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**Opracowanie technologii oczyszczania ścieków z
podziemnego zgazowania węgla z zastosowaniem metod
biologicznych**

Promotor: **prof.dr hab. Grażyna Plaza**

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Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych

Składam serdeczne podziękowania

Promotorowi **prof. dr hab. Grażynie Plaza**

za okazaną życzliwość, wyrozumiałość, motywację oraz nieocenioną pomoc
w trakcie przygotowania niniejszej rozprawy doktorskiej.

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**ŹRÓDŁA FINANSOWANIA
WSPÓŁPRACA**

I. Źródła finansowania

Badania, wchodzące w skład pracy doktorskiej, były realizowane w ramach projektu zatytułowanego: „Strategie oczyszczania wód powstających w procesie podziemnego zgazowania węgla i innych procesów, bazujące na sorbentach węglowych i bioremediacji” (akronim: **UCGWater+**) finansowanego z Funduszu Węgla i Stali (nr projektu 101033964) oraz z projektów Ministerstwa Nauki i Szkolnictwa Wyższego o numerach 5198/FBWIS/2021/2 i 5211/FBWIS/2021/2.



II. Współpraca



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**SPIS PUBLIKACJI WCHODZĄCYCH
W SKŁAD ROZPRAWY DOKTORSKIEJ**

III. Spis publikacji wchodzących w skład rozprawy doktorskiej

Artykuły wiodące:

1. **Jałowiecki Ł.**, Borgulat J., Strugała-Wilczek A., Jastrzębski J., Matejczyk M., Płaza G. Insights into bacterial diversity in industrial post-processing water from underground coal gasification (UCG) process. *Archives of Environmental Protection* 2024, 50(2), 32–41. <https://doi.org/10.24425/aep.2024.150550> (IF₂₀₂₄ = 1,5; MNiE = 100).
2. **Jałowiecki Ł.**, Strugała-Wilczek A., Ponikiewska K., Borgulat J., Płaza G., Stańczyk K. Constructed wetland as a green remediation technology for the treatment of wastewater from underground coal gasification process. *PLoS ONE* 2024, 19(3): e0300485 <https://doi.org/10.1371/journal.pone.0300485> (IF₂₀₂₄ = 2,9; MEiN = 100).
3. **Jałowiecki Ł.**, Borgulat J., Strugała-Wilczek A., Glaser M., Płaza G. Searching of phenol-degrading bacteria in raw wastewater from underground coal gasification process as suitable candidates in bioaugmentation approach. *Journal of Ecological Engineering* 2024, 25(2), 62-71. <https://doi.org/10.12911/22998993/176143> (IF₂₀₂₄ = 1,3; MEiN = 100).
4. **Jałowiecki Ł.**, Borgulat J., Strugała-Wilczek A., Stańczyk K., Jastrzębski J., Babis W., Płaza G. A look at biofilm on the coal waste-derived adsorbent enhanced wetland system. *Ecohydrology & Hydrobiology* 2025, <https://doi.org/10.1016/j.ecohyd.2024.12.005> (IF₂₀₂₄ = 2,7; MEiN = 100).

Artykuły uzupełniające:

1. Borgulat J., Ponikiewska K., **Jałowiecki Ł.**, Strugała-Wilczek A., Płaza G. Are wetlands as an integrated bioremediation system applicable for the treatment of wastewater from underground coal gasification processes? *Energies* 2022, 15: 4419. <https://doi.org/10.3390/en15124419> (IF₂₀₂₄ = 3.0; MEiN = 140).
2. Strugała-Wilczek A., **Jałowiecki Ł.**, Szul M., Borgulat J., Płaza G., Stańczyk K. A hybrid system based on the combination of adsorption, electrocoagulation, and wetland treatment for the effective remediation of industrial wastewater from underground coal gasification (UCG). *Journal of Environmental Management* 2024, 371: 123180. <https://doi.org/10.1016/j.jenvman.2024.123180> (IF₂₀₂₄ = 8.0; MEiN = 200).

Na rozprawę doktorską składają się:

- 4 opublikowane wiodące prace oryginalne o łącznym IF = 8,4 oraz o sumie pkt. MEiN = 400
- 2 opublikowane prace uzupełniające o łącznym IF = 13.0 oraz o sumie pkt. MEiN = 340.

Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych

CEL PRACY I KONCEPCJA BADAŃ

IV. Cel pracy i koncepcja badań

CEL BADAWCZY:

Ocena efektywności układu wetlandowego w usuwaniu zanieczyszczeń organicznych i nieorganicznych z wód poprocesowych z podziemnego zgazowania węgla (PZW).

