Archives of Environmental Protection
Vol. 50 no. 2 pp. 32–41

PL ISSN 2083-4772
DOI.10.24425/aep.2024.150550

Insights into bacterial diversity in industrial post-processing water from underground coal gasification (UCG) process

Łukasz Jałowiecki[1*], Jacek Borgulat[1], Aleksandra Strugała-Wilczek[2], Jan Jastrzębski[3], Marek Matejczyk[1], Grażyna Płaza[4]

[1] Institute for Ecology of Industrial Areas,Katowice, Poland
[2] Department of Energy Saving and Air Protection, Central Mining Institute, Katowice, Poland
[3] Faculty of Biology and Biotechnology, University of Warmia and Mazury in Olsztyn, Poland
[4] Silesian University of Technology, Poland

* Corresponding author's e-mail: l.jalowiecki@ietu.pl

Keywords: underground coal gasification process, post-processing wastewater, environmental engineering and microbiology

Abstract: This study represents the first culture-independent profiling of microbial diversity in post-processing wastewater from underground coal gasification (UCG) processes. Three types of post-processing wastewater, named W1, W2 and W3, were obtained from three UCG processes involving two types of coal and two gasification agents, namely oxygen-enriched air and oxygen. Very high concentrations of BTEX (benzene, toluene, ethylbenzene, xylene), polyaromatic hydrocarbons (PAHs), and phenol were detected in the wastewater, classifying it into the fifth toxicity class, indicating very high acute toxicity.

The values for the Shannon (H), Ace and Chao1 indices in W2 were the lowest compared to their values in W1 and W3. The dominate phyla were *Proteobacteria*, contributing 84.64% and 77.92% in W1 and W3, respectively, while *Firmicutes* dominated in W2 with a contribution of 66.85%. At the class level, *Gammaproteobacteria* and *Alphaproteobacteria* were predominant in W1 and W3, while *Bacilli* and *Actinobacteria* were predominant in W2. Among *Bacilli*, the *Paenibacillus* and *Bacillus* genera were the most numerous. Our results suggest that the main differentiating factor of the bacterial structure and diversity in the wastewater could be the gasification agent. These findings provide new insights into the shifting patterns of dominant bacteria in post-processing wastewater and illustrate the spread of bacteria in industrial contaminated wastewater.

Introduction

The issue of water pollution resulted from the underground coal gasification process (UCG) has been documented in the literature (Pankiewicz-Sperka et al. 2014, Kapusta et al. 2015, Zwain et al. 2021, Pankiewicz- Sperka et al. 2021, Grabowski et al. 2021). During the UCG process, significant quantities of both inorganic and organic pollutants are produced, leading to a serious environmental concern. Organic contaminants such as mono- and polycyclic aromatic hydrocarbons (BTEX, PAHs), and phenolic compounds have been identified as the primary constituents in post-processing water. Additionally, heavy metals, ammonia, and cyanides have been predominant among the inorganic impurities (Smoliński et al. 2013, Gawroński et al. 2022, Xu et al. 2017, Wiatowski et al. 2023).

Most pollutants from UCG wastewater are toxic to both prokaryotic and eukaryotic living organisms. Despite this, many microorganisms have been found to survive in such environments by utilizing the pollutants as sources of carbon and energy or by biotransformation of the organic and inorganic compounds (Kamika et al. 2016, Bassin et al. 2017). Pollutants and specific physico-chemical parameters (such as pH, oxygen levels, salinity, nutrients, pressure, anthropogenic effects) in heavily contaminated environments exert selective pressure on microbial communities, influencing their composition and diversity. Various environmental parameters, sometimes with extreme values representing unfavorable conditions, can affect microbial diversity and structure. The presence of certain contaminants in the environment can lead to significant alterations in indigenous microbial community structure, resulting in changes in species richness and dominance. Microorganisms quickly adapt to extreme environments and possess unique metabolic abilities, which are significant for research and application (Bedogni et al. 2020, Luo et al. 2020, Mauricio-Gutiérrez et al. 2020; Kochhar et al. 2022, Rappaport et al. 2022). In many cases, extreme environments serve as habitats for specific microorganisms, including novel, phylogenetic taxa.novel phylogenetic taxa."

Despite the available literature and modern methods, there are limited reports devoted to studying bacterial diversity in anthropogenic environments such as industrial effluents. To address this gap in the literature on industrial microbial diversity, we describe the diversity of microbial communities in UCG wastewater, targeting the V3-V4 hypervariable region of 16S rRNA gene.

The aim of the research was to characterize and compare the microbiomes of UCG-wastewaters, which represent extreme environments for the microbial life. In the study, metagenomic DNA was isolated from three post-processing waters, designated W1, W2 and W3, obtained from three UCG processes involving two types of coal and various gasification agents (air and oxygen). This study provides a comprehensive description of the microbiota present in UCG wastewater .

Materials and Methods

Underground coal gasification process and wastewater sampling

Three separate experimental simulations of UCG were carried out in a large-scale *ex-situ* installation located in the Barbara Experimental Mine in Mikołów (Poland). The experimental installation belonged to the Center for Clean Coal Technologies in GIG National Research Institute (Katowice, Poland). The processes were performed as previously described by Wiatowski et al. (2023). Some parameters and characteristics of the UCG experiments are presented in Table 1.

The raw wastewater generated during the coal gasification processes was collected in a 1 m^3 plastic tank (Mauser type). After mixing, the samples were trensferred into sterile 500 mL and 1000 mL bottles (SIMAX), and immediately transported to the laboratory at a temperature of 4°C. Wastewater was sampled in triplicate and pooled into a composite sample. Before analysis, the wastewater samples were filtered through a 0.45 µm pore diameter membrane filter under vacuum to remove coal tar and other undissolved residues. All filtrates were stored at 4°C before physico-chemical analysis. Table 2 presents some physico-chemical parameters and the toxicity of raw wastewater along with the appropriate methods and reference documents.

