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# Microbiological treatment of post-industrial water: Example of efficient bioremediation of the heavily polluted Kalina pond, Poland

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**Abstract:** The Kalina pond has been well known as a severely degraded area in the Silesia region, Poland. The environmental deterioration results from high contamination of water and bottom sediments with recalcitrant and toxic organic compounds, mainly phenol. The study was aimed at developing a bioremediation-based approach suitable for this type of polluted areas, involving microbiological treatment of water as a key and integral part of other necessary actions: mechanical interventions and the use of physical methods. During the initial biological treatment stage, autochthonous microorganisms were isolated from contaminated samples of water, soil and sediment, then subjected to strong selective pressure by incubation with the pollutants, and finally, cultivated to form a specialised microbial consortium consisting of five extremophilic bacterial strains. Consortium propagation and its biodegradation activity were optimised under variant conditions enabling bacteria to proliferate and to obtain high biomass density at large volumes allowing for the *in situ* application. After installing aeration systems in the pond, the consortium was surface-sprinkled to launch bioremediation and then both bacterial frequency and the contaminant level was systematically monitored. The complex remediation strategy proved efficient and was implemented on an industrial scale enabling successful remedial of the affected site. Treatment with the specifically targeted and adapted microbial consortium allowed for removal of most organic pollutants within a four-month season of 2022: the chemical oxygen demand (COD) value decreased by 72%, polyaromatic hydrocarbon (PAH) level by 97%, while the content of total phenols and other monoaromatic hydrocarbons (BTEX) dropped below the detection thresholds.

**Keywords:** autochthonous strains, bioremediation, landfill leachate, phenol, revitalisation, water treatment

## INTRODUCTION

Extensive industrialisation, urbanisation and increasing population pressure are the main causes of severe environmental pollution involving devastation of the biosphere, lithosphere and landscape deterioration, atmosphere and especially hydrosphere contamination. Anthropogenic pollutants have a major impact on various aspects of the economy and life. Their accumulation in water can cause endocrine disruption and genotoxicity in fish, as well as acute toxicity to aquatic biota, particularly water cilia, algae, and daphnia (Vasilachi *et al.*, 2021). They can damage agriculture by reducing soil fertility and the nitrogen-fixing capacity of plants, as well as by depleting the soil of nutrients and accelerating erosion processes. The disturbed biological balance of the soil can result in low crop yields or, in the case of severe contamination, can even render the land completely unsuitable for cultivation (Silva da *et al.*, 2020).

Environmental pollutants not only disturb the biological balance of wildlife, but also negatively affect human health (Singh *et al.*, 2020; Alori *et al.*, 2022). A wide range of organic chemicals such as polycyclic aromatic hydrocarbons (PAHs), pesticides or chlorinated derivatives have an ability to accumulate in human tissues (Jin *et al.*, 2017). Even small concentrations of such substances can cause cancer, pregnancy problems, neurobehavioural impairments and immunological or endocrine diseases (WHO, 2022). The hydrological ecosystem is the most damaged one from among all other areas of the biogeosphere. In Poland, poor water quality has been documented for 91.5% of rivers, 88.1% of lakes and 100% of transitional and coastal waters (Rączka, Skąpski and Tyc, 2021). These statistics support the urgent need for implementing water remediation and renewal actions.

The methods employed for neutralisation of contaminants can be divided into physico-chemical and biological ones (Kumar *et al.*, 2022). Among the physical techniques of water reclamation, the most commonly used are: the mechanical removal of bottom sediments, filtration, artificial oxygenation of the bottom layers, and lake flushing. The main chemical methods include coagulation, flotation and inactivation of biogenic substances, especially phosphorus (Chmist and Hämmerling, 2016). Although the physical and chemical reclamation methods are relatively fast and efficient, they tend to be expensive, laborious and often exert a strong, unfavourable environmental impact (Aparicio *et al.*, 2022). Nowadays, there is a growing understanding of the benefits that originate from the concerted use of both physical-chemical and biological approaches in the environmental clean-up projects. Such novel and advanced hybrid methods have recently been proposed, for example the combined use of sedimentation and biofiltration systems to reduce the discharge of pollutants into water bodies (Kupiec *et al.*, 2022). Biological methods have been used increasingly due to high remediation potential of plants and microorganisms Bioprocesses can be carried out either directly at the site of contamination, i.e. *in situ* (on-site) or after removal and transport of polluted material to a specialised facility, i.e. *ex situ* (off-site). Phytoremediation uses green plants to remove toxins, heavy metals or other pollutant chemicals and is considered a valuable solution for reclamation of relatively less contaminated areas such as transition zones between terrestrial and aquatic ecosystems, wetlands, or pre-treated wastewaters (Wei *et al.*, 2021). Bioremediation, in turn, can be defined as the process of transforming and degrading various contaminants into less toxic forms using microorganisms (Silva da *et al.*, 2020). It is usually optimised by two alternative methods: biostimulation or bioaugmentation. Biostimulation involves promoting the growth and metabolic activity of autochthonous microorganisms *in situ* by adjusting bioprocess conditions or supplementing mineral nutrients, cometabolites, or other necessary substances. This results in a significant microbiota proliferation accompanied by enhanced spontaneous biodegradation processes. Bioaugmentation is used when indigenous bacteria are not present or they are unable to initiate bioremediation reactions. In such a case, properly-selected, allochthonous microorganisms must be inoculated into the reclamation site (Curiel-Alegre *et al.*, 2022).

