www.czasopisma.pan.pl PAN www.journals.pan.pl
Chemical and Process Engineering: New Frontiers
DOI: 10.24425/cpe.2024.149459

The potential of *Stenotrophomonas maltophilia* KB2 for phenol degradation under exposure to heavy metal

Agnieszka Gąszczak[*], Elżbieta Szczyrba, Anna Szczotka

Polish Academy of Sciences, Institute of Chemical Engineering, Baltycka 5, 44-100 Gliwice, Poland

[*]Corresponding author: Agnieszka Gąszczak, gaszczak@iich.gliwice.pl

ORCID numbers:
Agnieszka Gąszczak 0000-0002-6122-6033
Elżbieta Szczyrba 0000-0002-1942-8408
Anna Szczotka 0000-0003-0730-1810

Abstract

The large diversity of chemical substances present in air, water, or soil makes it necessary to study their mutual impact on the effectiveness of microbiological decomposition of contaminants. This publication presents the results of the studies aimed at evaluating the effect of two biogenic heavy metals - zinc and copper - on the phenol biodegradation by the *Stenotrophomonas maltophilia* KB2 strain. The tests were carried out for concentrations of metals significantly exceeding the legally permitted wastewater values: for zinc up to 13.3 $g \cdot m^{-3}$, and copper up to 3.33 $g \cdot m^{-3}$. In the tested metal concentration range, phenol biodegradation by the *S. maltophilia* KB2 strain was not significantly influenced by the introduced dose of zinc. While the presence of copper inhibited both biomass growth and substrate degradation. Kinetic data of metal and phenol mixtures were analyzed and very good correlations were obtained for the proposed equations. An equation consistents with the Han and Levenspiel model was proposed for the system *S. maltophilia* KB2-phenol-copper, while an equation consistents with the Kai model for the system *St. maltophilia* KB2-phenol-zinc. The simultaneous presence of Zn and Cu ions in the culture resulted in a stronger inhibition of phenol biodegradation.

Keywords: phenol, heavy metal, biodegradation, kinetic equations, synergism

Accepted Article

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the version of record. Please cite this article as DOI: 10.24425/cpe.2024.149459.

Received: 20 September 2023 | Revised: 09 December 2023 | Accepted: 29 January 2024

©2023. The Author(s). This is an open-access article distributed under the terms of the Creative Commons Attribution (CC-BY 4.0, https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

1. INTRODUCTION

The rapid development of industry and agriculture generate varieties of waste, increased water consumption and the amount of discharged wastewater affecting aquatic ecosystems. Owing to the use of various raw materials and complexity of processes, wastewater generally contains many inorganic and organic pollutants like polycyclic aromatic hydrocarbons, nitrogen, phosphorous and sulphur, heavy metals as well as other emerging and harmful substances (Gaurav 2021, Butarewicz 2019) Wastewater can be treated using various methods that are generally classified as physical, chemical or biological. These techniques have both advantages and disadvantages, however the primary goal is high efficiency of pollution removal, so they are constantly being improved (Ahmed et al., 2021, Bibi et al., 2023, Khan et al., 2023, Maziotis and Molinos-Senante, 2023). Biological techniques have been continuously developed for several decades, which has made them effective, environmentally friendly, simple, and inexpensive solutions in many cases (Khalidi-Idrissi et al., 2023; Mohd, 2020, Hussain et al., 2021, Tang et al., 2023). Microorganisms (bacteria, fungi, yeasts) have unique predispositions that enable them to use enzymes to decompose xenobiotic substances into CO_2, H_2O, SO_2, NH_3, or other small molecules, and to synthesize the organic matter they need (Panigrahy et al., 2022; Priyadarshini et al., 2021,2022). Good results are achieved by modifying the conventional biological systems conjoining with physical, chemical, and other biological systems in hybrid technologies (Ahmed et al., 2021, Saidulu et al., 2021).

Frequently detected pollutants are phenol and its derivatives occurring in post-production wastewater from the petrochemical, textile, pharmaceutical, plastic, and chemical industries (Gadipelly et al., 2014; Tutić et al., 2023). Although there are natural sources of phenol, it is a priority pollutant that shouldn't be discharged directly into the environment. To meet the demand for phenol, approximately 7 billion kilograms of this organic compound are produced annually (Chen and Sun, 2023). Due to the enormous scale of this basic organic raw material use, phenol occurs in the air, water, soil, and bottom sediments. It can be detected in most industries wastewaters in various concentrations, ranging from a minimal 1 $mg \cdot L^{-1}$ to 7000 $mg \cdot L^{-1}$ (Mohd, 2020). Phenol is very toxic to neurons, the respiratory tract, the eyes, and the skin. Prolonged or repeated exposure to this organic substance may affect the condition and function of the kidneys and liver. Another mechanism for the toxicity of phenol may be the formation of phenoxyl radicals (Ataei et al., 2024). Undoubtedly, the effect of industrial wastewater treatment will be significantly influenced by the amount of phenol (Villegas et al., 2016), but also by the quantity and quality of accompanying substances, e.g. heavy metals. Some of the heavy metals (Fe, Cu, Co, Zn, Ni, Mn, Se, and Mo) are quintessential at low

Accepted Article

Accepted Article

concentrations for the normal physiological functions of living organisms. Specific amounts of them are required for the survival of organisms, while their high concentrations are toxic for humans, plants, and microorganisms. Other heavy metals, such as Ag, As, Cd, Pb, Hg, and Cr(VI) do not have a known biological function and are toxic in any quantity causing deleterious effects on the well-being and survival of living organisms (Gomathy and Sabarinathan, 2010; Abd Elnabi et al., 2023). Due to the ubiquity of metals in the environment, microorganisms have developed various ways of dealing with them, for example, active and passive uptake of metal ions, sequestration and immobilization of metals, chelation of metals outside the cell, solubilization by releasing organic acids (Sharma et al., 2023; Syed et al., 2023).

Copper is a micronutrient necessary for the proper functioning of humans and animals. The basic function is to participate in oxidation-reduction processes, where it occurs as a coenzyme and in the production of erythrocytes or the synthesis of hemoglobin. The sorption properties of soils towards copper are much higher than towards other trace cations. A constant increase in the content of this metal is observed especially in the orchard and horticultural soils, as well as near industrial areas, urban agglomerations, and near the road network (Abd Elnabi et al., 2023).