Isolation of metagenomic DNA and sequencing

After transportation to the laboratory, the raw wastewater samples were immediately passed through 0.2 µm polycarbonate membrane filters (Millipore, Germany) using a vacuum pump (Millipore, Germany). The filters were then stored at -80°C until DNA extraction. Metagenomic DNA (metDNA) was isolated using a commercial kit (MoBio Laboratories Inc., CA, United States) following the manufacturer's instructions. The quantity and quality of metDNA were determined using microspectrophotometry (BioSpectrometer, Eppendorf). With the A260/A280 ratio assessed. Then, metDNA samples were sent to Genomed (Warszawa, Poland) for library preparation and sequencing. Metagenomic analysis of microbial populations was performed based on the V3-V4 hypervariable region of the 16S rRNA gene. Amplification of the selected region and library preparation utilized specific sequences of 341F and 785R primers. PCR was carried out using Q5 Hot Start High-Fidelity 2x Master Mix, following the manufacturer's recommendations for reaction conditions. Sequencing was performed using 2 x 300 bp paired-end technology on the Illumnia MiSeq system with using Illumina's v. 3 kit. Automated preliminary data analysis was performed on a MiSeq sequencer using the MiSeq Reporter (MSR) v. 2.6 software. The analysis consisted of two steps: (1) automatic demultiplexing of samples, and (2) generation of fastq files containing the raw reads.

Bioinformatic analysis/NGS data processing

Raw sequence data was submitted in Sequence Read Archive (SRA) division of GenBank database (NCBI database), and they are under BioProject number PRJEB60074 (https://www.ncbi.nlm.nih.gov/bioproject/PRJ EB60074/).

Bioinformatics analysis, aiming to classify reads to the species level, was conducted using the QIIME 2 software package (DOI:10.1038/s41587-019-0209-9) based on the Silva 138 reference sequence database (DOI:10.1093/nar/

Table 1. Conditions for conducting the ex-situ underground coal gasification (UCG) processes (Wiatowski et al. 2023).

Parameters (units)	W1	W2	W3
Coal origin	"Piast-Ziemowit" mine (Poland)		"Wesoła" mine (Poland)
Gasifying agent	OEA	Oxygen	OEA
Installation pressure	Ambient	Ambient	Ambient
Coal block dimensions (m)	0.6 x 0.8 x 2.5	0.5 x 0.7 x 2.0	0.5 x 0.7 x 2.0
Mass of coal inside the reactor (kg)	1225	687	830
Experiment duration (h)	56	72	72
Amount of coal used (kg)	140.9	323.9	165.3
Wastewater produced (kg)	1018	1372	1197
Wastewater production rate (kg/h)	4.18	5.01	2.63
Wastewater outflow (kg/kg gasified coal)	1.66	1.12	1.14

OEA - oxygen-enriched air

Table 2. Physico-chemical and ecotoxicological characterization of UCG post-processing waters.

Parameters (units)	Methods used	W1	W2	W3
pH	Potentiometric	2.6	1.8	7.0
Conductivity (mS cm^{-1})	Conductometric	2610	8640	1530
Redox (mV)	Potentiometric (Ag/AgCl)	263	382	112
Ammonia NH_4 (mg L^{-1})	Flow injection analysis (FIA) with spectrophotometric detection	180	160	190
N-NO3 (mg L^{-1})	Spectrophotometric / ion chromatography (IC)	0.11	0.12	<0.1
N-NO2 (mg L^{-1})	Spectrophotometric / ion chromatography (IC)	<0.006	<0.002	0.016
Total nitrogen (mg L^{-1})	High-temperature combustion with chemiluminescent detection	150	120	150
BOD (mgO_2 L^{-1})	Electrochemical	360	550	170
COD (mgO_2 L^{-1})	Spectrophotometric	1130	1060	397
TOC (mgC L^{-1})	High-temperature combustion with IR detection	330	350	130
Chlorides (mg L^{-1})	Titrimetric/ion chromatography (IC)	516	1120	269
Sulfates (mg L^{-1})	Gravimetric / ion chromatography (IC)	154	106	130
Nitrates (mg L^{-1})	Spectrophotometric / ion chromatography (IC)	0.50	0.53	<0.5
Nitrites (mg L^{-1})	Spectrophotometric / ion chromatography (IC)	<0.006	<0.006	0.051
Total cyanides (mg L^{-1})	Continuous flow analysis (CFA) with spectrophotometric detection	6.7	42	5.1
Phenol index (mg L^{-1})	Continuous flow analysis (CFA) with spectrophotometric detection	94	165	41
Total phosphorus (mg L^{-1})	Inductively coupled plasma - optical emission spectrometry (ICP-OES)	0.12	0.10	<0.065
Sulfides (mg L^{-1})	Flow injection analysis (FIA) with spectrophotometric detection / spectrophotometric	<0.02	0.25	<0.02
Fe (mg L^{-1})	Inductively coupled plasma - optical emission spectrometry (ICP-OES)	3.64	22.7	0.12
Mn (mg L^{-1})		0.14	0.39	0.053
Sb (mg L^{-1})		0.24	<0.05	0.2
As (mg L^{-1})		<0.05	<0.05	<0.05
B (mg L^{-1})		0.28	1.75	0.26
Cr (mg L^{-1})		0.28	4.83	<0.005
Zn (mg L^{-1})		13.4	4.65	0.92
Al (mg L^{-1})		1.6	3.02	0.13
Ca (mg L^{-1})		2.12	1.34	0.86
Cd (mg L^{-1})		0.0078	0.0084	<0.001
Co (mg L^{-1})		0.0059	0.071	<0.005
Cu (mg L^{-1})		0.013	<0.01	0.026
Mg (mg L^{-1})		0.45	0.39	0.16
Mo (mg L^{-1})		0.0063	<0.005	0.011
Ni (mg L^{-1})		0.40	2.21	0.32
Pb (mg L^{-1})		1.25	3.06	<0.02
Se (mg L^{-1})		<0.05	<0.05	<0.05
Ti (mg L^{-1})		0.023	0.069	<0.005
Hg (mg L^{-1})	Cold-vapour atomic absorption spectrometry (CV-AAS) with the amalgamation technique	<0.0005	<0.001	<0.001
PAHs (µg L^{-1})	High-performance liquid chromatography (HPLC-FLD) after pressure liquid-solid extraction (SPE)	188.26	1731	2057
BTEX (µg L^{-1})	Headspace analysis with gas chromatography and mass detection (HS-GC-MS)	1357	732	604
Toxicity TU Toxicity class	Microtox	373.6 V	405.4 V	732 V

TU – Toxicity Unit

Table 3. Summary of the metagenomic sequencing and preprocessing results for each sample.