Bioremediation is regarded as particularly advantageous since it is cost-effective, highly efficient, and can be applied *in situ* with minimum risk of environmental interference (Bala *et al.*, 2022). However, aiming at the best possible performance of microorganisms, several limiting conditions must be considered prior to launching the process. Among the most prominent factors is the presence of toxic substances that can hamper microbiota growth and metabolic activity. Also, for certain compounds, low xenobiotic solubility, poor bioavailability or weak susceptibility to biodegradation may result in reduced bioremediation efficacy (Anekwe and Isa, 2023).

This article proves that for extremely contaminated sites, the most effective reclamation results can be achieved by employing mixed technologies that combine physical, chemical, and biological processes. The potential of bioremediation is explored and suggested as an efficient complementary treatment method for the highly polluted Kalina pond. The purpose of the study was to show the usefulness of such a complex remediation strategy, whose final stage was based on the application of unique, extremophilic, autochthonous bacteria isolated from polluted habitats.

## MATERIALS AND METHODS

### LOCATION OF THE RESEARCH OBJECT

The Kalina pond is located in the south of Poland, Silesian voivodeship, in Świętochłowice (50°16'47,4"N 18°55'39,5"E, Fig. 1). It is an anthropogenic, originally highly polluted reservoir of 5.3 ha, created before the First World War as a result of mining activities. The pond was fed by a now-defunct tributary of the Rawa River and by rainwater. There is also a waste disposal site adjacent to the eastern side of the reservoir, which was a source of influent pollution.


**Fig. 1.** Location map of the Kalina pond; source: own elaboration

### MATERIALS

All analyses were conducted using the samples collected from the Kalina pond and the surrounding area: water, bottom sediments, and the polluted soils from the adjacent sites (Fig. 2). The consortium used for bioremediation consisted of indigenous bacteria isolated from the samples as described below.

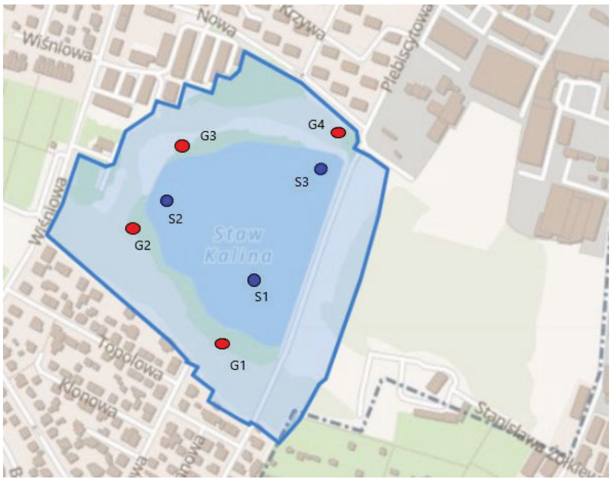


**Fig. 2.** Kalina pond sampling locations for water and bottom sediments (blue dots = sites S1–S3), and soil (red dots = sites G1–G4); source: own elaboration

## METHODS

### Isolation of bacteria, development of a microbial consortium and molecular identification of the strains

The samples of water and sediments of the Kalina pond as well as the soil of the surrounding area were analysed for the occurrence of microorganisms. Nineteen strains were originally isolated and cultivated to obtain microbiologically pure cultures. Next, the strains were integrated by mixing the monocultures to form a microbial consortium. Further cultivation and propagation were carried out in media containing Kalina pond water admixed with bottom sediments, which served as a growth limiting agent and provided sublethal environment. Determination of microbial population was analysed with the Koch plating method with the standard microbial media (Kaszycki *et al*., 2010).

After long-term incubation, the surviving strains of the consortium were isolated again and subjected to molecular proteomics identification with the Bruker Biotyper® analyzer, an automated next generation microbial identification system (Bruker Daltonics GmbH & Co. KG, Germany) performing the matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI TOF MS). The resultant proteomic data profiles were for each strain matched with a Bruker data base MBT IVD Library version K (release 2020). Microbial identification accuracy was evaluated with an identification factor (*IF*), calculated as a logarithmic score between 0 and 3 to quantify the similarity to appropriate reference spectra within database entries. Identity of particular strains was scored with the respective *IF* values and quantified into the following categories: high-confidence identification, for $IF \geq 2.0$; low-confidence identification, for *IF* ranging from 1.7 to 1.99, and lack of possibility to identify a microorganism, for $IF < 1.7$. All the matched microbial species of the study belonged to the first group and were referred to the NCBI (National Center for Biotechnology Information) taxonomy database. Finally, for each strain a specific NCBI taxonomy identification number (NCBI:txid no.) was assigned.

### Optimisation of bacterial growth and biodegradation activity

Optimisation of the consortium cultivation parameters was carried out in a laboratory test applying microbial liquid cultures in 300 $cm^3$ Erlenmeyer flasks rotary-shaken at 250 rpm, at the temperature of 24°C. The optimal oxygen concentration for bacterial growth was determined by adding 1.0% glucose to a consortium suspension diluted to a low microbial density of about $1.0 \cdot 10^4$ $cfu \cdot cm^{-3}$ (cfu = colony forming units). Five different aeration conditions were applied by using fine-bubble aeration micropumps: non-aerated, and 2.0, 4.0, 6.0, 8.0 mg $O_2 \cdot dm^{-3}$. The dynamics of bacterial population was analysed at the beginning of the experiment and after 1, 3, 7 and 14 days. The effect of oxygen concentration on bioremediation of pollutants by the tested consortium was also analysed. A high-density consortium at $1.0 \cdot 10^8$ $cfu \cdot cm^{-3}$ was added to the contaminated leachate at a ratio of 1:100. The effect for samples aerated (8 mg $O_2 \cdot dm^{-3}$) to the non-aerated ones (control) was compared. After two weeks, changes in concentrations of xenobiotic pollutants were determined.