Zinc is an essential element fulfilling several basic functions in the metabolic processes of living organisms. However, large doses of zinc cause damage to many biochemical processes and are deposited in the kidneys, liver, and sex glands. Zinc is widely used mainly as a component of alloys, in pressure casting, printing, and shipbuilding industries. Additionally, it is used in the production of electric tools, as well as in the tool, lighting, and metal industries. Most of the zinc produced is used for galvanizing to protect iron and steel from rusting. The highest concentrations of this metal occur in soils affected by emissions from non-ferrous metal smelters, and zinc also enters agricultural soils with fertilizers (Mitra et al., 2022)

Both phenol and heavy metals have been included on the US Environmental Protection Agency's (US EPA) list of 126 priority environmental pollutants. According to the regulation of the Minister of Maritime Economy and Inland Navigation from 2019 (Dz. U. 2019 poz. 1311) on the conditions to be met when discharging sewage into water or land, the highest permissible values of pollution indicators for industrial sewage are: for zinc 2 $mg\cdot dm^{-3}$ (all types of wastewater), and copper 0.1 $mg\cdot dm^{-3}$ (ceramics industry) and 0.5 $mg\cdot dm^{-3}$ (other types of wastewater). Biochemical processes are inhibited at zinc concentrations above 10 $mg\cdot dm^{-3}$, while nitrification processes are significantly inhibited at concentrations above 2 $mg\cdot dm^{-3}$ of zinc.

Numerous bacteria, fungi and yeasts such as Alcaligenes, Pseudomonas, Bacillus, Rhodococcus, Micrococcus, Cellulosimicrobium, Microbacterium, Flavobacterium, Methanospirillum, Aeromonas, Sphingobium, Aspergillus, Penicillium, Trichoderma, Streptomyces and Candida have been isolated and characterized as detoxifying the contaminants (Zhao et al., 2021, Zhang et al., 2023). New microorganisms are still being sought, and their potential in the treatment of contaminated water is widely researched due to the benefits that biodegradation offers (Miglani et al., 2022; Abd Elnabi et al., 2022). However, not all microorganisms that can degrade xenobiotics under the settled optimal laboratory conditions can be successfully applied in the remediation of contaminated wastewater or soil. Many of them are sensitive to periodically changing environmental conditions (temperature, pH, oxygen concentration, salinity) or the presence of coexisting pollutants, such as heavy metals.

Stenotrophomonas maltophilia – an opportunistic pathogen widespread in the environment, which can cause, among others, respiratory and bloodstream infections also belongs to the group of bacteria with the ability to degrade organic compounds. An important feature of S. *maltophilia* is its ability to form biofilms on moist surfaces, which enhances its resistance to antimicrobials (Brooke, 2021; García et al., 2023). Its ability to produce enzymes, nanoparticles, and inhibitory molecules that are useful not only in environmental protection but also in food production and agriculture makes it of wide interest in biotechnology. The potential of *S. maltophilia* to degrade xenobiotics has been exploited for several years (Dias et al., 2022; Chen et al., 2014, 2016; Wu et al., 2021; Alvarado-Gutiérrez et al., 2020).

Although the microbial degradation of phenol is well documented, the impact of co-pollutants are still not exhaustively investigated and described. The problem is the diversity and quantity of organic and inorganic compounds occurring in the environment and the lack of information about their interactions, hence it is so important to investigate the mutual influence and their impact on the effectiveness of biodegradation (Goutam Mukherjee et al., 2022; Štefanac et al., 2021). Assessment of the influence of two biogenic heavy metals – zinc and copper – on the phenol biodegradation by the *Stenotrophomonas maltophilia* KB2 strain will be presented below. The results of this study could be useful e.g. in optimizing operational conditions of existing plants.

Accepted Article

2. MATERIALS AND METHODS

2.1.Chemicals and medium

The bacteria lived in the mineral salts medium (MSM) containing: 3.78 g $Na_2HPO_4 \times 12H_2O$; 0.5 g KH_2PO_4; 5 g NH_4Cl; 0.2 g $MgSO_4 \times 7H_2O$; 0.01 g yeast extract; deionized water 1000 mL; enriched with 1 mL of TMS (Trace Mineral Solution). The phenol biodegradation in the presence of Cu^{2+} or Zn^{2+} ions was tested using a medium containing less phosphate salts to prevent precipitation. In preliminary tests, the optimal content of $Na_2HPO_4 \times 12H_2O$ (1.26 $g{\cdot}L^{-1}$) and KH_2PO_4 (0.167 $g{\cdot}L^{-1}$) was determined. The metal solution was prepared by soluble $ZnSO_4 \times 7H_2O$ or $CuSO_4 \times 5H_2O$ in deionized water.

2.2.Chemical analysis

The samples were collected from the bioreactor and centrifuged (15 000 rpm, 15 min, 4°C), filtered (2 μm pore diameter), and diluted with deionized water. The changes of substrate concentration in the liquid culture were determined by chromatographic analyses (Waters HPLC equipped with Waters 1525 gradient pump and two-wave detector UV-VIS Waters M2487, a reverse phase column (Spherisorb ODS 2, 5 μm, 150×4.6 mm), methanol and 1% acetic acid (40:60 v/v) with the flow rate of 1 mL•min-1); detection was carried out at wavelength 272 nm. Bacterial cell density was determined by measuring the absorbance at a wavelength $\lambda = 550$ nm (spectrophotometer HACH DR3900). The metal ion concentration was checked using colorimetric analysis (cuvette tests, Hach) at the beginning and end of the experiment.

2.3.Microorganisms

The *Stenotrophomonas maltophilia* KB2 strain is stored under number E-113197 in the VTT Collection in Finland. This strain was isolated from activated sludge of the wastewater treatment plant in Bytom – Miechowice, Poland, as described earlier (Guzik et al., 2009). It is known from previous studies that the KB2 strain has the ability to utilize different aromatic substrates as sole carbon and energy source, e.g. phenol, catechol, benzoic acid, protocatechuic acid, 4-hydroxybenzoic acid, and vanillic acid (Guzik et al., 2009). Guzik et al., 2012 demonstrated that strain KB2 was able to utilize 1100 $g{\cdot}m^{-3}$ phenol during 24 h of incubation. The abilities of *S. maltophilia* KB2 to degrade phenol effectively under suboptimal temperature, pH, and salinity were confirmed in Nowak et al., 2022. Tests on the sensitivity of catechol 2,3-dioxygenase isolated from *Stenotrophomonas maltophilia* KB2 cells demonstrated that the

Accepted Article

activity of this enzyme increased in the presence of Zn^{2+} ions by 18% but decreased to 0.5% in the presence of copper ions. Phenolic monooxygenase lost its activity after adding copper sulphate or iron chloride (Guzik et al., 2012).

The preparation of microorganisms for the kinetics studies was described in the publication Gąszczak et al., 2021.

2.4.Biodegradation experiments

Biodegradation tests were carried out in the stirred batch bioreactor (Biostat B fermenter, Sartorius, USA) equipped with temperature, pH, and O_2 sensors.

