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

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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 consistent with the Han and Levenspiel model was proposed for the system *S. maltophilia* KB2-phenol-copper, while an equation consistent with the Kai model for the system *S. 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

1. INTRODUCTION

The rapid development of industry and agriculture generates varieties of waste, increased water consumption and the amount of discharged wastewater that affects 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 (Butarewicz, 2019; Gaurav, 2021) 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 (Hussain et al., 2021; Khalidi-Idrissi et al., 2023; Mohd, 2022; 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; Tutićet al., 2023). Although there are natural sources of phenol, it is a priority pollutant that should not 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, 2022). 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 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 (Abd Elnabi et al., 2023; Gomathy and Sabarinathan, 2010). 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 (EPA, 2014) 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 (Zhang et al., 2023; Zhao et al., 2021). 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 (Abd Elnabi et al., 2022; Miglani 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 to be of wide interest in biotechnology. The potential of *S. maltophilia* to degrade xenobiotics has been exploited for several years (Alvarado-Gutiérrez et al., 2020; Chen et al., 2014; 2016; Dias et al., 2022; Wu et al., 2021).

Although the microbial degradation of phenol is well documented, the impact of co-pollutants has still not been 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.

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 of 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. (2013) demonstrated that strain KB2 was able to utilize 1100 g·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 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., 2013).

The preparation of microorganisms for the kinetics studies was described in 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·dm^{-3} (external compressor). The other environmental conditions were: 30 °C, pH = 7, and stirrer rotations at 300 rpm. Each cultivation was started with the OD = 0.2 which was equaling 61.3 g_{dcw}·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 ion 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:

$$\mu = \mu_{\max}\left(1 - \frac{M}{M_{\mathrm{crit}}}\right)^{n} \tag{1}$$

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; Manogaran et al., 2019; Shukor et al., 2018).

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

$$\mu = \mu_{\max} \cdot e^{-k\cdot M} \tag{2}$$

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

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

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

$$\text{(graph: }\log\left[\frac{\mu_{\max}\cdot S}{\mu\left(K_s + S + \dfrac{S^2}{K_{\mathrm{IS}}}\right)} - 1\right] = f(\log M))$$

(Nakamura and Sawada, 2000).

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

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

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 of 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 was studied previously and the results were described in the paper by Gąszczak et al. (2021). The following values of the Andrews equation parameters were reported: $\mu_m = 1.584\ \text{h}^{-1}$, $K_s = 185.4\ \text{g}\cdot\text{m}^{-3}$, $K_{\text{IS}} = 106.1\ \text{g}\cdot\text{m}^{-3}$.

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 $\text{g}\cdot\text{m}^{-3}$ and for copper concentrations ranging from 0.33 to 3.33 $\text{g}\cdot\text{m}^{-3}$, at the initial phenol concentration of 100 $\text{g}\cdot\text{m}^{-3}$. As shown in a previous publication (Gąszczak et al., 2021) 100 $\text{g}\cdot\text{m}^{-3}$ 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 $\text{g}\cdot\text{m}^{-3}$.

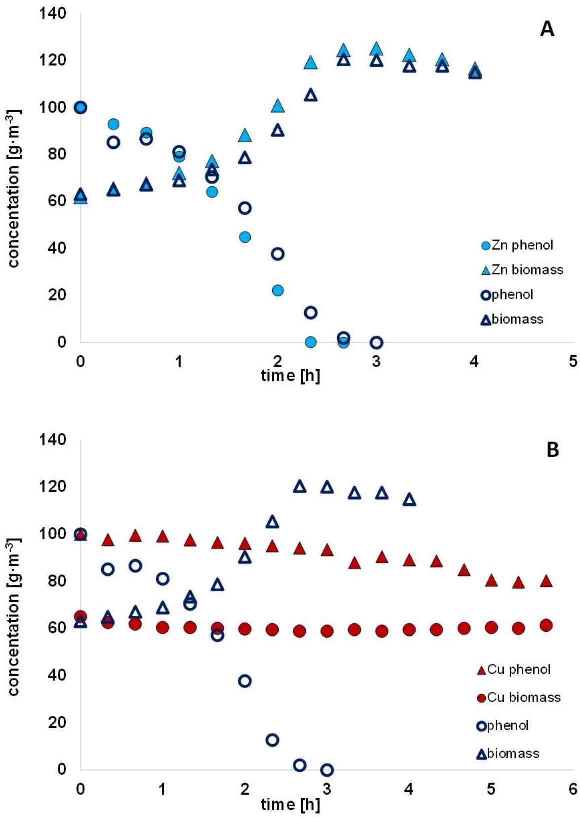

Figure 1. Example cultures with metal at 3.33 $\text{g}\cdot\text{m}^{-3}$ (full tracers) and without (empty tracers); circles – phenol concentration, triangles – biomass concentration; A) zinc, B) copper.