Preprocessed sequences	W1	W2	W3
Raw reads	162 900	189 704	221 974
Raw bases (Mbp)	49.00	57.10	66.80
Trimmed reads	130 800	147 946	180 152
Trimmed bases (Mbp)	29.45	33.30	40.55
Trimmed reads (%)	80.3	78.00	81.16
Trimmed bases (%)	60.10	58.32	60.70
Average length (bp)	225.15	225.2	224.8
Max length (bp)	301	301	301
GC content (%)	52.5	54.0	52.5
dada2 filtered sequences			
input sequences	130 800	147 946	180 152
filtered sequences	115 009	129 404	164 924
% of filtered	87.93	87.47	91.55
denoised	112 567	128 786	160 060
merged	105 403	127 328	143 161
% of megged	80.58	86.06	79.47
OTUs	100 233	125 264	127 757
% of OTUs	76.63	84.67	70.92

gkm864). The DADA2 package (DOI:10.1038/nmeth.3869) was also employed for filtering out sequences containing errors introduced during the sequencing process (denoising), as well as for merging paired reads to enhance the accuracy of sequencing. This process was performed in paired-end mode, allowing for subsequent merging of corresponding forward and reverse reads, dereplication (merging of identical, unique sequences while preserving their occurrence and quality profile), and chimera filtering.

Statistical analysis and data visualization

Statistical tools like Shannon diversity and Simpson indices, number of OTUs (Operation Taxonomic Units), Chao1 and Ace estimators were used for evaluation of α-diversity (Thukral 2023).

The top OTUs at various levels were used to generate a phylogenetic heatmap and to present the pattern of UCG post-processing wastewater bacterial community variation and distribution.

A redundancy analysis (RDA) was performed using R, and the data from the analysis were then transferred to Excel to create a graph illustrating the relationships among bacterial phyla and selected wastewater characteristics, including organic and inorganic pollutants. Venn diagrams of bacterial taxa were generated using SRplot.

Results and Discussion

Chemical and toxicological characterization of post-processing water

Wastewater from UCG processes exhibited very high concentrations of BTEX (benzene, toluene, ethylbenzene, xylene), polyaromatic hydrocarbons (PAHs) and phenol (Table 2). BTEX concentrations ranged from 604 to 1357 mg L^{-1}, while PAH concentrations ranged from 188.26 to 2057 mg L^{-1}. Benzene was the predominant BTEX compound, constituting between 35% (W1) and 74% (W3) of the total. Among PAHs, chrysene (18%), pyrene (14%), and benzo(β)fluoranthene (11%) predominated in W1; acenaphtalene (46%), pyrene (14%), and phenanthrene (10%) in W2; and naphtalene (61%) and benzo(α)pyrene (16.2%) in W3. Phenol concentrations were 94 mg L^{-1}, 165 mg L^{-1} and 41 mg L^{-1} in W1, W2 and W3, respectively. It is noteworthy that phenol toxicity levels range from 9 to 25 mg L^{-1} for both humans and aquatic life (Sharma and Bhattacharya 2017). Given the adverse health effects associated with phenolic compounds , the World Health Organization (WHO) has set the maximum permissible level for phenol in the environment at 0.01 mg L^{-1}.

All organic compounds detected in UCG wastewater pose high toxicity to the environment and living organisms, and their discharge leads to serious health risks to humans, animals, and aquatic systems. The majority of hydrocarbon pollutants are classified as persistent organic pollutants (POPs), exacerbating their impact on ecosystems and human health.

Among the inorganic contaminants, UCG wastewater exhibited high concentrations of nitrogen (120 – 150 mg L^{-1}), ammonia (160 -190 mg L^{-1}), and cyanide (5.1 – 42 mg L^{-1}). While the concentrations of detected metals were at low levels (Table 2), elevated levels were observed for some metals including B, Cr, Zn, Ni, and Al. The high concentrations of chemical compounds in the post-processing waters contributed to elevated values of BOD (biochemical oxygen demand) and COD (chemical oxygen demand) parameters. BOD and COD values ranged between 170 and 550 mg O_2 L^{-1} and from 397 to 1130 mg O_2 L^{-1}, respectively (Pankiewicz- Sperka et al.