The concentration of dissolved oxygen (DO) in the suspension was kept at 5 $mg \cdot dm^{-3}$ (external compressor). The other environmental conditions were: 30ºC, pH=7, and stirrer rotations at 300 rpm. Each cultivation was started with the OD=0.2 which was equaling 61.3 $g_{dcw} \cdot m^{-3}$. The culture was sampled at regular time intervals, only the metal concentration was checked at the beginning and end of the test.

2.5.Modelling kinetics of organic compound removal in the presence of metal

The effect of heavy metal ions presence on the bacterial growth rate or organic compound degradation rate is widely discussed in the literature. However, despite the importance of such research, the presented mathematical descriptions of these phenomena are insufficient. Several mathematical models that are developments of the Monod equation, have been found in the literature.

The Han and Levenspiel model considers the existence of a critical inhibitor concentration, above which the growth rate slows down or the reaction stops completely. (Gąszczak et al., 2021). The modified version of this model:

$$\mu = \mu_{max}(1 - \frac{M}{M_{crit}})^n \quad (1)$$

where M is the metal concentration, and M_{crit} is the critical metal concentration was applied to describe the organic compound biodegradation in an environment containing heavy metals (Gopinath et al., 2011; Shukor et al., 2018; Manogaran et al., 2019).

To describe the growth of Rhodococcus sp. n diesel fuel in the presence of zinc Kai et al. (Kai et al., 2020) used the equation:

$$\mu = \mu_{max} \cdot e^{-k \cdot M} \quad (2)$$

Equation 3 accounts for both the substrate inhibition and heavy metal ion inhibition:

Accepted Article

$$\mu = \frac{\mu_{max}\cdot S}{K_s+S+\frac{S^2}{K_{IS}}}\cdot\frac{K_{IM}}{K_{IM}+M^n} \tag{3}$$

where K_{IS} represents the substrate inhibition constant, K_{IM} represents the heavy metal inhibition constant, and n is an equation parameter. Constants K_{IM} and n can be determined graphically (graph: $\log\left[\frac{\mu_{max}\cdot S}{\mu\left(K_s+S+\frac{S^2}{K_{IS}}\right)}-1\right]=f(\log M)$) (Nakamura and Sawada, 2000).

Amor et al. used a simplified form of the Andrews equation (believing that Ks was irrelevant) to model the effect of cadmium, nickel, and zinc on rates of alkyl benzene biodegradation (Amor et al., 2001).

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{M}{K_{IM}\cdot\mu_{max}} \tag{4}$$

3. RESULTS

The large diversity of chemical substances released into the natural environment makes it necessary to investigate the interaction of organic and inorganic compounds and their influence on the effectiveness microbial decomposition of contaminants.

3.1.Phenol biodegradation

The frequently detected contaminants include phenol, its derivatives, and heavy metals. The kinetics of the phenol biodegradation by *Stenotrophomonas maltophilia* KB2 were studied previously and the results were described in the paper Gąszczak et al., 2021. The following values of the Andrews equation parameters were reported: μ_m = 1.584 h^{-1}, K_s = 185.4 $g\cdot m^{-3}$, K_{IS} = 106.1 $g\cdot m^{-3}$.

Accepted Article

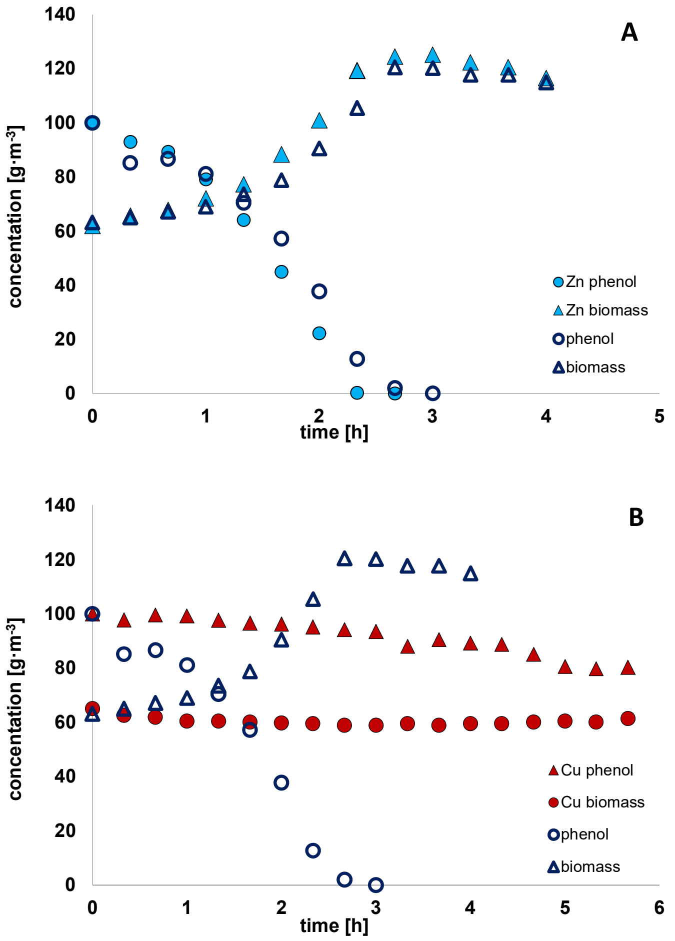

Fig.1. Example cultures with metal at 3.33 g·m^{-3} (full tracers) and without (empty tracers); circles – phenol concentration, triangles – biomass concentration; A) zinc, B) copper.

The tolerance of *S. maltophilia* KB2 strain to zinc and copper was assessed by evaluating changes in phenol and biomass concentrations in culture media amended with various concentrations of heavy metal ions. Two series of tests were carried out for the zinc concentrations ranging from 1.67 to 13.33 g·m^{-3} and for copper concentrations ranging from 0.33 to 3.33 g·m^{-3}, at the initial phenol concentration of 100 g·m^{-3}. As shown in a previous publication (Gąszczak et al., 2021) 100 g·m^{-3} is the phenol concentration at which the specific growth rate is close to the maximum value. Figure 1 shows the courses of exemplary cultures in the presence of zinc (A) or copper (B) at a concentration of 3.33 g·m^{-3}.

Even though both metals belong to the group of biogenic metals, their presence in the culture medium caused different effects. The presence of zinc, in the tested concentration range, had a slight effect on the course of phenol degradation, which was accelerated by several minutes (Figure 2A). Both the efficiency of phenol degradation and biomass growth decreased as the copper concentration increased. This relationship is illustrated in Figure 2B.