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.

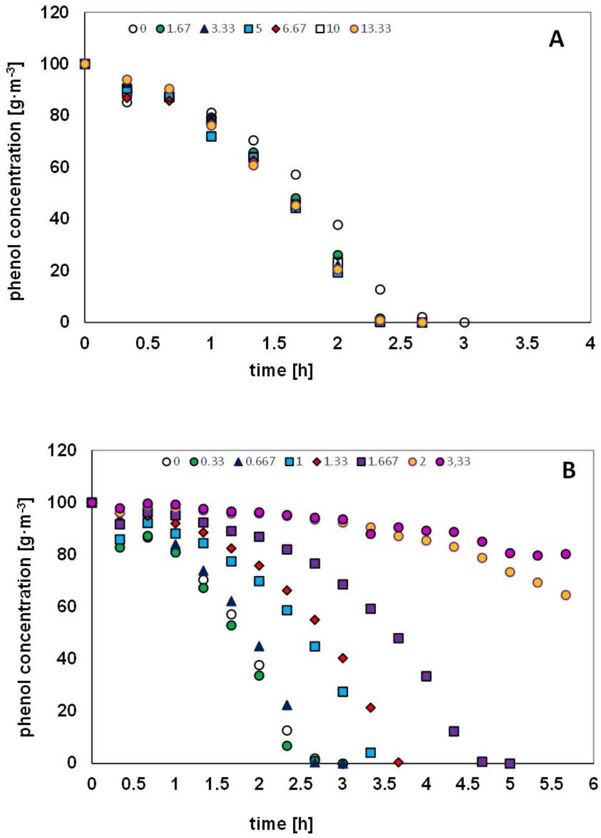

Figure 2. The effect of the metal concentrations [$\text{g}\cdot\text{m}^{-3}$] on phenol utilization; A) zinc and B) copper.

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 $\text{g}\cdot\text{m}^{-3}$, the phenol degradation time was significantly prolonged. The highest copper concentration tested was 3.33 $\text{g}\cdot\text{m}^{-3}$. 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 (Eqs. (1)–(4)). Methods for determining the parameters of the above-mentioned equations are described in the publication (Gąszczak et al., 2021).

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

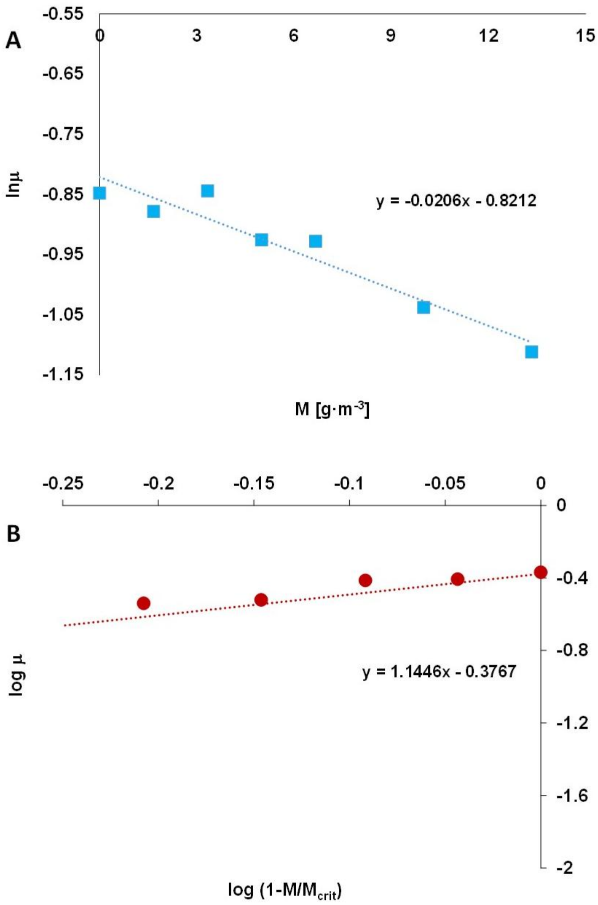

Figure 3. The method of determining the parameters of the Kai model (A) and Han and Levenspiel model (B).