CEL UTYLITARNY:

Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla (PZW) przy wykorzystaniu skutecznego i efektywnego systemu wetlandowego, zdolnego do usuwania szerokiego spektrum zanieczyszczeń zarówno związków organicznych i nieorganicznych, przy ograniczeniu zużycia energii i zasobów naturalnych.

CELE SZCZEGÓŁOWE:

1. Określenie gatunków roślin najlepiej przystosowanych do różnych warunków środowiskowych i rodzajów zanieczyszczeń, zdolnych do efektywnego usuwania zanieczyszczeń i tworzenia korzystnych warunków dla rozwoju mikroorganizmów.
2. Opracowanie systemu wetlandowego, dostosowanego do rodzaju zanieczyszczeń w ściekach z PZW.
3. Ocena efektywności usuwania zanieczyszczeń organicznych i nieorganicznych w opracowanym systemie wetlandowym na podstawie analiz fizykochemicznych i toksykologicznych.
4. Charakterystyka mikrobiologiczna wód poprocesowych wykorzystując metody mikrobiologii tradycyjnej i molekularnej.
5. Identyfikacja i charakterystyka bakterii występujących w zanieczyszczonych wodach poprocesowych i ocena ich aktywności w procesie bioaugmentacji ścieków koksowniczych: aspekt aplikacyjny otrzymanych wyników.
6. Porównanie efektywności układu wetlandowego w połączeniu z metodami fizykochemicznymi: elektrokoagulacja i adsorpcja w usuwaniu zanieczyszczeń.
7. Charakterystyka mikrobiologiczna biofilmu karbonizatu - odpadu z PZW wykorzystanego jako adsorbent w układzie wetlandowym.

PYTANIA BADAWCZE:

1. Jakie gatunki roślin i bakterii są najbardziej efektywne w usuwaniu określonych zanieczyszczeń ?
2. Jakie są optymalne parametry techniczne dla funkcjonowania systemu wetlandowego w skali półtechnicznej ?
3. Czy układ wetlandowy jest skuteczny w usuwaniu zarówno zanieczyszczeń organicznych, jak i nieorganicznych ?
4. Jakie są ograniczenia i możliwości zastosowania systemów wetlandowych w oczyszczaniu ścieków przemysłowych ?
5. Jakie są perspektywy rozwoju technologii wetlandowych, w różnych dziedzinach, takich jak oczyszczanie ścieków przemysłowych czy ochrona środowiska ?

HIPOTEZA BADAŃ:

Możliwe jest oczyszczenie ścieków z podziemnego zgazowania węgla (PZW) metodami biologicznymi.

OMÓWIENIE UZYSKANYCH WYNIKÓW
WPROWADZENIE

V. Wprowadzenie

Proces podziemnego zgazowania węgla (PZW) uważany jest za jedną z perspektywicznych technologii wykorzystania zasobów węgla nie nadających się do eksploatacji metodami tradycyjnymi. W procesie tym, w warunkach *in situ*, z węgla powstaje gaz syntezowy zwany syngazem. Syngaz jest mieszaniną metanu, wodoru i tlenków węgla, który jest wydobywany na powierzchnię i może mieć zastosowanie w energetyce i/lub przemyśle chemicznym, m.in. do produkcji paliw, energii elektrycznej, ciepła i/lub jako surowiec chemiczny. W trakcie badań teoretycznych i przy wdrożeniach rozwijających technologię PZW wykazano, że jest ona technicznie wykonalna i ekonomicznie, społecznie oraz środowiskowo opłacalna. Technologia podziemnego zgazowania węgla była przez wiele lat przedmiotem licznych badań pilotażowych i eksperymentów na terenach górniczych całego świata. Duże programy badawcze PZW rozpoczęły się w latach 50. XX wieku w Związku Radzieckim, a następnie, w latach 70. i 80. XX wieku w Stanach Zjednoczonych oraz w latach 80. XX wieku rozpoczęto intensywne badania w Chińskiej Republice Ludowej (Perkins, 2018). Obecnie, na całym świecie, w tym w Chinach, Indiach, Wielkiej Brytanii, Słowacji, Polsce, prowadzone są liczne badania w kierunku rozwoju technologii PZW, w celu umożliwienia jej szerokiego i komercyjnego zastosowania, a niektóre kraje, takie jak Australia, Wielka Brytania, Kanada, Nowa Zelandia i Stany Zjednoczone, wprowadzają już przepisy licencyjne dotyczące tego procesu (Couch, 2009).