Accepted Article

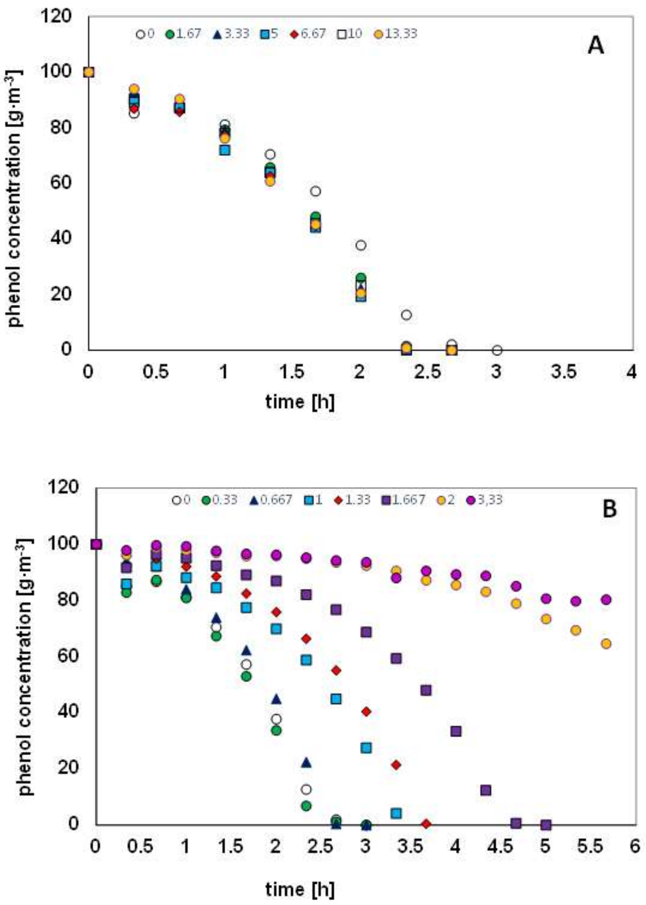

Fig. 2. The effect of the metal concentrations [g·m^{-3}] on phenol utilization; A zinc and B copper.

Copper turned out to be a strong inhibitor of phenol biodegradation by the KB2 strain. Small concentrations significantly slow down this process and even at a concentration of 1 g·m^{-3}, the phenol degradation time was significantly prolonged. The highest copper concentration tested was 3.33 g·m^{-3}. This dose of copper resulted in a negligible, in relation to the size of the inoculum, biomass growth, which was practically stopped. It can be assumed that the accumulation of Cu^{2+} ions in cells inhibited the activity of enzymes: catechol 2,3-dioxygenase and protocatechuate 3,4-dioxygenase (Guzik et al., 2009, Silva et al., 2012).

3.2.Modeling the Specific Growth Rate as a Function of Different Metal Concentrations

To study the kinetics of heavy metal inhibition, the phenol biodegradation experiments were carried out for various metal concentrations in a batch bioreactor. The specific growth rate was determined for each culture. The established experimental database was used to estimate the equation parameters for selected mathematical models (Equations 1-4). Methods for

Accepted Article

determining the parameters of the above-mentioned equations are described in the publication (Gąszczak et al., 2021).

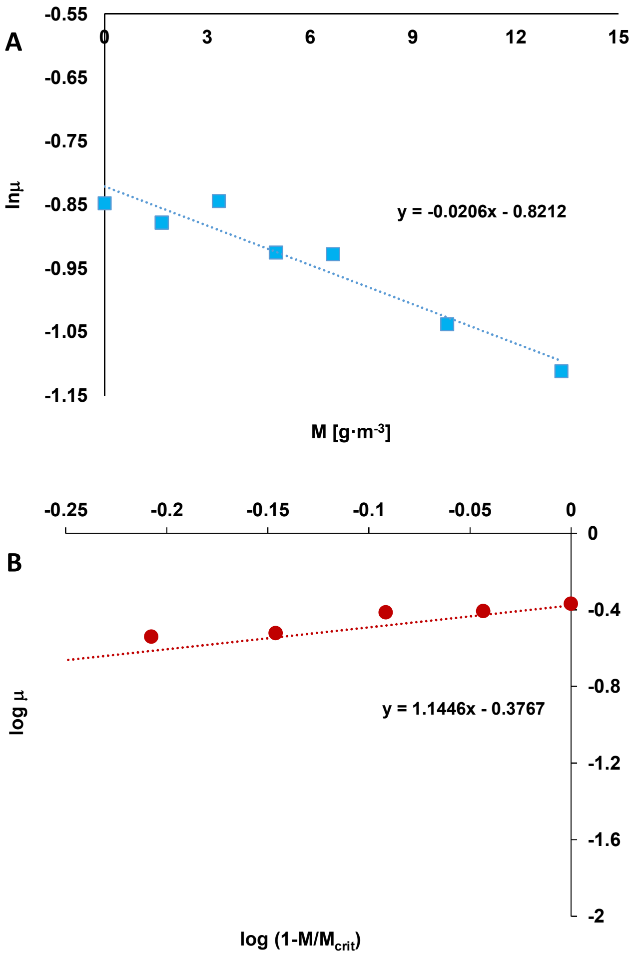

Fig. 3. The method of determining the parameters of the Kai model (A) and Han-Levenspiel model (B).

The best fit of the model and experimental data was obtained: for copper – with the Han-Levenspiel model, and for zinc – with the Kai model. Figure 3 illustrates the determination of the parameters of these models.

Kai model for Zn: $$\mu = 0.44 \cdot e^{-0.0206 \cdot M} \tag{5}$$

Han-Levenspiel model for Cu: $$\mu = 0.42\left(1 - \frac{M}{3.5}\right)^{1.1446} \tag{6}$$

Both Kai and Han-Levenspiel models showed good fitting: R^2=0.924 and R^2=0.948, respectively.

Accepted Article

Figure 4 shows the inhibitory effect of increasing metal concentrations on the specific growth rate of *S. maltophilia* KB2 strain, the experimental points, and fitted the Han–Levenspiel and Kai models.

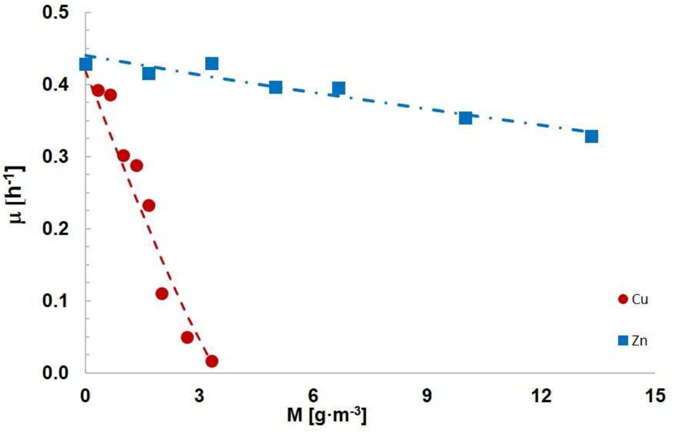

Fig. 4. Inhibitory effect of increasing concentrations of copper or zinc to the specific growth rate of *S.maltophilia* KB2 strain; phenol concentration 100 g·m^{-3} (●) Cu, (■)Zn.