Kai model for Zn:

$$\mu = 0.44 \cdot e^{-0.0206 \cdot M} \tag{5}$$

Han and Levenspiel model for Cu:

$$\mu = 0.42 \left(1 - \frac{M}{3.5}\right)^{1.1446} \tag{6}$$

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

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 and Levenspiel and Kai models.

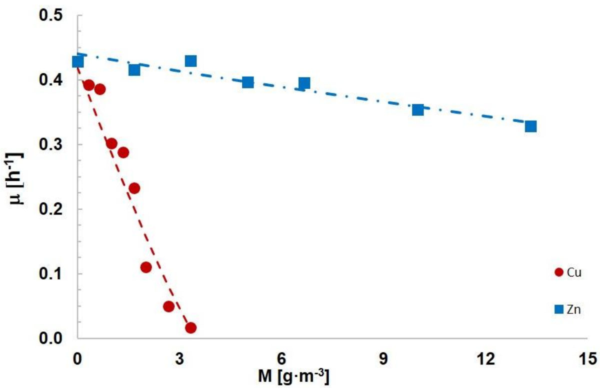

Figure 4. Inhibitory effect of increasing concentrations of copper or zinc to the specific growth rate of *S. maltophilia* KB2 strain; phenol concentration 100 g·m^{-3} (●) Cu, (■) Zn.

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 1 g·m^{-3}, while the zinc content was 3.33 or 6.67 g·m^{-3}.

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 (Fig. 6) 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

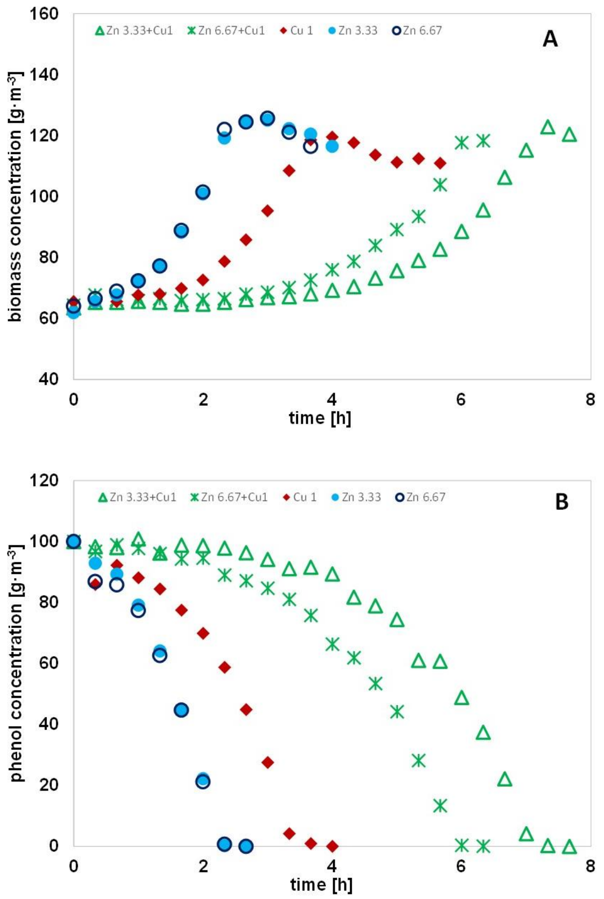

Figure 5. The effect of mixed heavy metals on the phenol degradation.

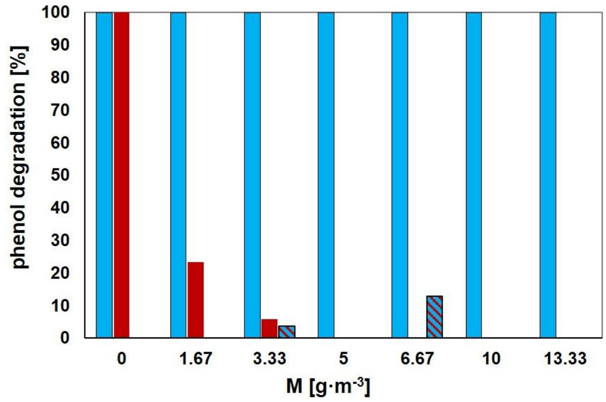

Figure 6. The effect of metal concentration on phenol degradation at 100 g·m^{-3}; Zn (blue bars), Cu (red bars), and Zn+Cu (blue/red pattern bars).

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 (2000) 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. (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).

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 presence of heavy metals 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.

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