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.
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 Mikolów (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 m³ plastic tank (Mauser type). After mixing, the samples were transferred 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 Illumina 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/PRJEB60074/).

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/gky1012).

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

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

OEA - oxygen-enriched air
<table>
<thead>
<tr>
<th>Parameters (units)</th>
<th>Methods used</th>
<th>W1</th>
<th>W2</th>
<th>W3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>Potentiometric</td>
<td>2.6</td>
<td>1.8</td>
<td>7.0</td>
</tr>
<tr>
<td>Conductivity (mS cm(^{-1}))</td>
<td>Conductometric</td>
<td>2610</td>
<td>8640</td>
<td>1530</td>
</tr>
<tr>
<td>Redox (mV)</td>
<td>Potentiometric (Ag/AgCl)</td>
<td>263</td>
<td>382</td>
<td>112</td>
</tr>
<tr>
<td>Ammonia NH(_4) (mg L(^{-1}))</td>
<td>Flow injection analysis (FIA) with spectrophotometric detection</td>
<td>180</td>
<td>160</td>
<td>190</td>
</tr>
<tr>
<td>N-NO(_3) (mg L(^{-1}))</td>
<td>Spectrophotometric / ion chromatography (IC)</td>
<td>0.11</td>
<td>0.12</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>N-NO(_2) (mg L(^{-1}))</td>
<td>Spectrophotometric / ion chromatography (IC)</td>
<td>&lt;0.006</td>
<td>&lt;0.002</td>
<td>0.016</td>
</tr>
<tr>
<td>Total nitrogen (mg L(^{-1}))</td>
<td>High-temperature combustion with chemiluminescent detection</td>
<td>150</td>
<td>120</td>
<td>150</td>
</tr>
<tr>
<td>BOD (mgO(_2) L(^{-1}))</td>
<td>Electrochemical</td>
<td>360</td>
<td>550</td>
<td>170</td>
</tr>
<tr>
<td>COD (mgO(_2) L(^{-1}))</td>
<td>Spectrophotometric</td>
<td>1130</td>
<td>1060</td>
<td>397</td>
</tr>
<tr>
<td>TOC (mgC L(^{-1}))</td>
<td>High-temperature combustion with IR detection</td>
<td>330</td>
<td>350</td>
<td>130</td>
</tr>
<tr>
<td>Chlorides (mg L(^{-1}))</td>
<td>Titrimetric/ion chromatography (IC)</td>
<td>516</td>
<td>1120</td>
<td>269</td>
</tr>
<tr>
<td>Sulfates (mg L(^{-1}))</td>
<td>Gravimetric / ion chromatography (IC)</td>
<td>154</td>
<td>106</td>
<td>130</td>
</tr>
<tr>
<td>Nitrates (mg L(^{-1}))</td>
<td>Spectrophotometric / ion chromatography (IC)</td>
<td>0.50</td>
<td>0.53</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Nitrates (mg L(^{-1}))</td>
<td>Spectrophotometric / ion chromatography (IC)</td>
<td>&lt;0.006</td>
<td>&lt;0.006</td>
<td>0.051</td>
</tr>
<tr>
<td>Total cyanides (mg L(^{-1}))</td>
<td>Continuous flow analysis (CFA) with spectrophotometric detection</td>
<td>6.7</td>
<td>42</td>
<td>5.1</td>
</tr>
<tr>
<td>Phenol index (mg L(^{-1}))</td>
<td>Continuous flow analysis (CFA) with spectrophotometric detection</td>
<td>94</td>
<td>165</td>
<td>41</td>
</tr>
<tr>
<td>Total phosphorus (mg L(^{-1}))</td>
<td>Inductively coupled plasma - optical emission spectrometry (ICP-OES)</td>
<td>0.12</td>
<td>0.10</td>
<td>&lt;0.065</td>
</tr>
<tr>
<td>Sulfides (mg L(^{-1}))</td>
<td>Flow injection analysis (FIA) with spectrophotometric detection / spectrophotometric</td>
<td>&lt;0.02</td>
<td>0.25</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Fe (mg L(^{-1}))</td>
<td>Inductively coupled plasma - optical emission spectrometry (ICP-OES)</td>
<td>3.64</td>
<td>22.7</td>
<td>0.12</td>
</tr>
<tr>
<td>Mn (mg L(^{-1}))</td>
<td></td>
<td>0.14</td>
<td>0.39</td>
<td>0.053</td>
</tr>
<tr>
<td>Sb (mg L(^{-1}))</td>
<td></td>
<td>0.24</td>
<td>&lt;0.05</td>
<td>0.2</td>
</tr>
<tr>
<td>As (mg L(^{-1}))</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>B (mg L(^{-1}))</td>
<td></td>
<td>0.28</td>
<td>1.75</td>
<td>0.26</td>
</tr>
<tr>
<td>Cr (mg L(^{-1}))</td>
<td></td>
<td>13.4</td>
<td>4.65</td>
<td>0.92</td>
</tr>
<tr>
<td>Zn (mg L(^{-1}))</td>
<td></td>
<td>1.6</td>
<td>3.02</td>
<td>0.13</td>
</tr>
<tr>
<td>Al (mg L(^{-1}))</td>
<td></td>
<td>2.12</td>
<td>1.34</td>
<td>0.86</td>
</tr>
<tr>
<td>Ca (mg L(^{-1}))</td>
<td></td>
<td>0.0078</td>
<td>0.0084</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cd (mg L(^{-1}))</td>
<td></td>
<td>0.0059</td>
<td>0.071</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Co (mg L(^{-1}))</td>
<td></td>
<td>0.013</td>
<td>&lt;0.01</td>
<td>0.026</td>
</tr>
<tr>
<td>Cu (mg L(^{-1}))</td>
<td></td>
<td>0.45</td>
<td>0.39</td>
<td>0.16</td>
</tr>
<tr>
<td>Mo (mg L(^{-1}))</td>
<td></td>
<td>0.0063</td>
<td>&lt;0.005</td>
<td>0.011</td>
</tr>
<tr>
<td>Ni (mg L(^{-1}))</td>
<td></td>
<td>0.40</td>
<td>2.21</td>
<td>0.32</td>
</tr>
<tr>
<td>Pb (mg L(^{-1}))</td>
<td></td>
<td>1.25</td>
<td>3.06</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Se (mg L(^{-1}))</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Ti (mg L(^{-1}))</td>
<td></td>
<td>0.023</td>
<td>0.069</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Hg (mg L(^{-1}))</td>
<td>Cold-vapour atomic absorption spectrometry (CV-AAS) with the amalgamation technique</td>
<td>&lt;0.0005</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PAHs (µg L(^{-1}))</td>
<td>High-performance liquid chromatography (HPLC-FLD) after pressure liquid-solid extraction (SPE)</td>
<td>188.26</td>
<td>1731</td>
<td>2057</td>
</tr>
<tr>
<td>BTEX (µg L(^{-1}))</td>
<td>Headspace analysis with gas chromatography and mass detection (HS-GC-MS)</td>
<td>1357</td>
<td>732</td>
<td>604</td>
</tr>
<tr>
<td>Toxicity TU Toxicity class</td>
<td></td>
<td>Microtox</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TU – Toxicity Unit</td>
<td></td>
<td>373.6 V</td>
<td>405.4 V</td>
<td>732 V</td>
</tr>
</tbody>
</table>
Insights into bacterial diversity in industrial post-processing water from underground coal gasification (UCG) process