3.3.The Effect of Mixed Heavy Metals on the Phenol Degradation

Synergism refers to a stronger toxic response from exposure to two or more chemicals than would be expected based on the sum of the effects of the individual chemicals. Synergistic properties should be taken into consideration when assessing the potential hazards of chemicals. A similar phenomenon is potentiation but in this case, one of the chemicals has no apparent ability to produce the toxic response on its own. In contrast, antagonism occurs when two or more substances in the mixture cause a weaker effect than when acting individually.

Since heavy metals are more likely to be present in a common environment as mixed heavy metals than as single ones, an attempt was made to analyse the effect of mixed zinc and copper on phenol degradation. Figure 5 shows the biomass growth and phenol degradation depending on the presence of single or mixed heavy metals. In the mixture, the copper concentration was 1g·m^{-3}, while the zinc content was 3.33 or 6.67 g·m^{-3}.

Accepted Article

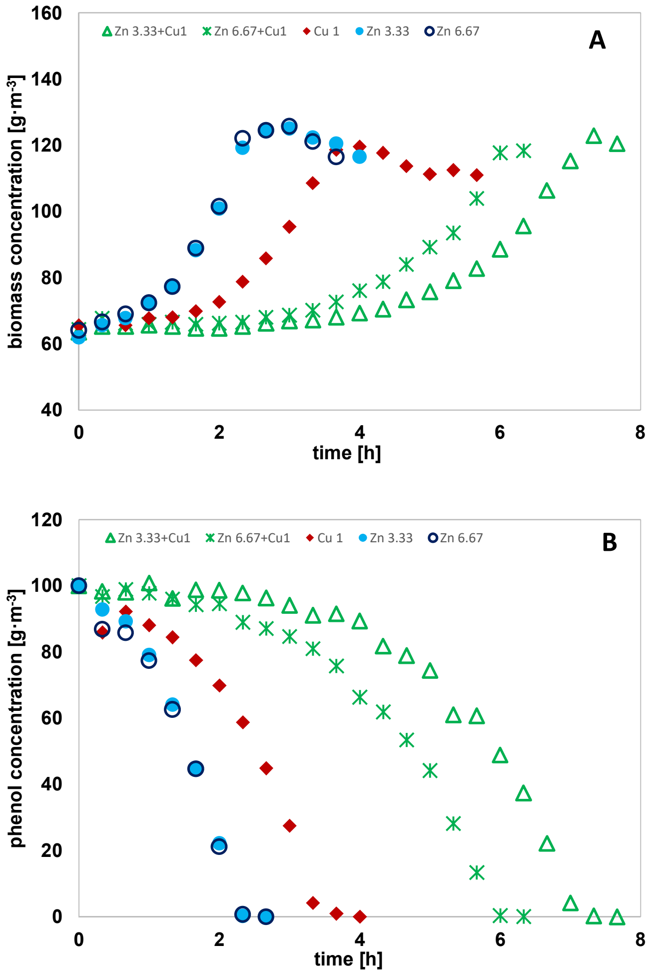

Fig. 5. The effect of mixed heavy metals on the phenol degradation

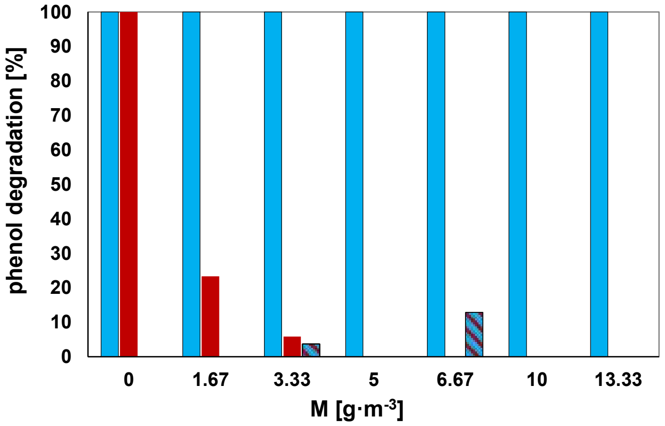

Fig. 6. The effect of metal concentration on phenol degradation at 100 g·m^{-3}; Zn (blue bars), Cu (red bars), and Zn+Cu (blue/red pattern bars).

During the experiments, a much stronger toxic effect was observed after the introduction of a mixture of Zn and Cu than in cultures containing only one metal (Fig.5). The graph (Figure 6)

Accepted Article

shows a comparison of the impact of the presence of zinc and copper ions on the phenol biodegradation. A dose of 100 g·m^{-3} was utilized in 100% by the *S.maltophilia* KB2 strain within 140 minutes. In the tested range of zinc concentrations, the degradation time of this dose of phenol did not change. As already mentioned, copper turned out to be a strong inhibitor of this process. Only 58.8% of the substrate was utilized at the same period (140 minutes), at a copper concentration of 1 g·m^{-3}, while at the highest tested concentration of copper ions (C_{Cu}=3.33 g·m^{-3}) only 5.8%. When, except copper at a concentration of 1 g·m^{-3}, zinc was also present in the culture medium, the obtained percentage of utilized phenol, depending on zinc concentration, was: 3,7% (C_{Zn}=3.33 g·m^{-3}) and 12,9 (C_{Zn}=6.67 g·m^{-3}). Noteworthy is the fact that a higher concentration of zinc resulted in a greater amount of utilized phenol at the same time. Is this phenomenon synergism or potentiation? The key seems to be the interpretation of the effects induced by the presence of zinc in culture. Considering Figure 2A, it can be concluded that zinc is not toxic to *S. maltophilia* KB2, so it would be a potentiation. However, in Figure 4 you can see a decrease in the value of the specific growth rate with increasing zinc concentration. Therefore, it seems justified to call the effect caused by the simultaneous introduction of copper and zinc ions synergism. To better understand this phenomenon, more research needs to be conducted.