In an attempt to accelerate and optimise the efficiency of bacterial biomass growth, the following ten carbon sources were tested: sucrose 0.1% w/v; 0.5% w/v, glucose 0.1% w/v, trehalose 0.5% w/v, the so-called, activator: acetic acid + glucose (3:1) 0.5% w/v (Xu *et al*., 2020), acetic acid + sucrose (3:1) 0.5% w/v, sodium acetate + sucrose (3:1) 0.5% w/v, sodium acetate 0.5% w/v, methanol 0.2% w/v, and sorbitol 0.5% w/v. Each of the listed substances was added directly to a highly diluted consortium (biomass density of $1.0 \cdot 10^2$ $cfu \cdot cm^{-3}$) inoculated to a leachate from the Kalina pond. The effect of temperature on the consortium bioremediation efficiency was evaluated by measuring changes in the COD parameter upon cultivation at five different thermal conditions: 6, 11, 24, 30 and 37°C. First, the consortium was centrifuged and the biomass resuspended as described above. Then, the samples were cultivated at the given temperatures for seven days.

### Production of microbial biomass and application of the consortium *in situ*

The propagation of the consortium was carried out in three stages. Initially, on a laboratory scale, in flasks and fermenters up to 60 $dm^3$, then on a semi-technical scale in plastic containers of a 1000 $dm^3$ volume, and finally, in large reinforced-concrete tanks, where the temperature was controlled using energy produced with solar panels. The consortium was supplemented with sucrose (0.1% w/v), casein peptone (0.025% w/v), yeast extract (0.025% w/v) and mineral salt mixture (5 $cm^3 \cdot (100\ cm^3)^{-1}$). Once a density of $10^8$ $cfu \cdot cm^{-3}$ was reached, the biomass of the consortium was transferred directly into the Kalina pond. The process was initiated in May 2022 and then repeated five times till October 2022. A volume of 150,000 $dm^3$ of microbial suspension was introduced into the pond each month, giving a total volume of the inoculated bacterial consortium of 900,000 $dm^3$. Once a month, a water sample was collected from the pond for the control of the bioremediation process.

### Analyses of xenobiotic content

Chemical oxygen demand (COD) index and phenol concentrations were determined with an automated, spectrophotometric analytical module Hach-Lange (*Hach-Lange GmbH,* Germany) equipped with a Hach DR 5000 UV-Vis Spectrophotometer and a Dry LT200 Thermostat as a mineralisation unit, using the appropriate cuvette LCK tests and following the manufacturer's instructions. Aromatic hydrocarbon content (BTEX = benzene, toluene, ethylbenzene, xylenes, and PAHs = polyaromatic hydrocarbons) was analysed as a commissioned service in a certified laboratory of Eurofins Environment Testing Polska Sp. z o.o., using standardised methods: PN-ISO 11423-1:2002 (HS-GC-MS) for BTEX and PN-EN ISO 17993:2005 (LC-FLD) for PAHs.

### Statistical analysis

The results were subjected to a statistical analysis using the ANOVA module of Statistica 13.5 (TIBCO Software Inc., Palo Alto, CA, USA). The significance of differences was assessed employing a Tukey HSD test at an assumed probability level of $p < 0.05$.

## RESULTS

### CHARACTERISTICS OF THE RESEARCH AREA

In the period between the two world wars, the Kalina pond was used for fishing, as a swimming pool and recreational facility. As regards the source of extreme water pollution, the Chemical Plant Hajduki Company, established in 1888 and serving as a high-temperature tar distillery, is blamed for generating toxic waste end discharging hazardous contaminants. The company was involved in the production of benzene naphthalene, anthracene, together with washing and impregnating oils. It was also the first factory in Poland extensively extracting and processing phenolic compounds. Until 1990, technological wastes and furnace slag were stored onto a heap adjacent to the Kalina pond. It is estimated that each year approximately 25,000 Mg of waste was deposited, including 13,000 Mg of lime sludge from the caustication of sodium carbonate and resin production, 6,000 Mg of paraffinic acids generated during the refining of benzene and naphthalene, and 5,000 Mg of waste from the cleaning machinery and tar tanks. The growing waste dump area covered 5.1 ha and was 23–25 m high. The compounds accumulated there, mainly phenols, continuously migrated as leachates and caused the resultant pollution of the Kalina pond waters (Tkaczyk, Pietrzak and Kołak, 2005).