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.)
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.

Figure 1. Taxonomic hierarchy and relative abundance of bacteria in investigated post-processing wastewaters (top of 8 most common taxons from each wastewater).
Insights into bacterial diversity in industrial post-processing water from underground coal gasification (UCG) process

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

<table>
<thead>
<tr>
<th>Samples</th>
<th>Number of OTU</th>
<th>Observed features</th>
<th>Shannon</th>
<th>Simpson</th>
<th>Ace</th>
<th>Chao1</th>
</tr>
</thead>
<tbody>
<tr>
<td>W1</td>
<td>100,233.00</td>
<td>747.00</td>
<td>6.255</td>
<td>0.945</td>
<td>747.886</td>
<td>747.833</td>
</tr>
<tr>
<td>W2</td>
<td>125,264.00</td>
<td>235.00</td>
<td>3.982</td>
<td>0.859</td>
<td>235.383</td>
<td>235.09</td>
</tr>
<tr>
<td>W3</td>
<td>127,757.00</td>
<td>1,138.00</td>
<td>6.022</td>
<td>0.845</td>
<td>1,139.506</td>
<td>1,139.894</td>
</tr>
</tbody>
</table>

**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 *Woesearchaeales* 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 *Thermoplasmata*. 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 this 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. Phylogenetic 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 *Nevska* 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 javensis*, *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 (Jalowiecki et al. 2024). From the 100 strains isolated from UCG wastewaters, three - *Penibacillus pasadenensis* SAFN-007, *Paenidibacillus huminicu* 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, *Penibacillus* and *Bacillus* species were dominant in UCG post-processing waters. The following bacteria were dominated in the post-processing wastewater: *Paenibacillus glucanolyticus* P69, *Bacillus altitudinis* VMFR48, *Bacillus nealsoni* LE3, *Bacillus pseudomycoides* S2015-2C, *Bacillus* sp. CMAA 1185, *Bacillus stratosphericus* TR4, *Margaliticia shackletoni* LMG-18435, *Oceanobacillus picturae* B5, *Bacillus lehmnii* W5, *Bacillus accipidicus* LE4, *Bacillus stearothermophilus* LE2, *Bacillus subtilis* LE3, *Bacillus pseudomycoides* S2015-2C, *Bacillus* sp. CMAA 1185, *Bacillus stratosphericus* TR4, *Margaliticia 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.
Insights into bacterial diversity in industrial post-processing water from underground coal gasification (UCG) process

Figure 2. The redundancy analysis (RDA) order diagrams showing the relationships between the bacterial phylum (the taxa 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.


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\textsubscript{4}\textsuperscript{2-}, CN\textsuperscript{-}, Cl\textsuperscript{-}, 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\textsuperscript{-} and Cl\textsuperscript{-}, 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


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