Most works on environmental pollution by heavy metals describe their harmfulness and removal methods. Much fewer publications have been devoted to the interaction of pollutants, e.g. xenobiotics + heavy metals, and the problems occurring during their removal. Nakamura and Sawada studied phenol biodegradation by *Acinetobacter calcoaceticus* AH strain in a solution containing heavy metals such as zinc or copper ions. The model they proposed could satisfactorily express the inhibitory effects of substrate and heavy metals and it showed fair agreement with the experimental data. Similar research was carried out by Zhang et al. (Zhang et al., 2022), who isolated a new strain of *Bacillus cereus* ZBW3. The addition of Zn^{2+} slowed down the rate of phenol degradation, however, the complete degradation occurred within 72 hours. Conversely, the presence of Cu^{2+} ions resulted in the complete inhibition of phenol degradation, as their accumulation in cells hindered the activity of catechol dioxygenase (Zhang et al., 2022). The co-culture consisting of Arthrobacter sp. strain AQ5-15 and Arthrobacter sp. strain AQ5-06 exhibited phenol degradation capabilities up to 1.7 g/L within a week. The examination of resistance to heavy metals found that the disintegration of phenol by this co-culture was completed in the presence of As, Al, Co, Cr Cu, Ni, Pb, and Zn at 1.0 ppm concentrations. However, the activity of Arthrobacter strains was inhibited by Cd, Ag, and Hg (Subramaniam et al., 2021).

Accepted Article

A literature review of the effects of pollutant co-interactions showed that they depend on the nature of the pollutants and their concentrations or proportions in the mixture (Nlemolisa et al., 2020). The research by Batkhuyag et al. (2021) investigated the additive inhibitory effects of heavy metals on the utilization of phenol by various microorganisms, namely *Alicycliphilus denitrificans* K601, *Alicycliphilus* sp. R-2461, uncultured *Alicycliphilus* sp., and *Acidovorax aerodenitrificans*. The inhibitory effects of binary heavy metal mixtures on phenol-using microorganisms were found to follow a particular order based on synergistic interactions. This order was as follows: (Cd + Pb) ≈ (Cd + Cu) > (Zn + Pb) > (Zn + Cd) > (Pb + Cu) > (Zn + Cu).

4. CONCLUSIONS

What is the impact of heavy metals presence on the degradation of phenol by the *Stenotrophomonas maltophilia* KB2 strain? Due to such different courses of the processes depending on the introduced element, it is not possible to give a concise answer to this question. The slight increase in copper concentration resulted in a significant decrease in phenol degradation efficiency, whereas higher zinc concentrations had no practical effect on phenol biodegradation. The most appropriate equation to describe the system *S. maltophilia* KB2-phenol-copper utilises the Han and Levenspiel model. Likewise, for the system *S. maltophilia* KB2-phenol-zinc, an equation consistent with the Kai model was found to be the most suitable. The simultaneous presence of Zn and Cu ions in the culture resulted in a stronger inhibition of phenol biodegradation. At present, there is no generally accepted kinetic equation to describe the multiple effects of the introduction of co-pollutants. However, there is an urgent need to develop a new model for microbial growth and pollutant degradation that takes into account multiple substrates and inhibitors in an actual industrial setting. Understanding the biodegradation requirements and kinetics of microbial growth is imperative for designing and scaling up efficient bioreactor systems and optimizing technological processes to meet high quality standards.

REFERENCES

1. Abd Elnabi M.K., Elkaliny N.E., Elyazied M.M., Azab S.H., Elkhalifa S.A., Elmasry S., Mouhamed M.S., Shalamesh E.M., Alhorieny N.A., Abd Elaty A.E.; et al.,2023.Toxicity of Heavy Metals and Recent Advances in Their Removal: A Review. *Toxics*, 11, 580. DOI: 10.3390/toxics11070580

Accepted Article

2. Ahmed S.F., Mofijur M., Nuzhat S., Chowdhury A.T., Rafa N., Alhaz Uddin Md., Inayat A., Mahlia T.M.I., Ong H., Chia W.Y., Show P., 2021. Recent developments in physical, biological, chemical, and hybrid treatment techniques for removing emerging contaminants from wastewater. *Journal of Hazardous Materials*, 416, 125912, DOI:10.1016/j.jhazmat.2021.125912.
3. Alvarado-Gutiérrez M.L., Ruiz-Ordaz N., Galíndez-Mayer J., Curiel-Quesada E., Santoyo-Tepole F., 2020. Degradation kinetics of carbendazim by *Klebsiella oxytoca*, *Flavobacterium johnsoniae*, and *Stenotrophomonas maltophilia* strains. *Environmental Science and Pollution Research*, 27, 28518–28526. DOI:10.1007/s11356-019-07069-8.
4. Amor L., Kennes C., Veiga M.C., 2001. Kinetics of inhibition in the biodegradation of monoaromatic hydrocarbons in the presence of heavy metals. *Bioresource Technology,* 78, 181–185..DOI: 10.1016/S0960-8524(00)00182-6.
5. Ataei M, Maghsoudi A.S., Hassani Sh., 2024. *Phenol,* In: Editor(s): Philip Wexler, Encyclopedia of Toxicology, Fourth Edition, Academic Press, Pages 521-526, ISBN 9780323854344, DOI: 10.1016/B978-0-12-824315-2.00168-8.
6. Batkhuyag N., Matyakubov B., Mang N.Z.L., Lee T., 2021. Additive inhibitory effects of heavy metals on phenol-utilizing microorganisms. *Environmental Engineering Research*, 27, 210342–0. DOI: 10.4491/eer.2021.342.
7. Bibi A., Bibi Sh., Abu-Dieyeh M., Mohammad A. Al-Ghouti M., 2023. Towards sustainable physiochemical and biological techniques for the remediation of phenol from wastewater: A review on current applications and removal mechanisms. *Journal of Cleaner Production*, 417, 137810, DOI: 10.1016/j.jclepro.2023.137810.
8. Brooke, J.S., 2021. Advances in the microbiology of *Stenotrophomonas maltophilia*. *Clin. Microbiol. Rev.*, 34, e00030-19. DOI: 10.1128/CMR.00030-19.
9. Butarewicz A., Rosochacki S. J., Wrzaszcz E., 2019. Toxicity of sewage from industrial wastewater treatment plants. *Journal of Ecological Engineering*, 20(2), 191-199. DOI: 10.12911/22998993/99060.
10. Chen S., Sun L., 2023. Screening of efficient phenol-degrading bacteria and analysis of their degradation characteristics. *Sustainability,* 15, 6788. DOI: 10.3390/su15086788
11. Chen S., Yin H., Tang S., Peng H., Liu Z., Dang Z., 2016. Metabolic biotransformation of copper–benzo[a]pyrene combined pollutant on the cellular interface of *Stenotrophomonas maltophilia*. *Bioresource Technology,* 204, 26–31. DOI: 10.1016/j.biortech.2015.12.068
12. Chen S., Yin H., Ye J., Peng H., Liu Z., Dang Z., Chang J., 2014. Influence of co-existed benzo[a]pyrene and copper on the cellular characteristics of *Stenotrophomonas maltophilia*

Accepted Article

Accepted Article

during biodegradation and transformation. *Bioresource Technology*, 158, 181–187. DOI: 10.1016/j.biortech.2014.02.020