W Polsce, pierwsze próby eksperymentalnych procesów PZW przeprowadzono w Głównym Instytucie Górnictwa (GIG) w latach 2007-2010. Po 2010 roku, w Centrum Czystych Technologii Węglowych GIG, zaprojektowano i zbudowano zaawansowaną eksperymentalną instalację do symulacji procesu PZW w warunkach *ex situ*. Zespół naukowców z GIG-u przeprowadził liczne procesy podziemnego zgazowania węgla wykorzystując różne rodzaje węgla, od półantracytu do ortolignitu, oraz różne czynniki gazyfikujące i różne parametry techniczne procesu (Wiatowski i wsp., 2015; Mocek i wsp., 2016; Kapusta, 2021; Wiatowski i wsp., 2023). Proces gazyfikacji węgla przeszedł długą drogę od pierwszych prób w byłym Związku Radzieckim do opracowania złożonych instalacji z zintegrowaną gazyfikacją w układzie łączonym lub wychwytywaniem i składowaniem CO₂ (Edgar i wsp., 1981; Burton i wsp., 2006; Liu i wsp., 2007; Perkins, 2018; Xu i wsp., 2021).

Główne zalety PZW to przede wszystkim, jest to proces przyjazny środowisku i daje możliwość wielokierunkowego wykorzystania gazu zarówno do produkcji energii elektrycznej, jak i dla sektora chemicznego. PZW eliminuje potrzebę wydobycia węgla oraz zagrożenia dla

górników i degradację środowiska, nie wymaga wysokonakładowej infrastruktury. Sprawia również, że głębokie lub trudno dostępne pokłady węgla stają się użytecznymi zasobami energetycznymi. Jak oszacowano tylko jedna szóstą do jednej ósmej światowych rezerw węgla nadaje się do ekonomicznego wydobycia (Wiatkowski i wsp., 2023). Natomiast, wśród wad PZW należy wymienić przede wszystkim: brak pełnej kontroli procesu eksploatacji, negatywny wpływ na środowisko naturalne obejmujące zanieczyszczenie warstw wodonośnych produktami spalania oraz osiadanie powierzchni terenu, działanie trujące i własności wybuchowe powstających substancji gazowych, ograniczone możliwości monitorowania skutków eksploatacji i reagowania na powstające dla środowiska zagrożenia.

Ryzyko środowiskowe technologii PZW związane jest głównie z potencjalnym negatywnym wpływem procesu na wody podziemne oraz wody powierzchniowe. Zagrożenie środowiska występuje zarówno w czasie prowadzenia procesu, jak i po jego zakończeniu, gdzie powstają liczne zanieczyszczenia organiczne i nieorganiczne. W procesie podziemnego zgazowania węgla powstają i przechodzą do środowiska wodnego, w tym do wód poprocesowych, liczne zanieczyszczenia organiczne oraz nieorganiczne. Wiele reakcji chemicznych, heterogenicznych i homogenicznych, zachodzących w strefach utleniania, redukcji i pirolizy wpływają na uwalnianie zanieczyszczeń z węglowych smół i popiołów wytwarzanych w procesie uwęglania. Ścieki przemysłowe i szlam z PZW zawierają znaczną ilość węglowodorów alifatycznych i aromatycznych, w tym benzen, węglowodory poliaromatyczne i fenole, ale także amoniak, cyjanki i metale (Fergusson, 2015; Grabowski i wsp., 2021).

Ścieki z PZW, wytwarzane głównie podczas parowania i pirolizy węgla, chłodzenia gazu i procesów oczyszczania gazu, mają podobny skład do ścieków koksowniczych i wymagają złożonego oczyszczania mechanicznego, chemicznego i biologicznego ze względu na wysoką zawartość licznych zanieczyszczeń organicznych i nieorganicznych (Osmólski, 2013; Sobolewski i Ściążko, 2016). Należy podkreślić, że jakość i ilość otrzymanych ścieków PZW różni się w zależności od wielu czynników, takich jak: typ instalacji PZW, parametry procesu (temperatura i ciśnienie), rodzaj gazyfikowanego węgla i jego właściwości, rodzaj czynnika gazyfikującego i zastosowanej technologii gazyfikacji. Jak podano w literaturze, średnia ilość „wyprodukowanych” ścieków wynosi około 0,30 kg na 1 kg węgla (Wiatkowski i wsp., 2023). W czasie prowadzenia procesu powstają liczne zanieczyszczenia w postaci organicznych związków aromatycznych m.in.: benzen, toluen, etylobenzen, ksyleny, fenole oraz wielopierścieniowe węglowodory aromatyczne (WWA). Dodatkowo uwalnianie są