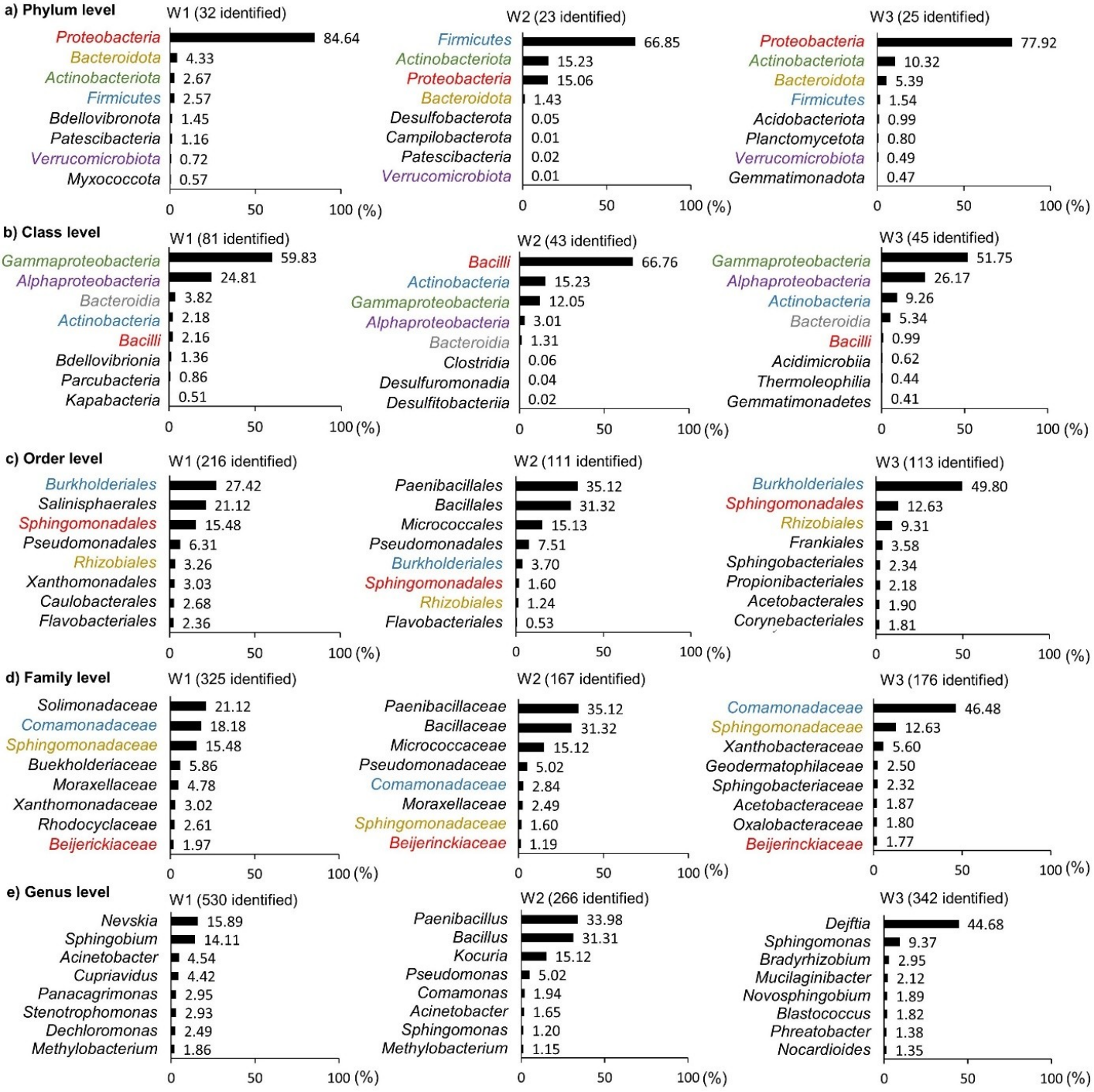

Figure 1. Taxonomic hierarchy and relative abundance of bacteria in investigated post-processing wastewaters (top of 8 most common taxons from each wastewater).

2014). Toxicological analyses revealed the high toxicity of UCG post-processing water due to the presence of refractory and inhibitory compounds. As a result, the wastewater was classified as cytotoxic and genotoxic waste.

Table 2 reveals chemical differences among post-processing waters obtained from three underground coal gasification processes, where two types of coal and various gasification agents (air and oxygen) were used. Similar results were reported by Pankiewicz-Sperka et al. (2021), who performed the UCG experiments using semi-anthracite and bituminous coal under different pressures (20 and 40 bars). These results confirm that the chemical composition of post-processing water depends on the type of coal and gasification. Among the chemicals detected, organic compounds such as phenol, BTEX, and PAHs were identified as the most significant contaminants in UCG post-processing wastewater. A high variability of phenol concentration, ranging from 500–3000 mg L^{-1}, was observed due to fluctuations in pre-treatment performance.

Table 2 presents the toxicity of wastewater evaluated by toxicity unit (TU). The TU values were 373, 405 and 732 for W1, W2 and W3, respectively. According to the classification proposed by Persoone et al. (2003), these values correspond to the fifth toxicity class, indicating very high acute toxicity.

Table 4. Alpha diversity indices of bacterial communities in analyzed samples.

Samples	Number of OTU	Observed features	Shannon	Simpson	Ace	Chao1
W1	100 233.00	747.00	6.255	0.945	747.886	747.833
W2	125 264.00	235.00	3.982	0.859	235.383	235.09
W3	127 757.00	1 138.00	6.022	0.845	1139.506	1139.894

Microbial community diversity

Table 3 presents a summary of results from sequencing and preprocessing. A total of 409,337 filtered sequences were obtained from the UCG post-processing wastewater, accounting for 89% of the raw reads.

Archaea were detected in W1 and W2. In W1, about 4 reads were classified as Archaea, representing 0.003521% of the total reads. All of these reads were related to *Woesarchaeles* family. In W2, only 3 reads were classified as Archaea, accounting for 0.002115% of the total reads. These reads were related to the phylum *Nanoarchaeota* and *Thermoplasmatota*. As Archaea appeared to be sporadic, they were omitted from our analysis, and only bacterial communities were characterized.

The total number of OTUs was 353,254, with 76.63 %, 84.67% and 70.92% found in W1, W2 and W3, respectively.

Alpha-diversity analyses were performed to assess the diversity among the W1, W2 and W3 samples, and the results are presented in Table 4. Microbial community richness and diversity were assessed using alpha diversity estimators, including the Ace, Chao 1, Simpson, and Shannon indices. Generally, W2 exhibited the lowest values for the Shannon (H), Ace, and Chao1 indices, indicating reduced microbial community diversity and richness compared to W1 and W3. Specifically, the Chao1 and Ace indices were approximately 70-80% lower in W2. Conversely, W3 displayed the highest diversity, followed by W1, as indicated by the Shannon, Chao1 and Ace estimators. The values of Shannon's diversity index, ACE and Chao1 metrics indicated that W2 had lower species richness than the other samples.

The observed microbial richness and diversity, based on all indices, followed the increasing order: W2<W1<W3. These metrics were influenced by the number of OTUs present. Variations in contaminant concentrations, particularly phenol, BTEX, and PAHs, in the post-processing wastewater, impacted microbial diversity. Additionally, parameters of the underground coal gasification process, such as oxygen, which served as a gasifying agent in the second process, likely influenced bacterial diversity. In underground coal gasification processes no. 1 and no. 3, oxygen-enriched air was utilized as the gasifying agent. Comparing the diversity estimators values obtained in thius study with those reported in the literature for wastewater treatment plants, they were found to be low. This observation suggests that diversity and richness in polluted environments are specific and depend on the type of pollution (Liu et al. 2019, Yang et al. 2020).

Microbial community structure

This study presents the first culture-independent profiling of microbial diversity in UCG post-processing wastewater. Phylogenic diversity and relative abundance among W1, W2, and W3 are depicted in Figure 1. The dominant phylum observed was *Proteobacteria*, constituting 84.64% and 77.92% of the microbial community in W1 and W3, respectively. While W1 and W3 shared similar dominant phyla, their proportion differed.

In W2, *Firmicutes* dominated, comprising 66.85% of the microbial community. At the class level, *Gammaproteobacteria* and *Alphaproteobacteria* were predominant in W1 and W3, while *Bacilli* and *Actinobacteria* were predominant in W2. Significant differences in taxonomic abundance were observed between W1 and W3, starting from the order level The distinctions in bacterial community structures among W1, W2, and W3 are presented in Figures 2 and 3.

As the analyses showed the unidentified species ranged between 86.3% in W2 and 96% in W3.

In W1, the percentage of unidentified species was similar to that in W3, and amounted to 93.35%. In contrast, identified species accounted for less than 5% of total classified reads, with the remaining percentage termed as 'spare', ranging between 4.11% in W3 and 13.73% in W2. Most of the identified species in the wastewater samples were uncultured. For example, in W1, uncultured *Nevskia* represented 15.82% of the identified species. Other significant uncultured species were found in *Burkholderiales* (14.26%) and *Sphingobium* (13.2%).

In W2, the most dominant species were *Penibacillus* (26.51%), *Bacillus* (25.34%), and *Actinobacteria* (15.12%). Keystone species included *Paenibacillus glucanolyticus, Paenibacillus favisporus, Bacillus horneckiae*, and *Kocuria* spp. These bacteria listed above possess specific metabolic properties that facilitate their survival in extreme environments and could be valuable in biotechnological applications (Karn et al. 2011, Grady et al. 2016, Timkina 2020). Conversely, in W3, the most dominant bacteria were *Delftia* (44.68%) and *Sphingomonas* (6.56%).

