which was noted in the study of gram-negative bacteria and microscopic fungi. It is believed that these three extracellular enzymes play the most important role in the degradation of environmental bisphenol A.

The corresponding exogenous enzymatic activity was detected in the culture fluid of all 4 microorganisms studied. According to the data of experimental studies, reliable changes in Mn-dependent peroxidase, lignin peroxidase and laccase activities were observed at all the studied concentrations of bisphenol A, which were 10 and 100 times higher than the maximum permissible limits. Probably, the presence of bisphenol A in the environment led to the synthesis of low- and high-molecular weight components and activation of enzymes involved in the decomposition of polutants (Ma Y. et al., 2019).

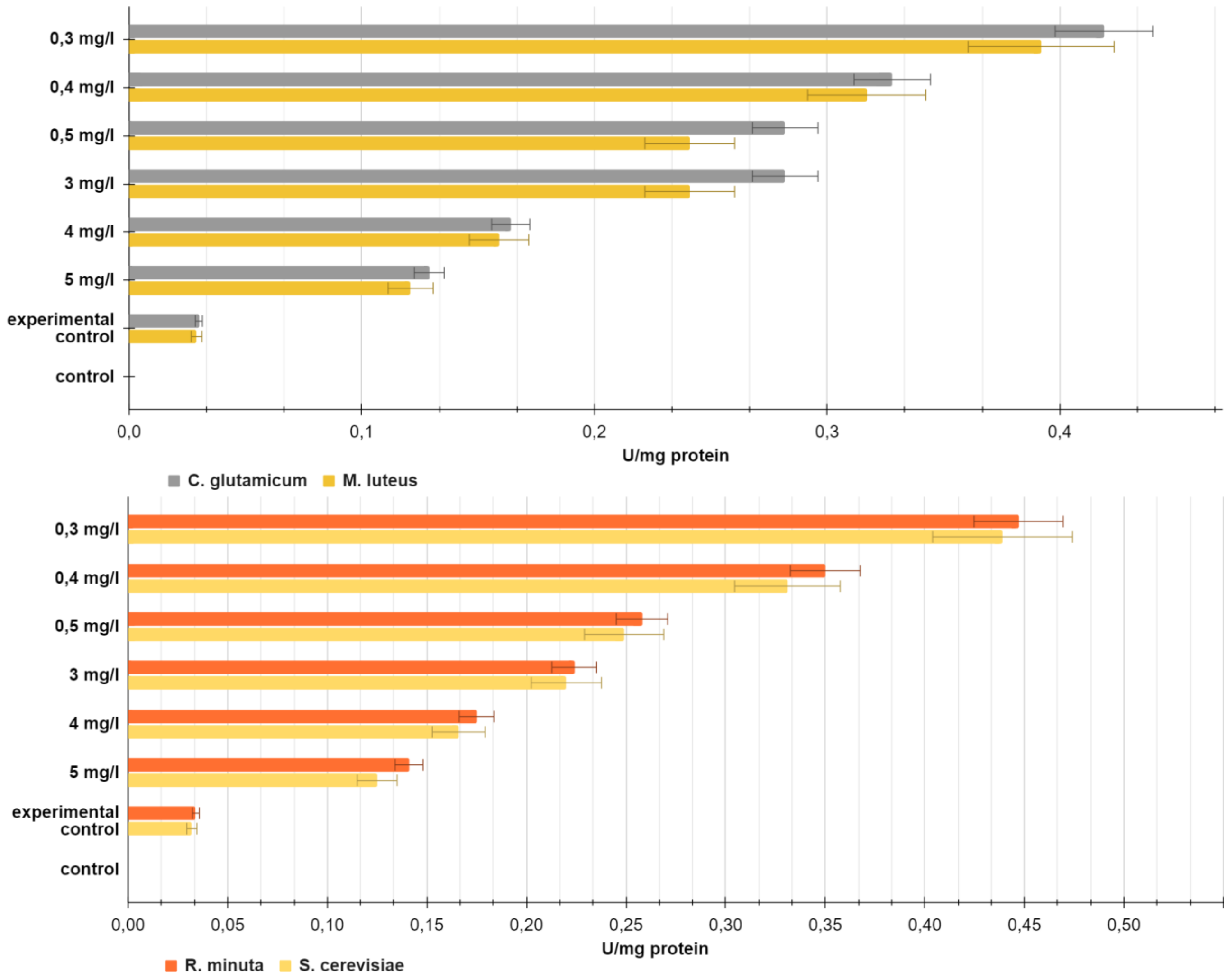

Fig. 2. Ligninperoxidase activity of microorganisms in culture media containing BPA after 24 hours of cultivation

These trends confirm the decrease in the enzymatic activity of the studied bacteria and microscopic fungi under the influence of the chemical polutant due to the possible development of destructive cell processes as a result of exposure to high concentrations of bisphenol A. Among them, there may be an increase in the content of reactive oxygen species (ROS), increased lipid peroxidation and DNA damage (Durovcova I. et al., 2022).