13. Dias P.R.P., Paiva T.O., de Oliveira A.M., de Magalhães J.C., 2022. Biodegradation of phenol by *Pseudomonas aeruginosa*, *Acinetobacter* sp. And *Stenotrophomonas maltophilia* isolated of the sludge activated of a steel industry, *International Journal of Development Research*, 12, (04), 55571-55574.
14. Dz. U. 2019 poz. 1311. Rozporządzenie Ministra Gospodarki Morskiej i Żeglugi Śródlądowej z dnia 12 lipca 2019 r. w sprawie substancji szczególnie szkodliwych dla środowiska wodnego oraz warunków, jakie należy spełnić przy wprowadzaniu do wód lub do ziemi ścieków, a także przy odprowadzaniu wód opadowych lub roztopowych do wód lub do urządzeń wodnych. Available at: https://isap.sejm.gov.pl/isap.nsf/download.xsp/WDU20190001311/O/D20191311.pdf
15. Gadipelly C., Pérez-González A., Yadav G.D., Ortiz I., Ibáñez R., Rathod V.K., Marathe K.V., 2014. Pharmaceutical Industry Wastewater: Review of the Technologies for Water Treatment and Reuse. *Ind. Eng. Chem. Res*. 53, 11571–11592. DOI: 10.1021/ie501210j
16. García G., Girón J.A., Yañez J.A., Cedillo M.L., 2022. *Stenotrophomonas maltophilia* and Its Ability to Form Biofilms. *Microbiology Research* 14, 1–20. DOI: 10.3390/microbiolres14010001
17. Gomathy M., Sabarinathan K.G., 2010. Microbial mechanisms of heavy metal tolerance- a review. *Agricultural Reviews* 31 (2), 133-138
18. Gopinath K.P., Kathiravan M.N., Srinivasan R., Sankaranarayanan S., 2011. Evaluation and elimination of inhibitory effects of salts and heavy metal ions on biodegradation of Congo red by Pseudomonas sp. Mutant. *Bioresource Technology*, 102, 3687–3693. DOI: 10.1016/j.biortech.2010.11.072
19. Goutam Mukherjee A., Ramesh Wanjari U., Eladl M.A., El-Sherbiny M., Elsherbini D.M.A., Sukumar A., Kannampuzha S., Ravichandran M., Renu K., Vellingiri B., Kandasamy S., Valsala Gopalakrishnan A., 2022. Mixed Contaminants: Occurrence, Interactions, Toxicity, Detection, and Remediation. *Molecules*, 27, 2577. DOI: 10.3390/molecules27082577
20. Guzik U., Greń I., Wojcieszyńska D., Łabużek S., 2009. Isolation and characterization of a novel strain of *Stenotrophomonas maltophilia* possessing various dioxygenases for monocyclic hydrocarbon degradation. *Braz J Microbiol.,* 40, 285-291.
21. Guzik, U., Hupert-Kocurek, K., Sałek, K., Wojcieszyńska, D., 2012. Influence of metal ions on bioremediation activity of protocatechuate 3,4-dioxygenase from

Stenotrophomonas maltophilia KB2. *World J. Microbiol. Biotechnol.*, 29, 267–273. DPI: 10.1007/s11274-012-1178-z

22. Guzik U.; Wojcieszyńska D.; Greń I.; Hupert-Kocurek K., 2010. Activity of Catechol Dioxygenases in the Presence of Some Heavy Metal Ions: Bioremediation of an Environment Polluted with Aromatic Compounds. *Ochrona Środowiska* , *32*, 9–13
23. Hussain A., Kumari R., Sachan S.G., Sachan A., 2021.Biological wastewater treatment technology: advancement and drawbacks. *Microb. Ecol. Wastewater Treat. Plants*, 175-192, DOI: 10.1016/B978-0-12-822503-5.00002-3
24. Kai E.X.; Johari W.L.W.; Habib S.; Adeela N.; Ahmad S.A.; Shukor M.Y., 2020.The growth of *Rhodococcus* Sp. on diesel fuel under the effect of heavy metals and different concentrations of zinc. *Adv. Polar Sci.*, *31*, 132–136
25. Khalidi-Idrissi A, Madinzi A, Anouzla A, Pala A, Mouhir L, Kadmi Y, Souabi S., 2023. Recent advances in the biological treatment of wastewater rich in emerging pollutants produced by pharmaceutical industrial discharges. *Int. J. Environ. Sci. Technol.* (Tehran). 16,1-22. DOI: 10.1007/s13762-023-04867-z.
26. Khan N., López-Maldonado E., Majumder A., Singh S., Varshney R., J.R. López, P.F. Méndez, Ramamurthy P., Khan M., Khan A., Mubarak N., Amhad W., Shamshuddin S., Aljundi I., 2023, A state-of-art-review on emerging contaminants: Environmental chemistry, health effect, and modern treatment methods. *Chemosphere*, 344, 140264, DOI: 10.1016/j.chemosphere.2023.140264.
27. Manogaran M., Othman A.R., Shukor M.Y., Halmi M.I.E., 2019. Modelling the Effect of Heavy Metal on the Growth Rate of an SDS-degrading *Pseudomonas sp.* Strain DRY15 from Antarctic soil. *Bioremed. Sci. Technol. Res.,* 7, 41–45. doi.org/10.54987/bstr.v7i1.463
28. Maziotis A. and Molinos-Senante M., 2023. A comprehensive eco-efficiency analysis of wastewater treatment plants: estimation of optimal operational costs and greenhouse gas emissions. *Water Research*, 243,120354, DOI: 10.1016/j.watres.2023.120354.
29. Mitra S., Chakraborty A., Tareq A., Emran T., Nainu F., Khusro A., Idris A., Khandaker M., Osman H., Alhumaydhi F., Simal-Gandara J., 2022. Impact of heavy metals on the environment and human health: Novel therapeutic insights to counter the toxicity. Journal of King Saud University - Science, 34, 2022, 101865, doi.org/10.1016/j.jksus.2022.101865.
30. Miglani R., Parveen N., Kumar A., Ansari, Mohd A., Khanna S., Rawat G., Panda A.K., Bisht S.S., Upadhyay J., Ansari M.N., 2022. Degradation of Xenobiotic Pollutants: An

Accepted Article

Environmentally Sustainable Approach. *Metabolites* 12, 818. DOI: 10.3390/metabo12090818