In our previous studies, we employed a straightforward cultivation procedure and unique biochemical selection to gain insights into the specific properties of bacteria (Jałowiecki et al. 2024). From the 100 strains isolated from UCG wastewaters, three - *Paenibacillus pasadensis* SAFN-007, *Peanibacillus humicus* Au34, and *Staphylococcus warneri* DK131 - demonstrated the capacity to degrade phenol and exhibited specific biochemical properties. These specific properties make the isolated bacterial strains good candidates for bioremediation of phenol-contaminated environments. Among the identified bacteria, *Paenibacillus* and *Bacillus* species were dominant in UCG post-processing waters. The following bacteria were dominated in the post-processing wastewater: *Paenibacillus glucanotylicus* P69, *Bacillus altitudinis* VMFR48, *Bacillus nealsoni* LE3, *Bacillus pseudomycoides* S2015-2C, *Bacillus sp.* CMAA 1185, *Bacillus stratosphericus* TR4, *Margalitia shackletoni* LMG-18435, *Oceanobacillus picturae* B5,

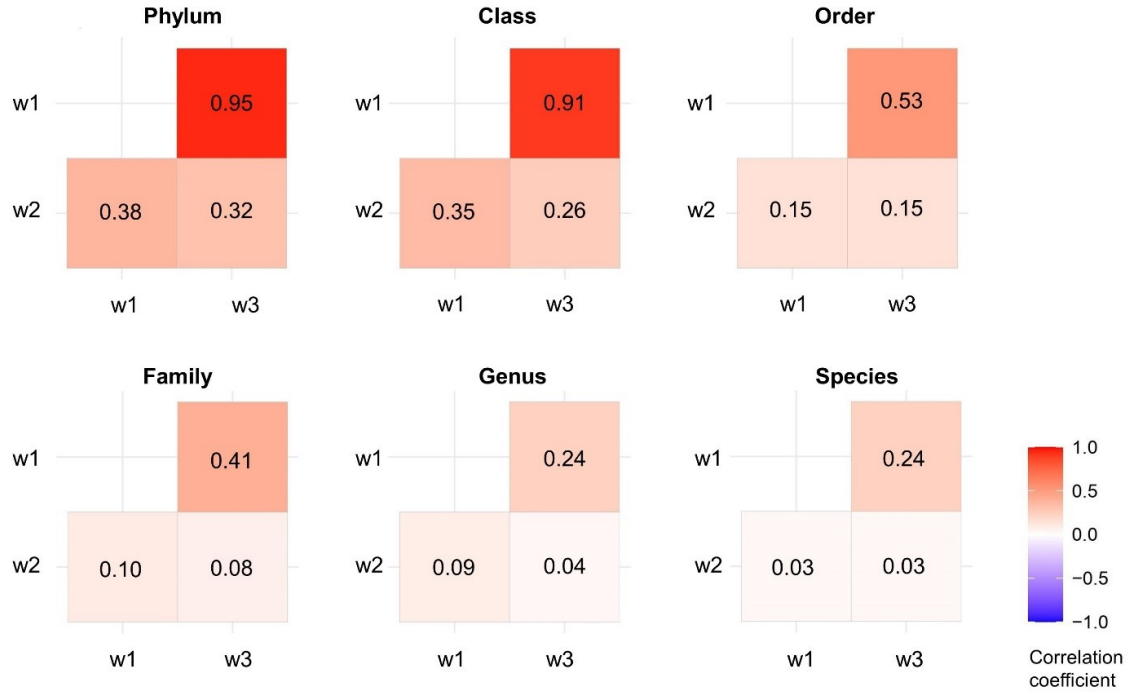

Figure 2. Correlation matrices of the contribution of individual OTUs between the analyzed sample groups (W1, W2 and W3) for six taxonomic levels (Phylum, Class, Order, Family, Genus and Species).
The level of correlation is shown on a scale from 0 (no correlation between samples: white color on the scale) to 1 (highest correlation between samples, with a value of 1 meaning that the samples have identical taxonomic composition: red color on the scale). The blue color on the legend's scale and a value of -1 indicate negative correlation, which in this case is impossible and is treated as a control.