It was noted that the highest enzymatic activity is inherent in rhodotorulas under the influence of all experimental concentrations of BPA.

The use of a liquid unified mineral-salt medium, which is limited by the carbon source, made it possible to investigate the probability of bisphenol A utilisation by bacteria and microscopic fungi as the only source of carbon in the culture medium. We noted the following regularities: the efficiency of bisphenol A detoxification by microorganisms depended on the duration of their cultivation; the efficiency of bisphenol A utilisation by enzymes of lower eukaryotes was generally higher than that of prokaryotes; the most efficient biotransformer of bisphenol A was R. minuta was the most efficient biotransformer of bisphenol A, especially at low concentrations, at which rhodotorola convert at least twice as much hazardous substrate as corynebacteria.

Conclusions. *R. minuta* has the greatest ability to utilise bisphenol A, as it actively transforms the xenobiotic in the culture medium. *S. cerevisiae, C. glutamicum* and *M. luteus* possess laccase, Mn-dependent peroxidase and lignin peroxidase activities, which allows their use as bioremediants of bisphenol A-contaminated environments, including those containing industrial or household waste.

References:

1. Bodor A., Bounedjoum N., Feigl G. et al. Exploitation of extracellular organic matter from *Micrococcus luteus* to enhance ex situ bioremediation of soils polluted with used lubricants. *Journal of Hazardous Materials*. 2021; 417: 125996. https://doi.org/10.1016/j.jhazmat.2021.125996
2. Daassi D., Prieto A., Zouari-Mechichi H. et al. Degradation of bisphenol A by different fungal laccases and identification of its degradation products. *International Biodeterioration & Biodegradation*. 2016; 110: 181–188. https://doi.org/10.1016/j.ibiod.2016.03.017
3. Durovcova I., Kyzek S., Fabova J. et al. Genotoxic potential of bisphenol A. *Environmental Pollution*. 2022; 306: 119346.
4. Eio E. J., Kawai M., Niwa C. et al. Biodegradation of bisphenol A by an algal-bacterial system. *Environmental Science and Pollution Research*. 2015; 22 (19): 15145–15153. https://doi.org/10.1007/s11356-015-4693-2
5. Eltoukhy A., Jia Y., Nahurira R. et al. Biodegradation of endocrine disruptor Bisphenol A by Pseudomonas putida strain YC-AE1 isolated from polluted soil, Guangdong, China. *BMC Microbiology*. 2020; 20 (1): 1–14. https://doi.org/10.1186/s12866-020-1699-9
6. Ingale S., Patel K., Sarma H., Joshi S. J. Bacterial Biodegradation of Bisphenol A (BPA). *Biotechnology for Sustainable Environment*. Singapore. 2021: 95–110. https://doi.org/10.1007/978-981-16-1955-7_4
7. Jarboui R., Houda Baati H., Fetoui F. Yeast performance in wastewater treatment: case study of *Rhodotorula mucilaginosa*. *Environmental Technology*. 2012; 33 (8): 951–960. https://doi.org/10.1080/09593330.2011.603753
8. Karimi M., Hassanshahian M. Isolation and characterization of phenol degrading yeasts from wastewater in the coking plant of Zarand, Kerman. *Brazilian Journal of Microbiology*. 2016; 47 (1): P. 18–24. https://doi.org/10.1016/j.bjm.2015.11.032
9. Kim D., Kwak J. I., An Y.-J. Effects of bisphenol A in soil on growth, photosynthesis activity, and genistein levels in crop plants (Vigna radiata). *Chemosphere*. 2018; 209: 875–882. https://doi.org/10.1016/j.chemosphere.2018.06.146
10. Kosior E., Crescenzi I. Solutions to the plastic waste problem on land and in the oceans. *Plastic Waste and Recycling*. 2020: 415–446. https://doi.org/ 10.1016/B978-0-12-817880-5.00016-5
11. Kyrila G., Katsoulas A., Schoretsaniti V. et al. Bisphenol A removal and degradation pathways in microorganisms with probiotic properties. *Journal of Hazardous Materials*. 2021; 413: 125363. https://doi.org/10.1016/j.jhazmat.2021.125363
12. Ma Y., Liu H., Wu J. et al. The adverse health effects of bisphenol A and related toxicity mechanisms. *Environmental Research*. 2019; 176: 108575. https://doi.org/10.1016/j.envres.2019.108575
13. Molina-Lopez A. M., Bujalance-Reyes F., Ayala-Soldado N. et al. An Overview of the Health Effects of Bisphenol A from a One Health Perspective. *Animals*. 2023; 13 (15): 2439. https://doi.org/10.3390/ani13152439
14. Moussavi G., Abbaszadeh Haddad F. Bacterial peroxidase-mediated enhanced biodegradation and mineralization of bisphenol A in a batch bioreactor. *Chemosphere*. 2019; 222: P. 549–555. https://doi.org/10.1016/j.chemosphere.2019.01.190
15. Myburgh M. W., Zyl van W., Modesti M. et al. Enzymatic hydrolysis of single-use bioplastic items by improved recombinant yeast strains. *Bioresource Technology*. 2023: 129908. https://doi.org/10.1016/j.biortech.2023.129908
16. Ray D., Anand U., Jha N.K. et al. The soil bacterium, *Corynebacterium glutamicum*, from biosynthesis of value-added products to bioremediation: A master of many trades. *Environmental Research*. 2022. P. 113622. https://doi.org/10.1016/j.envres.2022.113622.
17. Rubin A. M., Seebacher F. Bisphenols impact hormone levels in animals: A meta-analysis. *Sci Total Environ.* 2022; 828: 154533. https://doi.org/10.1016/j.scitotenv.2022.154533.
18. Sarma H., Nava A. R., Manriquez A. M. E. et al. Biodegradation of bisphenol A by bacterial consortia isolated directly from river sediments. *Environmental Technology & Innovation*. 2019; 14: 100314. https://doi.org/ 10.1016/j.eti.2019.01.008
19. Tarafdar A., Sirohi R., Balakumaran P. A. et al. The hazardous threat of Bisphenol A: Toxicity, detection and remediation. *Journal of Hazardous Materials*. 2022; 423: 127097. https://doi.org/10.1016/j.jhazmat.2021.127097
20. Welden N. A. The environmental impacts of plastic pollution. *Plastic Waste and Recycling*. 2020: 195–222. https://doi.org/10.1016/B978-0-12-817880-5.00008-6