As a consequence of industrial degradation, the waters of Kalina pond contained no dissolved oxygen. For this reason, as well as due to extreme toxicity caused by the presence of pollutants there was almost no biological life except for some indigenous bacteria. For a more detailed information on the affected area, hydrogeological profile of the pond and its acute ecotoxicity see Kaszycki *et al.* (2022). At the reservoir bottom, a 20–40 cm layer of a dense waste sediment was formed (Photo 1), in which hundreds to thousands $mg \cdot dm^{-3}$ of phenols were detected. The COD index for this sediment exceeded 10,000 mg $O_2 \cdot dm^{-3}$ (Tkaczyk, Pietrzak and Kołak, 2005). Both in the water and in the air above and around Kalina pond, elevated concentrations of other persistent xenobiotics could be detected: xylene, benzene, naphthalene, toluene and a number of polycyclic aromatic hydrocarbons (PAHs) (Siwek, 2015).

Due to an increasing need for recovery of the devastated area, the complex remediation and revitalisation plan for the Kalina pond was launched and led by Municipality of Świętochłowice under the project POIS.02.05.00-00-0105/16 (Gmina Świętochłowice, 2017). A contract for the comprehensive clean-up of the basin was signed in 2020 between the city of Świętochłowice and REMEA Sp z.o.o., Warsaw, Poland, Menard

**Photo 1.** Highly polluted bottom sediments collected in 2020 from the Kalina pond before launching remediation actions (phot.: *P. Kaszycki*)

Polska Sp.z.o.o., Warsaw, Poland and Remea société par actions simplifiée à associé unique, Nanterre, France (REMEA and Menard, 2020). In the initial stages of the reclamation work, a sealed wall was built to screen against further leakage of pollutants from the neighbouring heap into the pond, an excess sediment was removed from the bottom with the refuller pumps and then diffuser aeration systems were installed at the bottom of the reservoir to provide the water with missing oxygen (Photo 2). Next, the removed sludges were neutralised with the thermal desorption technique. The subsequent remediation stages involved the *in situ* microbiological treatment of the pond waters and residual bottom sediments that still contained hazardous concentrations of phenolic and other hydrocarbon xenobiotics.

**Photo 2.** The Kalina pond – view on the reclaimed area with the visible effect of action of the installed aeration nozzle system (phot.: *K. Starzec*)

### MICROBIAL CONSORTIUM DEVELOPMENT, CULTIVATION OPTIMISATION AND BIODEGRADATION ACTIVITY TESTING

The bacterial biocenosis formed by all the nineteen initially isolated strains was subjected to a long-term strong selective pressure caused by incubation with sublethal concentrations of toxic xenobiotics occurring in Kalina waters and sediments. Under such conditions only extremophilic microorganisms survived and finally five strains were identified in the stabilised consortium, that is *Bacillus clausii* RK1, *Bacillus pumilus* RK2, *Oceanobacillus profundus* RK3, *Arthrobacter grandavensis* RK4 and *Bacillus fordii* RK5. Prior to the use of the consortium for application *in situ*, it was necessary to obtain a sanitary/hygienic certificate allowing for save environmental applications. A unique certificate for the consortium named "REMEA-K1" was released by a competent institution, the National Institute of Public Health – National Research Institute (PZH, Poland) (Atest Higieniczny, 2021).

Interestingly, the consortium was tolerant to the tested wide range of oxygen supplied to the medium and no significant effect of aeration on bacterial proliferation was observed. In the case of non-aerated culture, after two weeks, a considerably high degradation potential was observed: removal of 40% of phenols applied at initial concentration of 500 mg·dm$^{-3}$, 90% of PAHs with an initial concentration of 9 mg·dm$^{-3}$ and 19% decrease of COD with a starting level at 5,000 mg $O_2$·dm$^{-3}$ were achieved. Such an activity was most probably due to the fact that at least some of the constituent strain isolates were derived from the bottom sediments, i.e. anaerobic environment, and therefore it might be expected that low oxygen concentration was not a limiting factor for microbial metabolism. On the other hand, in the presence of 8 mg·dm$^{-3}$ oxygen, a significant increase in bioremediation efficiency was documented, reaching, respectively, 100, 98 and 43% (Fig. 3).

In search for the optimal carbon source for stimulation of biomass growth, in most of the tested variants bacterial population reached $10^7$–$10^8$ cfu·cm$^{-3}$ after one day of cultivation (Fig. 4). For the cases of sodium acetate and trehalose a hampered growth was recorded at the first stage of culture. In turn, a lower biomass yield (approx. $10^6$ cfu·cm$^{-3}$ reached after seven days) was shown upon supplementation of the "activator", i.e. acetic acid

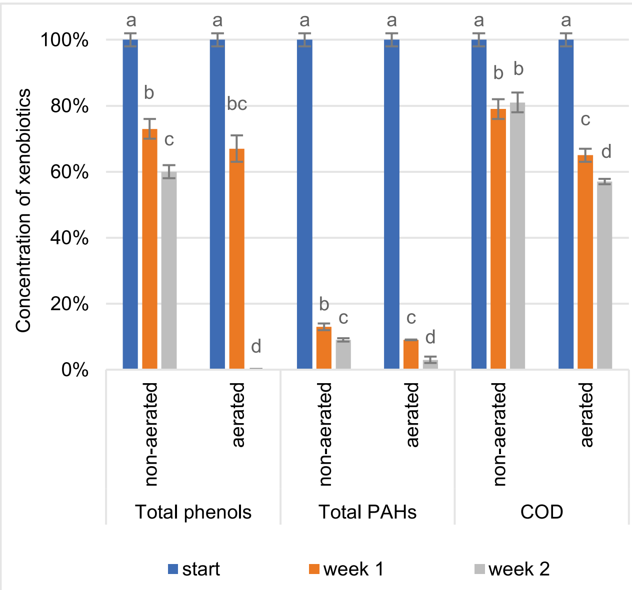


**Fig. 3.** Concentration of organic pollutants (phenols, PAHs and COD) remaining after 2-week biodegradation with a microbial consortium; different letters indicate significant statistical differences at $p < 0.05$; the analysis was performed separately for each class of compounds. The aerated samples contained 8 mg·dm$^{-3}$ of oxygen; source: own study