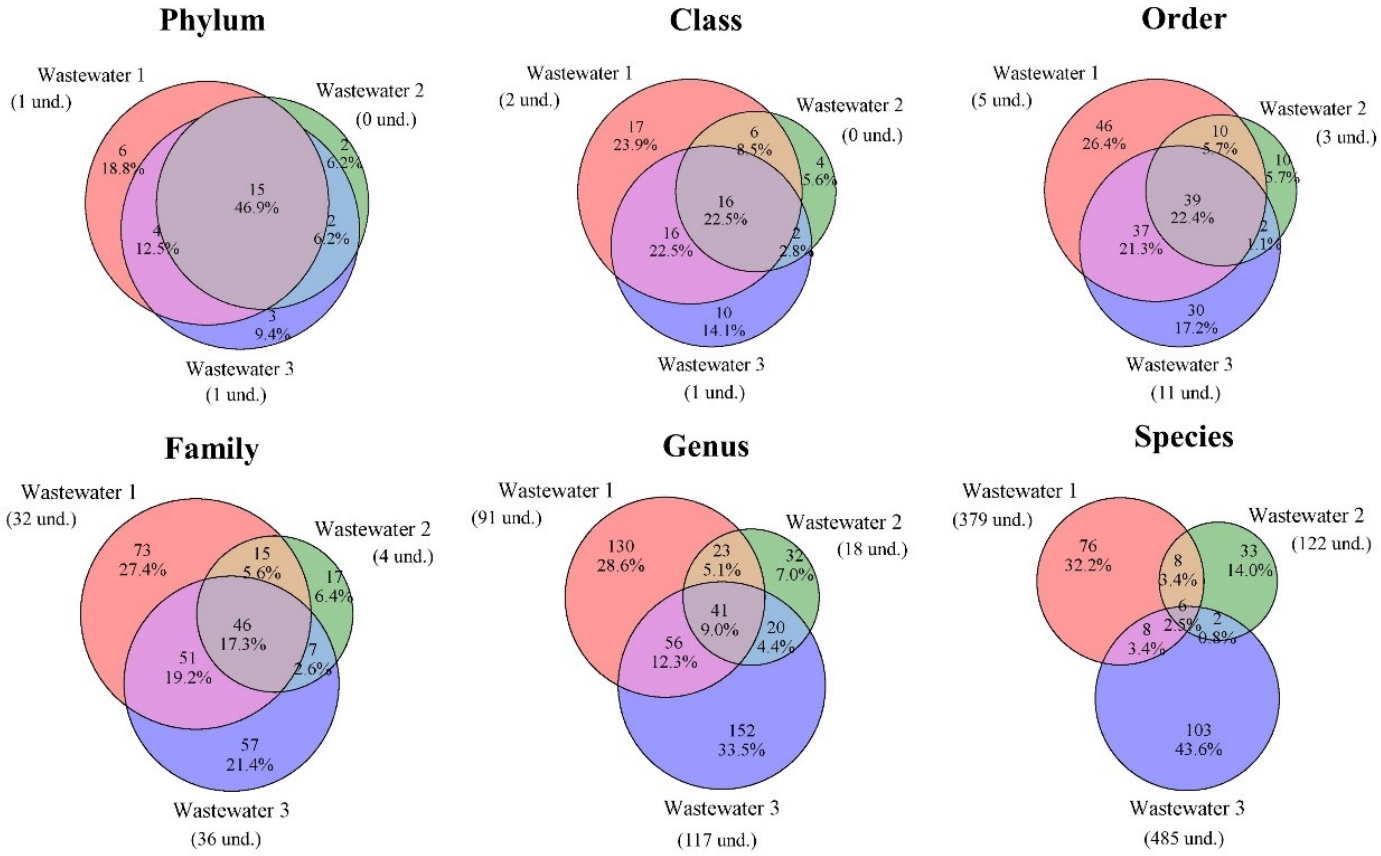

Figure 3. Venn diagrams showing the proportion of common bacterial taxa at different taxa levels in all analysed wastewaters. The number of unidentified reads that were not classified at the respective taxonomic level is shown in brackets.

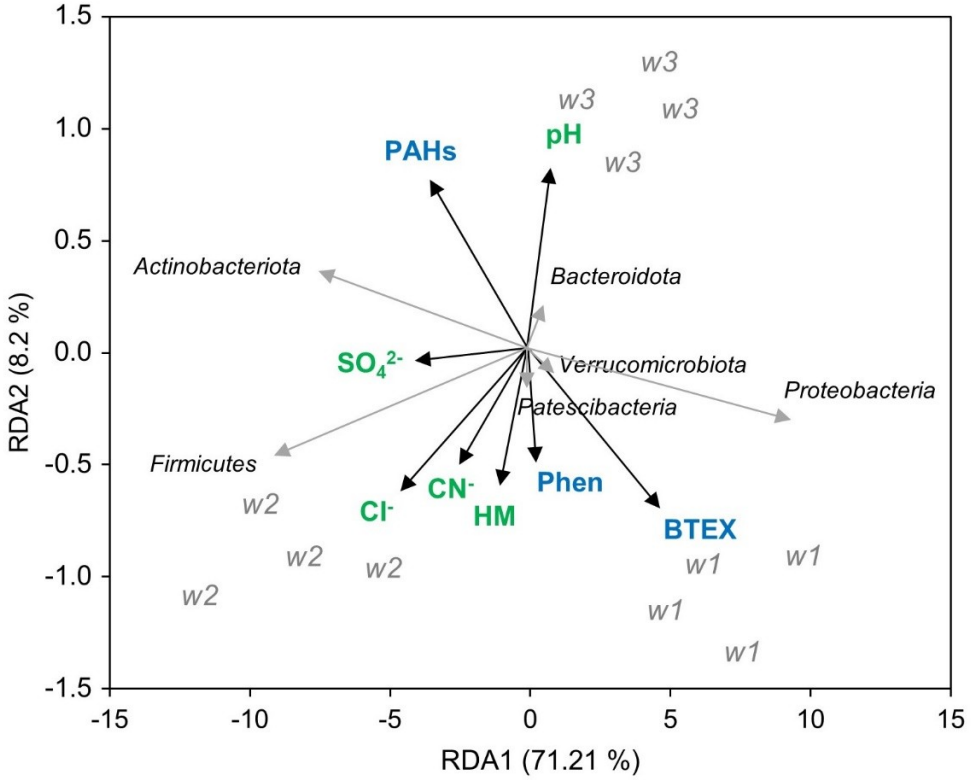

Figure 2. The redundancy analysis (RDA) order diagrams showing the relationships between the bacterial phylum (the taxons with the highest abundance which are found in each wastewater) and selected wastewater characteristics (green labels indicate inorganic substances, blue – organic compounds). Arrows indicate the direction and magnitude of the variables. Abbreviations: HM – heavy metals, Phen – phenols, PAHs – polycyclic aromatic hydrocarbons, BTEX – benzene, toluene, ethylbenzene and xylene

Paenibacillus cineris BB, *Paenibacillus favisporus* U3, *Paenibacillus humicus* Au34, *Paenibacillus humicus* Sp29, *Paenibacillus pasadensis* SAFN-007, *Paenibacillus cineris* cu1-7, *Peanibacillus cellulositrophicus* KACC 16577 and *Staphylococcus warneri* DK131.

When comparing the results obtained from molecular analysis (metagenomic approach) and traditional microbial culture tests, certain similarities can be seen. Both types of experiments detected the genera *Paenibacillus* and *Bacillus*. Strains of *Paenibacillus* and *Bacillus* are known to produce various enzymes and useful molecules, including exo-polysaccharides (EPS), biosurfactants, and enzymes such as oxygenases, dehydrogenases, amylases, cellulases, hemicellulases, lipases and lignilolytic enzymes (Grady et al. 2016). Considering the physiological and biochemical properties of *Paenibacillus* and *Bacillus* strains, they exhibit the ability to degrade aliphatic and aromatic organic pollutants (Guisado et al. 2015, Mauricio-Gutiérrez et al. 2020, Jayapal et al. 2023). Both genera, *Paenibacillus* and *Bacillus*, are Gram-positive, endospore-forming bacteria capable of thriving in both aerobic and anaerobic conditions.

Figure 4 shows the relationship between selected physicochemical parameters of wastewater and the dominant bacterial phylum. Acute angles were observed among SO_4^{2-}, CN^-, Cl^-, and heavy metals (HM), as well as between PAHs and pH, suggesting a potentially synergetic impact of these variables on the bacterial community. However, the conducted analysis showed that the effect of these factors on phyla such as *Bacteroidota*, *Verrucomicrobiota* and *Patescibacteria* may have a weak influence on their abundance. In contrast, *Firmicutes* phylum showed a positive correlation with anions CN^- and Cl^-, which were present at high levels in wastewater 2 (W2). Additionally, W2 exhibited the highest salinity, as evidenced by the elevated electrical conductivity values. Many *Firmicutes* are capable of producing spores, enabling them to withstand extreme conditions such as high salinity (Fimlaid and Shen 2015). *Proteobacteria* emerged as one of the most abundant bacteria phyla in the examined UCG wastewater, particularly in wastewaters with high BTEX levels. Previous research has indicated that many *Proteobacteria* possess higher BTEX degradation activity (Chen et al. 2022).