ОСОБЛИВОСТІ УТИЛІЗАЦІЇ БІСФЕНОЛУ А ФЕРМЕНТАМИ RHODOTORULA MINUTA, SACCHAROMYCES CEREVISIAE, CORYNEBACTERIUM GLUTAMICUM, MICROCOCCUS LUTEUS

М.В. Воронка, Л. М. Васіна

Чернівецький національний університет імені Юрія Федьковича
вул. Коцюбинського, 2, Чернівці, 58012
e-mail: l.vasina@chnu.edu.ua

Однією з найсерйозніших проблем сьогодення є забруднення навколишнього середовища пластиком та його похідними. Відомо, що бісфенол А (BPA) – широко розповсюджена сполука, що використовується у виробництві різноманітних полімерних матеріалів. При потраплянні у воду, грунт, повітря, їжу та напої спричиняє чисельні токсичні ефекти в організмі. Бісфенол А порушує функціонування ендокринної, репродуктивної, імунної, нервової, дихальної систем (Kosior E., Crescenzi I., 2020). При поступанні в тваринний організм аліментарним шляхом, BPA може поглинатися клітинами шлунково-кишкового тракту та печінки, де піддається біотрансформації з утворенням бісфенол А-глюкуроніду, рідше бісфенол А-сульфату (Durovcova I. et al., 2022).

Мало відомо про вплив BPA на розвиток мікроорганізмів, повідомлялося про можливість перетворення полютанту ферментами ряду грамнегативних бактерій та мікроскопічних грибів (Ingale S. et al., 2021).

На сьогодні активно ведеться пошук ефективних та економічно доцільних стратегій, які б дозволяли видалити ксенобіотик з навколишнього середовища та запобігти його потраплянню в організм людини через трофічні ланцюги. Однією з сучасних передових технологій є біодеградація. Завдяки ефективним позаклітинним ферментним системам лігнінолітичних, нелігнінолітичних мікроскопічних грибів і бактерій можлива біотрансформація BPA з утворенням нетоксичних продуктів (Daassi D. et al., 2016).

Нами досліджувалася можливість біодеградації бісфенолу А мікроорганізмами Rhodotorula minuta, Saccharomyces cerevisiae, Corynebacterium glutamicum, Micrococcus luteus. Встановлено, що культивування мікроорганізмів на середовищах, які містили концентрації бісфенолу, що перевищували його ГДК у довкіллі, відзначалося зниженням накопичення їх біомаси та колонієутворення. Внесення 0,3; 0,4; 0,5; 3; 4; 5 мг/л бісфенолу А у культуральне середовище призводило до підвищення лакказної, Mn-пероксидазної та лігнінпероксидазної активностей, найбільшою мірою R. minuta. Здатність до утилізації полютанту в середовищі досліджуваними мікроорганізмами – відрізняється. Проведення досліджень, що свідчили про опосередковану можливість перетворювати бісфенол А зазначеними грампозитивними бактеріями та грибами, показали, що ферментативні системи R. minuta утилізували, в середньому, 13 % внесеного ксенобіотику, а M. luteus – близько 6 % БФА.

Ключові слова: бісфенол А, ксенобіотики, оксидоредуктази, біодеградація

Отримано редколегією 17.10.2023 р.