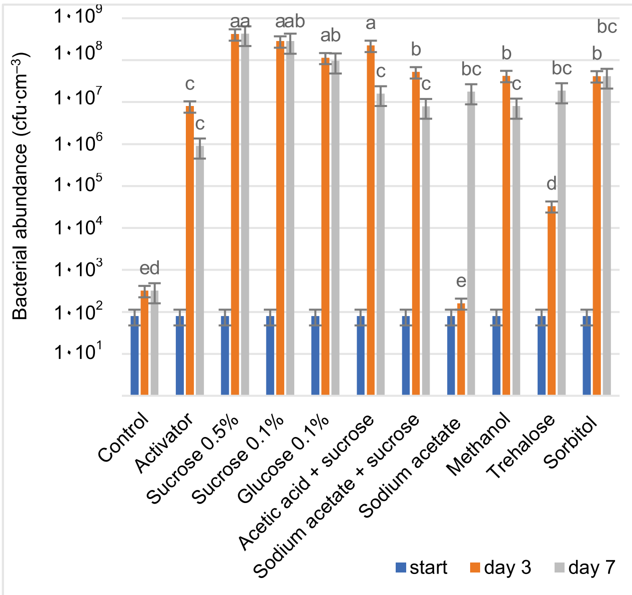


**Fig. 4.** Bacterial biomass growth upon 7-day cultivation as depended on supplementation with variant carbon sources; different letters indicate significant statistical differences at $p < 0.05$; source: own study

+ glucose (3:1) 0.5% w/v. At the same time no biomass growth was observed in the control sample, not admixed with any additional carbon supplement. This is an important finding since bacteria were incubated in the medium containing high load of organic matter present in the Kalina pond water (the COD value was about 900 mg $O_2$·dm$^{-3}$). Finally, sucrose proved to be the most efficient carbon source, causing intense growth of the culture whose biomass density exceeded $10^8$ cfu·cm$^{-3}$.

As regards the temperature optimum of bioremediation process, statistically significant decrease of COD after 7-day incubation was observed only at 24°C (data not shown). Such a result strongly suggests that biological treatment of water with the microbial consortium consisting of environmental mesophilic strains should be carried out during summer. This is because Poland is located in a moderate climate zone and the most favourable temperature of water, higher than 15°C and reaching about 25°C is maintained in the period between spring and early autumn.

## *IN SITU* BIOREMEDIATION OF THE KALINA POND

Since the laboratory tests proved that bioremediation was the most efficient in the presence of oxygen, an active water aeration system (see: Photo 2) was applied for stimulation of biological removal of xenobiotics. In May 2022, after reaching the level of 1 mg·dm$^{-3}$ of dissolved oxygen, at average daily temperatures exceeding 15°C, the pre-grown consortium was first applied to launch the bioprocess. The manner of the surface-sprinkling of the consortium employing fire-brigades is shown in Photo 3. The pond was then systematically bioaugmented monthly for the next five months as described earlier.

After the first application of the consortium, bacterial abundance analysis in the Kalina pond waters revealed microorganism frequency of 2·$10^5$ cfu·cm$^{-3}$. Then, bacterial population density gradually increased by 10-fold, together with the oxygen level tending to rise up to 5 mg·dm$^{-3}$. The highest degradation

**Photo 3.** Application of the microbial consortium to the Kalina pond with the use of high-performance pumps (phot.: *E. Stańkowska*, *P. Surma*)

rate of contaminants was observed between late July and early August 2022, which probably correlated with the higher (optimal) water temperature in the Kalina pond. For phenol and its derivatives, relatively rapid removal kinetics were observed: starting with the initial level of total phenols at 165 mg·dm$^{-3}$, a 61% degradation yield was achieved within the first month (till June) and 88% removal rate till July 2022. Later, this group of pollutants was below detection thresholds. Biodegradation efficiency of the microbial consortium is presented graphically in Figures 5–7 which show the decrease of the COD parameter, removal of BTEX and PAHs, respectively. For the case of COD, representing the total of organic pollutants, within the first four months after consortium application a 72% removal yield was achieved, and the final analysis carried out in February 2023 revealed an 86% decrease of this parameter (Fig. 5). The content of BTEX monoaromatics dropped to the values below the threshold of quantification (<6 μg·dm$^{-3}$) already in August 2022 (Fig. 6) whereas the PAH concentration decreased by more than 97% compared to the initial level (Fig. 7). Table 1 presents selected water quality parameters as determined before the biotreatment stage and after microbiological remediation.