The structure of bacterial communities is strongly influenced by environmental variables such as nutrients and contaminants concentration, temperature, pH, etc. Variations in microbial community compositions can significantly impact their functional role in environmental processes. Post-processing waters from the underground coal gasification process serve as an example of an extreme environment conducive to the isolation of unique bacteria with specific metabolic properties.

Many wastewaters from various industries contain a mixture of recalcitrant, hazardous, and highly toxic compounds, leading to adverse effects. Consequently, establishing treatment methods capable of handling the complex mixture of compounds associated with industrial wastewaters is imperative. Industrial wastewaters pose significant challenges that necessitate effective treatment. One method involves the application of microorganisms with the capability to remove pollutants. Bioaugmentation-assisted bioremediation has emerged as a sustainable technology for the ecorestoration

of heavily polluted environments. Microorganisms can utilize most the toxic pollutants as substrates and possess the ability to degrade or metabolize them. Therefore, selecting a suitable strain is essential for the success of bioaugmentation (Nwankwegu et al. 2022). The addition of supplementary microorganisms with their associated biodegradation capacities and specific properties can enhance the quality of the wastewater. The selected strain(s) or their consortia must be able to withstand specific environmental conditions imposed during treatment process, including pH, dissolved oxygen, temperature, high pollutant concentrations, nutrient availability, and microbial pressures (Muter 2023). Thus, understanding the structure of microorganisms under extreme conditions is one of the challenges in developing effective wastewater treatment strategies.

Bioaugmentation relies on the selection and isolation of relevant strains from the indigenous population, which is favoured for increasing the success of bioaugmentation. This approach increases the likelihood of strains adapting to survival in the selected environment. Moreover, the success of bioaugmentation is increasingly associated with the effective incorporation of inoculated strains into the contaminated environment. This success is influenced, among other factors, by the selection of appropriate strains, their introduction to extreme environments, and finally, their ability to survive and thrive in these conditions.

Conclusions

The results of the study contribute to our understanding of microbial ecology in contaminated environments. Bacterial communities in W1, W2, and W3 are predominantly composed of *Proteobacteria* and *Firmicutes.* Bacteria isolated from the specific and extreme environments hold significant potential for biotechnological applications and provide insights into various environmental and ecophysiological functions. Overall, the study enhances our knowledge of microbial diversity in extreme environments, such as industrial wastewater heavily contaminated by hydrocarbons. These findings are valuable for developing green strategies to clean up contaminated environments where environmental degradation is prevalent. Understanding the microbial structure in polluted environments is essential for the development of remediation technologies, especially bioaugmentation-assisted bioremediation. This approach has proven to be effective, fast, and affordable for eco-restoration in heavily polluted environments. By applying microbial science in bioremediation processes, the selection of microbes or their consortia with degradation potential and specific product production for biotechnological applications is simplified.

Acknowledgements

The research was conducted as part of the UCG WaterPlus project entitled: „Coal- and bio-based water remediation strategies for underground coal gasification and beyond" supported by the EU Research Fund for Coal and Steel, under the Grant Agreement no. 101033964, and by the Polish Ministry of Education and Science under the contracts no. 5198/FBWiS/2021/2 and 5211/FBWiS/2021/2.

Conflicts of Interest

The authors declare no conflict of interest.

References

Bassin, J.; Rachid, C.; Vilela, C. Cao, S.; Peixoto, R. & Dezotti, M. (2017). Revealing the bacterial profile of an anoxic-aerobic moving-bed biofilm reactor system treating a chemical industry wastewater, *International Biodeterioration & Biodegradation*, 120, pp. 152–160. DOI:10.1016/j.ibiod.2017.01.036

Bedogni, G.L.; Massello, F. L.; Giaveno, A.; Donati, E.R. & Urbieta, M.S. (2020). A deeper look into the biodiversity of the extremely acidic copahue volcano - Río Agrio system in Neuquén, Argentina, *Microorganisms*, 8, 58. DOI:10.3390/microorganisms8010058

Chen, T.; Wu, Y.; Wang, J. & Philippe, C. F. X. (2022). Assessing the biodegradation of btex and stress response in a bio-permeable reactive barrier using compound-specific isotope analysis, *International Journal of Environmental Research and Public Health*, 19, 8800. DOI:10.3390/ijerph19148800

Fimlaid, K. A. & Shen, A. (2015). Diverse mechanisms regulate sporulation sigma factor activity in the *Firmicutes*, *Current Opinion in Microbiology,* 24, pp. 88-95. DOI:10.1016%2Fj.mib.2015.01.006

Gawroński, S., Łutczyk, G.; Szulc, W. & Rutkowska, B. (2022). Urban mining: Phytoextraction of noble and rare earth elements from urban soils, *Archives of Environmental Protection,* 48, 2, pp. 24-33. DOI:10.24425/aep.2022.140763

Grabowski, J., Korczak, K. & Tokarz, A. (2021). Aquatic risk assessment based on the results of research on mine waters as a part of a pilot underground coal gasification process, *Process Safety and Environmental Protection*, 148, pp. 548-558. DOI:10.1016/j.psep.2020.10.003

Grady, E.N., MacDonald, J., Richman, A. & Yuan, Z.C. (2016). Current knowledge and perspectives of *Paenibacillus*: a review. *Microbial Cell Factories*, 15, 203. DOI:10.1186/s12934-016-0603-7

Guisado, I.M., Purswani, J., Gonzales-Lopez, J. & Pozo, C. (2015). Physiological and genetic screening methods for isolation of methyl-tert-butyl-ether-degrading bacteria for bioremediation purposes, *International Biodeterioration and Biodegradation*, 97, pp. 67-74. DOI:10.1016/j.ibiod.2014.11.008

Jałowiecki, Ł., Borgulat, J.; Strugała-Wilczek, A., Glaser, M. & Płaza, G. (2024). Searching of phenol-degrading bacteria in raw wastewater from underground coal gasification process as suitable candidates in bioaugmentation approach, *Journal of Ecological Engineering*, 25, pp. 62–71. DOI:10.12911/22998993/176143