31. Mohd A., 2020. Presence of phenol in wastewater effluent and its removal: an overview. *International Journal of Environmental Analytical Chemistry*, 102, 1362–1384. DOI: 10.1080/03067319.2020.1738412
32. Nakamura Y., Sawada T., 2000. Biodegradation of phenol in the presence of heavy metals. *J. Chem. Technol. Biotechnol.*, 75, 137–142. DOI: 10.1002/(SICI)1097-4660(200002)75:2<137::AID-JCTB194>3.0.CO;2-0
33. Nlemolisa O.R., Nwanyanwu C.E., Akujobi C.O., Ihenetu F.C., Nwokorie R.C., Obasi C.C., Kemka U.N., Uzoho K.H., Nwoke M.C., 2020. Toxicity of Binary Mixtures of Phenol, Zinc, and Cadmium to Yeast Strains Isolated from Hydrocarbon Impacted Soil. *OALib 07*, e6201,1–15. DOI: 10.4236/oalib.1106201
34. Nowak A., Wasilkowski D., Mrozik A., 2022. Implications of Bacterial Adaptation to Phenol Degradation under Suboptimal Culture Conditions Involving *Stenotrophomonas maltophilia* KB2 and *Pseudomonas moorei* KB4. *Water*, 14, 2845. DOI: 10.3390/w14182845
35. Panigrahy N., Priyadarshini A., Sahoo M.M., Verma A.K., Daverey A., Sahoo N.K., 2022. A comprehensive review on eco-toxicity and biodegradation of phenolics: Recent progress and future outlook. *Environmental Technology & Innovation*, 27, 102423. DOI: 10.1016/j.eti.2022.102423
36. Priyadarshini A., Mishra S., Sahoo M.M., Rout P.R., Sahoo N.K., 2022. Effect of nutrient and culture conditions on enhanced biodegradation of phenolic pollutants: A review on recent development and future prospective. *Environmental Quality Mgmt*, 32, 161–176. DOI: 10.1002/tqem.21934
37. Priyadarshini A., Sahoo M.M., Raut P.R., Mahant, B., Sahoo N.K., 2021. Kinetic modelling and process engineering of phenolics microbial and enzymatic biodegradation: A current outlook and challenges. *Journal of Water Process Engineering*, 44, 102421. DOI: 10.1016/j.jwpe.2021.102421
38. Saidulu D., Gupta B., Gupta A., Ghosal P., 2021. A review on occurrences, eco-toxic effects, and remediation of emerging contaminants from wastewater: Special emphasis on biological treatment based hybrid systems. *Journal of Environmental Chemical Engineering*, 9, 4,2021,105282, DOI: 10.1016/j.jece.2021.105282.
39. Sharma M., Agarwal S., Agarwal Malik R., Kumar G., Pal D.B., Mandal M., Sarkar A., Bantun F., Haque S., Singh P., Srivastava N., Gupta V.K., 2023. Recent advances in

Accepted Article

microbial engineering approaches for wastewater treatment: a review. *Bioengineered*, 14, 2184518. DOI: 10.1080/21655979.2023.2184518

40. Shukor M.Y., Gusmanizar N., Rusnam, 2018. Modelling the Effect of Heavy Metals on the Growth Rate of Enterobacter sp. Strain Neni-13 on SDS. *J Environ Microbiol Toxicol*, 6, 24–27. DOI: 10.54987/jemat.v6i1.403
41. Silva A. S., Camargo F. A. D. O., Andreazza R., Jacques R. J. S., Baldoni D. B., & Bento F. M., 2012. Enzymatic activity of catechol 1, 2-dioxygenase and catechol 2, 3-dioxygenase produced by *Gordonia polyisoprenivorans*. *Quimica Nova*, 35, 1587-1592.
42. Subramaniam K., Ahmad S.A., Convey P., Shaharuddin N.A., Khalil K.A., Tengku-Mazuki T.A., Gomez-Fuentes C., Zulkharnain A., 2021. Statistical Assessment of Phenol Biodegradation by a Metal-Tolerant Binary Consortium of Indigenous Antarctic Bacteria. *Diversity* 13, 643. DOI: 10.3390/d13120643
43. Syed Z., Sogani M., Rajvanshi J., Sonu K., 2023. Microbial Biofilms for Environmental Bioremediation of Heavy Metals: a Review. *Appl Biochem Biotechnol*, 195, 5693–5711. DOI: 10.1007/s12010-022-04276-x
44. Štefanac T.; Grgas D.; Landeka Dragi˘cevi´c T., 2021. Xenobiotics—Division and Methods of Detection: A Review. *J. Xenobiot*., 11, 130–141. DOI: 10.3390/jox11040009
45. Tang H., Liu Y., Liu X., Zhang A., Yang R., Han Y., Liu P., He H., Li Z.,2023. Regulation methods and enhanced mechanism on the efficient degradation of aromatics in biochemical treatment system of coal chemical wastewater. *Journal of Environmental Management*, 348, 119358, DOI: 10.1016/j.jenvman.2023.119358.
46. Tutić M., Miloloža M. Cvetnić V,. Martinja, L., Furač, M., Markić Š., Ukić T., Bolanča, Kučić Grgić D., 2023. An Overview of Coking Wastewater Characteristics and Treatment Technologies, *Kem. Ind*. 72 (5-6) 349–358.
47. U.S. Environmental Protection Agency, 2014 Toxic and Priority Pollutants Under the Clean Water Act | Effluent Guidelines | US EPA U.S. Washington
48. Villegas L.G.C., Mashhadi N., Chen M., Mukherjee D., Taylor K.E., Biswas N., 2016. A Short Review of Techniques for Phenol Removal from Wastewater. *Curr Pollution Rep*, 2, 157–167. DOI: 10.1007/s40726-016-0035-3
49. Wu X., Zhang C., An H., Li M., Pan X., Dong F., Zheng Y., 2021. Biological removal of deltamethrin in contaminated water, soils, and vegetables by *Stenotrophomonas maltophilia* XQ08. *Chemosphere* 279, 130622. DOI: 10.1016/j.chemosphere.2021.130622

Accepted Article

50. Zhang J., Zhou X., Zhou Q., Zhang J., Liang, J., 2022. A study of highly efficient phenol biodegradation by a versatile *Bacillus cereus* ZWB3 on aerobic condition. *Water Science and Technology*, 86, 355–366. DOI: 10.2166/wst.2022.209
51. Zhang J., Bing W., Hu T., Zhou X.,, Liang J., Li Y.,2023. Enhanced biodegradation of phenol by microbial collaboration: Resistance, metabolite utilization, and pH stabilization. *Environmental Research*, 238, Part 2, 117269, DOI: 10.1016/j.envres.2023.117269.
52. Zhao T., Gao Y., Yu T., Zhang Y., Zhang Z., Zhang l., Zhang L., 2021. Biodegradation of phenol by a highly tolerant strain *Rhodococcus ruber* C1: Biochemical characterization and comparative genome analysis. *Ecotoxicology and Environmental Safety*, 208, 111709, DOI: 10.1016/j.ecoenv.2020.111709.

Accepted Article