znaczne ilości metali ciężkich z węgla kamiennego oraz z popiołów powstających podczas zgazowania (Grabowski i wsp., 2021; Pankiewicz-Sperka i wsp., 2021). Do niebezpiecznych zanieczyszczeń zaliczyć należy również amoniak oraz cyjanki. Stężenia poszczególnych zanieczyszczeń w ściekach PZW zmieniają się znacznie wraz z postępującymi fazami procesu. Niektóre parametry fizykochemiczne wahają się w znacznych zakresach, np. pH waha się od 4,9 do 7,8, ChZT od 48 do 5060 mg O₂/L i BZT5 od 300 do 4373 mg O₂/L, stężenie azotu amoniakowego waha się od 11 do 7800 mg N/L, a poziom siarczanów wynosi od 33 do 3220 mg/L. Stwierdzono również wysokie stężenia fenolu od 2 do 3090 mg/L oraz WWA i BTEX odpowiednio, w zakresie 360 - 1912 µg/L i 56 - 5483 µg/L. We wszystkich ściekach zaobserwowano stężenia cyjanków na poziomie od 0,5 do 5,7 mg/L (Kapusta i wsp., 2019; Pankiewicz-Sperka i wsp., 2021; Borgulat i wsp., 2022).

Ścieki w PZW stanowią swoistą mieszaninę różnych zanieczyszczeń i w związku z tym opracowanie opłacalnych i przyjaznych dla środowiska procesów usuwania niebezpiecznych i ogniotrwałych związków organicznych ze ścieków jest uważane za priorytetowe wyzwanie dla zrównoważonego rozwoju przemysłu węglowego i pomyślnego zastosowania technologii PZW w transformacji energetycznej. W literaturze można znaleźć przykłady propozycji zintegrowanych systemów inżynierskich, obejmujących zaawansowane metody oczyszczania ścieków z PZW (Burton i wsp., 2006; Yadav i wsp., 2021; Grabowski i wsp., 2021; Meena i wsp., 2021).

Spośród różnych procesów fizycznych, fizykochemicznych i biologicznych, metody biologiczne, w głównej mierze oparte na naturalnych procesach biochemicznych okazały się skuteczne w usuwaniu wielu zanieczyszczeń zawartych w różnych ściekach i odpadach przemysłowych (Maiti i wsp., 2019; Singh i Singh, 2019; Shoukat i wsp., 2019; Gupta i wsp., 2019; Meena i wsp., 2021a; Meena i wsp., 2021b; Jayapal i wsp., 2023). W wyniku licznych badań opracowano holistyczne podejście wykorzystujące aktywność mikroorganizmów oraz różnych gatunków roślin lądowych i wodnych w biodegradacji zanieczyszczeń, połączenie fitoremediacji i bioremediacji. Takimi przykładami są obiekty techniczne z roślinnością hydrofitową wykorzystywane do oczyszczania i zagospodarowania ścieków zwane wetlandami. Są one niskokosztowymi, zrównoważonymi, łatwymi w obsłudze i przyjaznymi dla środowiska technologiami skutecznego usuwania różnych związków organicznych i nieorganicznych bazujących na procesach zachodzących w naturze określane jako NBS (ang. nature-based solutions) (Metcalf i wsp., 2018; Masi i wsp., 2018; Parde i wsp., 2021; Vymazal i wsp., 2021; Vymazal, 2022; Waly i wsp., 2022). Funkcjonują one jako biofiltry wykorzystując

naturalne procesy oparte na interakcjach między mediami, takimi jak żwir, piasek, gleba oraz roślinami i mikroorganizmami. W szczególności, mikroorganizmy uważane są za kluczowy czynnik w usuwaniu zanieczyszczeń ze ścieków (Nagda i wsp., 2021).

Wiedza o zbiorowiskach mikroorganizmów funkcjonujących w ekosystemach wetlandowych ma istotne znaczenie w monitorowaniu procesów oczyszczania. Ich rola jest również kluczowa w funkcjonowaniu i wzroście roślinności oraz w utrzymaniu zrównoważonego rozwoju ekosystemów wetlandowych, m.in. w obiegu pierwiastków biogennych. Systemy wetlandowe są przykładem „ekoprzemysłu”, który koncentruje się na opracowywaniu przyjaznych dla środowiska technologii oczyszczania z wykorzystaniem naturalnych procesów zachodzących przy udziale mikroorganizmów i roślin (fitotechnologia). Wielofunkcyjność CWs wynika przede wszystkim z ich "naturalnego charakteru".