Jayapal, A., Chaterjee, T. & Sahariah, B.P. (2023). Bioremediation techniques for the treatment of mine tailings: A review, *Soil Ecology Letters*, 5, 220149. DOI:10.1007/s42832-022-0149-z

Kamika, I., Azizi, S. & Tekere, M. (2016). Microbial profiling of South African acid mine water samples using next generation sequencing platform, *Applied. Microbiology and Biotechnol*ogy, 100, pp.6069–6079. DOI:10.1007/s00253-016-7428-5

Kapusta, K. & Stańczyk, K. (2015). Chemical and toxicological evaluation of underground coal gasification (UCG) effluents. The coal rank effect, *Ecotoxicology and Environmental Safety*, 112, pp. 105– 113. DOI:10.1016/j.ecoenv.2014.10.038

Karn, S.K., Chakrabarti, S.K. & Reddy, M.S. (2011). Degradation of pentachlorophenol by *Kocuria* sp. CL2 isolated from secondary

sludge of pulp and paper mill, *Biodegradation*, 22, pp. 63-69. DOI:10.1007/s10532-010-9376-6

Kochhar, N., Kavya, I.K., Shrivvastava, S., Ghosh, A., Rawat, V.S., Sodhi, K.K. & Kumar, M. (2022) Perspectives on the microorganisms of extreme environments and their applications, *Current Research Microbial Sc*iences. 3, 100134. DOI:10.1016/j.crmicr.2022.100134

Liu, F., Hu, X., Zhao, X., Guo, H. & Zhao, Y. (2019). Microbial community structures' response to seasonal variation in a full-scale municipal wastewater treatment plant, *Environmental Engineering Science*, 36, pp. 172-178. DOI:10.1089/ees.2018.0280

Luo, Z., Ma, J., Chen, F., Li, X., Zhang, Q. & Yang, Y. (2020). Adaptive development of soil bacterial communities to ecological processes caused by mining activities in the Loess Plateau, China, *Microorganisms*, 8, 477. DOI:10.3390/microorganisms8040477

Mauricio-Gutiérrez, A., Machorro-Velázquez R., Jiménez-Salgado, T.;Vázquez-Crúz C., Sánchez-Alonso, M.P. & Tapia-Hernández, A. (2020). *Bacillus pumilus* and *Paenibacillus lautus* effectivity in the process of biodegradation of diesel isolated from hydrocarbons contaminated agricultural soils, *Archives of Environmental Protection*, 46, 4, pp. 59–69. DOI:0.24425/aep.2020.135765

Muter, O. (2023). Current trends in bioaugmentation tools for bioremediation: A critical review of advances and knowledge gaps, *Microorganisms*, 11, 710. DOI:10.3390/microorganisms11030710

Nwankwegu, A.S., Zhang, L., Xie, D., Onwosi, C.O., Muhammad, W.I., Odoh, C.K., Sam, K. & Idenyi, J.N. (2022). Bioaugmentation as a green technology for hydrocarbon pollution remediation. Problems and prospects. *Journal of Environmental Management*, 304, 114313. DOI:10.1016/j.jenvman.2021.114313

Pankiewicz-Sperka, M., Kapusta, K., Basa, W. & Stolecka, K. (2021). Characteristics of water contaminants from underground coal gasification (UCG) process - effect of coal properties and gasification pressure, *Energies,* 14, 6533. DOI:10.3390/en14206533

Pankiewicz-Sperka, M., Stańczyk, K., Płaza, G., Kwaśniewska, J. & Nałęcz-Jawecki, G. (2014). Assessment of the chemical, microbiological and toxicological aspects pf post-processing water from underground coal gasification, *Ecotoxicology and Environmental Safety*, 108, pp. 294-301. DOI:10.1016/j.ecoenv.2014.06.036

Persoone, G., Marsalek, B., Blinova, I., Torokne, A., Zarina, D., Manusadzianas, L. (2003). A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters, *Environmental Toxicology*, 18, pp. 395–402. DOI:10.1002/tox.10141.

Rappaport, H.B. & Oliverio, A.M. (2023). Extreme environments offer an unprecedent opportunity to understand microbial eukaryotic ecology, evolution, and genome biology, *Nature Communication*, 14, 4959. DOI:10.1038/s41467-023-40657-4

Sharma, S. & Bhattacharya, A. (2017) Drinking water contamination and treatment techniques. *Appied Water Science* 7, pp. 1043-1067. DOI:10.1007/s13201-016-0455-7

Smoliński, A.. Stańczyk, K.. Kapusta, K. & Howaniec, N. (2013). Analysis of the organic contaminants in the condensate produced in the in situ underground coal gasification process, *Water Science and Technol*ogy, 67, pp. 644-650. DOI:10.2166/wst.2012.558

Thukral, A.K. (2017). A review on measurement of alpha diversity in biology, *Agricultural Research Journal,* 54, 1. DOI:10.5958/2395-146X.2017.00001.1

Timkina, E., Drabova, L., Palyova, A,, Rezanka, T., Matatkova, O. & Kolouchova, I. (2020). *Kocuria* strains from unique radon spring water from Jachymov Spa, *Fermentation*, 8, 35. DOI:10.3390/fermentation8010035

Wiatowski, M., Kapusta, K., Strugała-Wilczek, A., Stańczyk, K., Castro-Muñiz, A., Suárez-García F. & Paredes, J.I. (2023). Large-scale experimental simulations of in situ coal gasification in terms of process efficiency and physicochemical properties of process by-products, *Energies*, 16, 4455. DOI:10.3390/en16114455

Xu, B., Chen, L., Xing, B., Li, Z., Zhang, L., Yi, G., Huang, G. & Mohanty, M.K. (2017). Physicochemical properties of Hebi semi-coke from underground coal gasification and its adsorption for phenol, *Process Safety Environmental Prot*ection, 107, pp. 147–152. DOI:10.1016/j.psep.2017.02.007

Yang, Y., Wang, L., Xiang, F., Zhao, L. & Qiao, Z. (2020). Activated sludge microbial community and treatment performance of wastewater treatment plants in industrial and municipal zones, *International Journal of Environmental Research and Public Health*, 17, 436. DOI:10.3390/ijerph17020436

Zwain, H., Al-Marzook, F., Nile, B., Ali Jeddoa, Z., Atallah, A., Dahlan, I. & Hassan, W. (2021). Morphology analysis and microbial diversity in novel anaerobic baffled reactor treating recycled paper mill wastewater, *Archives of Environmental Protection*, 47, 4, pp. 9–17. DOI:10.24425/aep.2021.139498