Regardless of the attempt to effectively remove the pollutant load, the main objective of the Kalina pond remediation project was to restore biological life in the reservoir. Following the completion of microbiological treatment, the studies showed gradual increase in biodiversity. Upon biodegradation of the

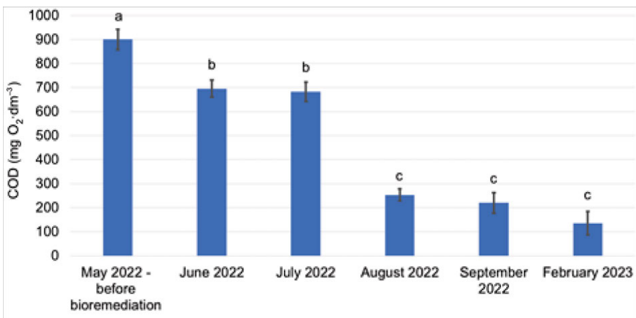


**Fig. 5.** Changes of the chemical oxygen demand (COD) of the Kalina pond water as observed during bioremediation process with the microbial consortium; different letters indicate significant statistical differences at $p < 0.05$; source: own study

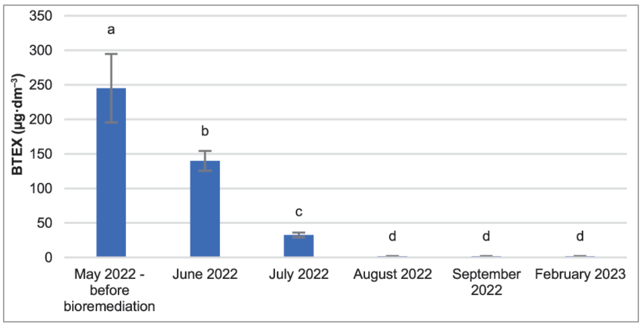


**Fig. 6.** Concentration changes of benzene, toluene, ethylbenzene, and xylenes (BTEX) in the Kalina pond during bioremediation process; different letters indicate significant statistical differences at $p < 0.05$; source: own study

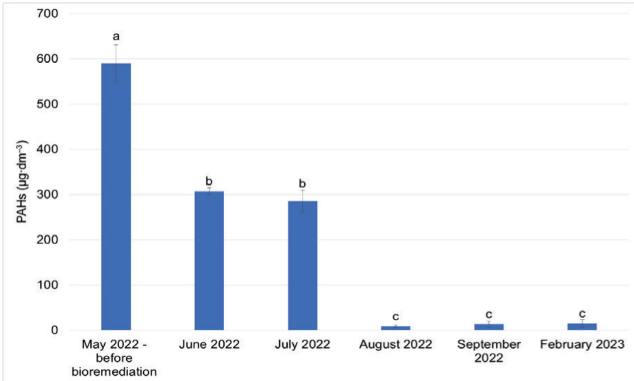


**Fig. 7.** Concentration changes of polyaromatic hydrocarbons (PAHs) in the Kalina pond during bioremediation process; different letters indicate significant statistical differences at $p < 0.05$; source: own study

**Table 1.** Kalina pond water quality parameters

| Parameter | Unit | Value | |
|---|---|---|---|
| | | before bioremediation (May 2022) | after bioremediation (February 2023) |
| Oxygen content | mg·dm$^{-3}$ | 0.0 | 2.5 |
| Redox potential | mV | –200 | +140 |
| Conductivity | μS·cm$^{-1}$ | 3050 | 2350 |
| COD | mg·dm$^{-3}$ | 900 | 120 |
| TOC | mg·dm$^{-3}$ | 240 | 64 |
| Phenol | mg·dm$^{-3}$ | 165 | nd |
| PAHs | μg·dm$^{-3}$ | 590 | nd |
| BTEX | μg·dm$^{-3}$ | 245 | 16 |
| pH | – | 7.77 | 7.90 |

Explanations: COD = chemical oxygen demand, TOC = total organic carbon, PAHs = polyaromatic hydrocarbons, BTEX = benzene, toluene, ethylbenzene, xylenes, nd = non-detectable. Source: own study.

nuisance compounds, the specialised bacteria of the applied microbial consortium died off and then the reduced toxicity enabled water repopulation with numerous species of environmental microbes as well as the higher organisms. Various bacteria and microscopic fungi emerged as well as protozoans including slipper and sedentary or free-swimming periphytes. Facilitated growth of algae led to a pronounced development of the green algae belonging to the phylum *Charophyta*. Also, a high population of *Daphnia* was observed together with some initial amphibians such as toads as well as beetles like the yellow-bellied swimmer. The pond became a habitat for waterfowl, and finally, it was artificially stocked with fish (two hundred carp).

## DISCUSSION

The present work shows the applicability of the combined, sequential use of mechanical and physical operations followed by microbiological remediation in reclamation and revitalisation of polluted surface waters. The study concerned the case of anthropogenically-degraded Kalina pond, where the final stage of environmental rehabilitation involved biological treatment with the selected bacterial strains. These microorganisms were applied *in situ* as a synergistically acting consortium and they proved highly effective in terms of biodegradation of recalcitrant and toxic pollutants. The results demonstrate a complete, successful and fully industrial-scale biotechnological clean-up project that enabled elimination of such hazardous chemicals as phenols, BTEX and PAHs. A high-yield removal of these xenobiotics was achieved within a relatively short time compared to other remediation projects. Note that biodegradation of the mentioned compounds is difficult because of their toxic effect on the organisms, which limits any possible biostimulation actions on the natural microbiota. In such a case, the use of highly-specialised consortia applied as a dense pre-grown biomass to inoculate the polluted site seems to be the only possible solution. This type of bioremediation is called bioaugmentation (Muter, 2023). The use of indigenous microorganisms adapted to the specific environmental conditions, especially the presence of contaminants, is a very beneficial approach. The autochthonous strains usually reveal high tolerance to the particular set of xenobiotics and very often have the ability to actively biotransform or degrade these compounds (Kaszycki *et al.*, 2010; Tiku *et al.*, 2010; Supel, Petryszak and Kaszycki, 2013; Li *et al.*, 2020; Kaszycki *et al.*, 2022).

Optimisation of the bioprocess parameters is usually carried out in model systems, after which bioremediation tests can be scaled up to the semi-technical stage to ensure satisfactory results under environmental working conditions (Kaszycki *et al.*, 2010). Therefore, for the Kalina pond leachates, preliminary parameter optimisation needed to be carried out prior to the water clean-up *in situ* and it is described in an earlier work of Kaszycki *et al.* (2022). In this study, optimal process conditions were established upon *in vitro* testing of the microbial biomass growth and bioremediation potential. As regards the temperature optimum, the best biodegradation results were observed at 24°C. This can be explained by the fact that all the bacterial strains were obtained from environmental habitats and they preferred moderate temperatures for efficient growth and metabolism. Based on the literature data it is known that at low temperatures, the solubility of organic compounds in water and thus their availability is usually reduced, which results in a delayed start of biodegradation reactions and poor xenobiotic removal rate (Bher *et al.*, 2020). Under such conditions, the fluidity and permeability of the microbial cell membrane are lowered as well, thus leading to a reduced uptake of nutrients and contaminants. For the reasons given above a decreased temperature is considered to slow down the degradation of pollutants; however, it does not stop this process entirely (Yadav *et al.*, 2012). Microbial metabolism accelerates along with increasing environmental temperature up to the optimal value at which the cell growth rate reaches its maximum. Higher temperatures are associated with greater enzymatic activities and faster biotransformation kinetics; however, the temperature optima are species-specific. It was shown that the rate of hydrocarbon degradation could double or even triple as the temperature increased by 10°C, provided that the other conditions were also optimal (Yadav, Shrestha and Hassanizadeh, 2012). Most hydrocarbon-degrading microorganisms are mesophilic and they proliferate best at moderate temperatures, that is within the range of 20–35°C. It is therefore assumed that the most efficient bioremediation process occurs at temperature of 20–30°C (Adedeji *et al.*, 2020; Bhandari *et al.*, 2021). For temperature exceeding the threshold values, proteins, enzymes and nucleic acids become denatured and inactive, which leads to inhibition of biodegradation as observed by Yadav, Shrestha and Hassanizadeh (2012) and Mahgoub *et al.* (2023). These authors also noted that for lower temperatures, i.e. below 10°C, xenobiotic transformations slowed considerably. Moreover, based on the laboratory and field studies, they concluded that the efficiency of biodegradation process depended on the temperature more strongly than on the changes in microbial populations. For the case of phenol, the highest degradation rates were achieved for *Serratia* sp. at 30°C (Aisami *et al.*, 2020), *Klebsiella oxytoca* at 37°C (Mahgoub *et al.*, 2023), and *Pseudomonas aeruginosa* KBM13 at 40°C (Ghaima, Rahal and Mohamed, 2017).

As for testing the influence of bacterial culture aeration on the performance of the microbial consortium, the results of this work showed that the presence of oxygen in the medium had no significant effect on biomass growth, while it considerably accelerated degradation of contaminants. It is well known that oxygen serves as the most favourable final electron acceptor in the cellular metabolism and therefore, its presence may be crucial to obtain satisfactory results in biotransformation and biodegradation of xenobiotics (Supel, Petryszak and Kaszycki, 2013; Kaszycki *et al.*, 2022). The last aspect of bioremediation optimisation was to determine the most suitable carbon source for propagation of bacterial biocenosis applied for treatment of the Kalina pond. The fastest biomass growth was achieved using sucrose. It should be mentioned here that many other studies point to glucose rather, as the carbon source best absorbed by bacteria, easily integrated into the basic metabolic pathways and biotransformed into other metabolites (for examples see: Kiefer, Heinzle and Wittmann (2002) and Molina-Ramírez *et al.* (2017)). For the case of sucrose (a disaccharide) metabolism, a slightly higher amount of energy input as well as the presence of invertase is necessary; however, this growth substrate is cheaper and easier to obtain commercially than glucose.

The problem of surface water remediation has recently attracted much interest due to the growing pressure for recovery of environmentally devastated areas. In Poland, several ponds and lakes have been subjected to reclamation and/or revitalisation. Among these, eutrophic reservoirs were the most common types of water bodies in need of restoration. So far, effective removal or inactivation of biogenic nutrients employing biological or combined physicochemical/biological methods was described, among others, for the lakes: Maltańskie, Uzarzewskie, Swarzędzkie, Rusałka (Dondajewska *et al.*, 2020), as well as for the Kamienna Góra dam reservoir (Mazur and Sitarek, 2020), and the Słoneczko reservoir (Mazurkiewicz *et al.*, 2020). Recently, Kupiec *et al.* (2022) have described an innovative large-scale, sedimentation/biofiltration system employing specialised microorganisms to limit the inflow of pollutants, especially biogenic nutrients, into the Jelonek Lake of the Gniezno Lake District. In turn, for several other lakes, e.g. Pruszków, Muchawka, Starachowice or Oleśnica, the use of microbial consortia enabled reduction of the amount of bottom sediment matter and led to improvement of the overall water condition by refining the oxygen profile or reducing turbidity (Mazur, Jakubiak and Santos, 2023).

However, the available literature data on industrial-scale remediation projects of waters severely polluted with toxic organic substances is scarce. Most of the reports focus either on laboratory model bioremediation testing or on obtaining robust microorganism isolates and elucidation of biodegradation mechanisms of particular xenobiotics. There are several papers dealing with the issue of contamination and toxicity of the Kalina pond. In a series of thorough studies by Michalska *et al.*, the Kalina pond leachates were shown to strongly negatively affect the consortial structure, physiology and activity of activated sludge microorganisms upon discharge to the nearby wastewater treatment plant. These effluents also dramatically reduced the operating parameters of the laboratory-scale sequencing batch reactor (Michalska *et al.*, 2019). The activated sludge performance could be improved by bioaugmentation with the strains showing high tolerance and biodegradation activity towards phenol compounds (Michalska *et al.*, 2020b). The addition of two environmental isolates (yet not obtained from the Kalina pond waters): *Pseudomonas putida* OR45a and *P. putida* KB3 to the activated sludge could diminish the toxic effects of the Kalina pond leachate. This was evidenced by a reduction in the biomass stress factor, improvement of phenol degradation efficiency, and a decrease in COD (Michalska *et al.*, 2020a). Zamorska and Kiełb-Sotkiewicz (2021) investigated the potential of commercially available microbial consortia, called “biopreparations”, in treating waters from the Kalina pond, but only on a laboratory scale. They achieved a total organic carbon (TOC) reduction by 68% within two weeks. In turn, Noszczyńska *et al.* (2021) were able to isolate the strain *Acinetobacter* sp. K1MN from the Kalina pond leachate. The strain proved to be an efficient bisphenol A degrader in model solutions.

In the studies of efficient phenol biodegraders, Mishra and Kumar (2017) conducted experiments on a strain of *Pseudomonas putida* MTTC 1194 and achieved degradation rate of 1000 ppm phenols within 162 h and 500 ppm of catechol in 94 h. Sarwade and Gawai (2014) tested biodegradation of phenols using a strain of *Bacillus badius* D1. After 48 h of bacterial culture in the presence of a xenobiotic at an initial concentration of 1500 ppm, the strain degraded 85% of the phenolic pollutants. Wang *et al.* (2007) used immobilised *Acinetobacter* sp. cells which neutralised more than 99% of phenols at initial concentration of 500 ppm and survived in the presence of phenols at the level of 1100 ppm. Mei *et al.* (2019) used a pH-tolerant strain of *Cobetia* sp. SASS1. With an initial phenolic concentration of 1500 $mg \cdot dm^{-3}$ and a COD of 2239 mg $O_2 \cdot dm^{-3}$, the authors achieved complete degradation of phenols and a decrease in the COD parameter to a value of 181.6 mg $O_2 \cdot dm^{-3}$. Panigrahy, Barik and Sahoo (2020) studied bioremediation of phenolic contaminants by *Pseudomonas citronellolis* NS1 isolated from coke oven wastewater. The wastewater used in testing contained 1045 $mg \cdot dm^{-3}$ of phenols and its COD value was 3600 mg $O_2 \cdot dm^{-3}$. The resultant decrease in COD was 95.5%, and the degradation of phenols was almost complete within 90 h of running the culture. Poi *et al.* (2017) used a consortium for bioremediation of phenolic pollutants consisting of 22 bacterial strains, of which 12 belonged to the *Bacillus* genus, 5 to *Pseudomonas*, 3 to *Acinetobacter*, and individual ones to *Alcaligenes*, *Brevibacillus* and *Arthrobacter*. Over a period of 104 days, they obtained a decrease in phenolic content from an initial concentration of 407 $mg \cdot dm^{-3}$ to concentrations below the detection threshold while the COD parameter decreased by 77% from 11,503 to 2,630 mg $O_2 \cdot dm^{-3}$.

It is to note here that the polluted water from the Kalina reservoir treated in the presented study contained, apart from phenolics, numerous other contaminants of different chemical structure and environmental hazard. Still, the applied reclamation approach proved efficient and the treatment with the unique microbial consortium consisting of bacterial autochthons enabled successful remedial of large volumes of water in the anthropogenically degraded basin. Within four months of active remediation, most of organic pollutants were removed thus bringing the possibility of full environmental recovery and revitalisation of the Kalina pond.

## CONCLUSIONS

The complex remediation strategy based on the subsequent use of physical-chemical methods and followed by microbiological treatment was shown to enable successful revitalisation of the post-industrial Kalina pond, extremely polluted with toxic organic compounds, especially phenols as well as other mono- and polyaromatic hydrocarbons. A large-scale *in situ* application of a unique microbial consortium enabled efficient and fast biodegradation of xenobiotics. The consortium consisted of five specialised autochthonous strains capable of biochemical transformation of the contaminants. These extremophilic bacteria were isolated from the samples collected at the area of the pond and then cultured under selective conditions in the presence of pollutants. Active bioremediation led to the reduced ecotoxicity which, in turn, allowed for restoration of biological life and considerable increase in biodiversity. The described biotechnological approach remains in line with the current trends in surface water reclamation projects and can be proposed as a feasible and cost-effective solution for rehabilitation of other anthropogenically-degraded reservoirs.

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## CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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