Jak opisano w literaturze, wiele ścieków przemysłowych oczyszczano za pomocą procesów biologicznych, wśród których wetlandy, jako technologia oczyszczania oparta na naturze, okazała się dobrym rozwiązaniem (Benny, 2020; Hassan i wsp., 2021; Kiflay i wsp., 2021; Aregu, 2022; Vymazal, 2022). Zalety oczyszczalni wetlandowych, jako zielonej i zrównoważonej technologii, zostały omówione przez wielu badaczy i zaowocowały licznymi zastosowaniami opisanymi w literaturze (Metcalf i wsp., 2018; Nguyen i wsp., 2019; Sandoval i wsp., 2019; Saeed i wsp., 2019; Benny i Chakraborty, 2020; Rana i wsp., 2020; Singh i Chakraborty, 2020; Hassan i wsp., 2021; Kiflay i wsp., 2021; Aregu, 2022; Berego i wsp., 2022; Younas i wsp., 2022). Przeprowadzona analiza literatury wykazała, że oczyszczalnie wetlandowe były wykorzystywane do oczyszczania ścieków i odpadów z przemysłu węglowego, ale tylko w nielicznych przypadkach (Nguyen i wsp., 2019; Shi i wsp., 2022).

Waloryzacja zanieczyszczonych ścieków przemysłowych, również w kontekście czystych technologii węglowych, do których należy proces PZW, wydaje się być jednym z filarów nowoczesnego i odpowiedzialnego przemysłu. Wetlandy wzbudzają ostatnio duże zainteresowanie badaczy w zakresie oczyszczania i usuwania antropogenicznych zanieczyszczeń ze ścieków. Pomimo szerokiego zainteresowania, to jednakże dokładniejsze informacje o funkcjonowaniu i procesach zachodzących w wetlandach są nadal nieznane. Nadal potrzebna jest wiedza na temat projektowania i funkcjonowania wydajnych i skutecznych systemów wetlandowych w oczyszczaniu ścieków przemysłowych.

W niniejszej pracy doktorskiej podjęto badania dotyczące oczyszczania ścieków przemysłowych w ekosystemach wetlandowych, zwłaszcza w zakresie usuwania zarówno zanieczyszczeń organicznych, jak i nieorganicznych w trudnych matrycach, jakimi są ścieki

z procesów PZW. Systemy wetlandowe stanowią wielofunkcyjne podejście do opracowania skutecznej remediacji w oczyszczaniu ścieków przemysłowych, w tym ścieków z procesów gazyfikacji węgla. Można spodziewać się, że odpowiednio zaprojektowane układy wetlandowe mogą oczyszczać ścieki pochodzące z podziemnego zgazowania węgla, jednak należy zauważyć, że wysokie stężenia niektórych zanieczyszczeń, które często towarzyszą procesowi PZW, np. WWA, mogą wymagać opracowania nowych parametrów technologicznych takich jak: dodatkowe napowietrzenie systemu czy też wydłużenie czasu retencji hydraulicznej.

Poznanie mechanizmów oczyszczania i na tej podstawie opracowanie metod oczyszczania ścieków z PZW stanowi wyzwanie dla biotechnologii i inżynierii środowiska, a tylko nieliczne doniesienia naukowe opisują metody oczyszczania ścieków z PZW (Nguyen i wsp., 2019; Shi i wsp., 2022). Opracowanie metody oczyszczania ścieków z PZW zmniejsza ryzyko tegoż procesu, a jednocześnie jest zgodne z zasadami zrównoważonego rozwoju i zielonej gospodarki. Obiekty hydrofitowe (wetlandy) ze względu na swoje specyficzne właściwości, m.in.: szerokie spektrum usuwanych zanieczyszczeń, odporność na wahania składu i ilości ścieków, wydają się być ogromną szansą również dla rozwiązania problemów oczyszczania ścieków poprzemysłowych, w tym z PZW. Dlatego zasadnym było podjęcie badań dotyczących oczyszczania wód poprocesowych z PZW przy użyciu systemu wetlandowego.

METODOLOGIA I SCHEMAT BADAŃ

VI. Metodologia i schemat badań

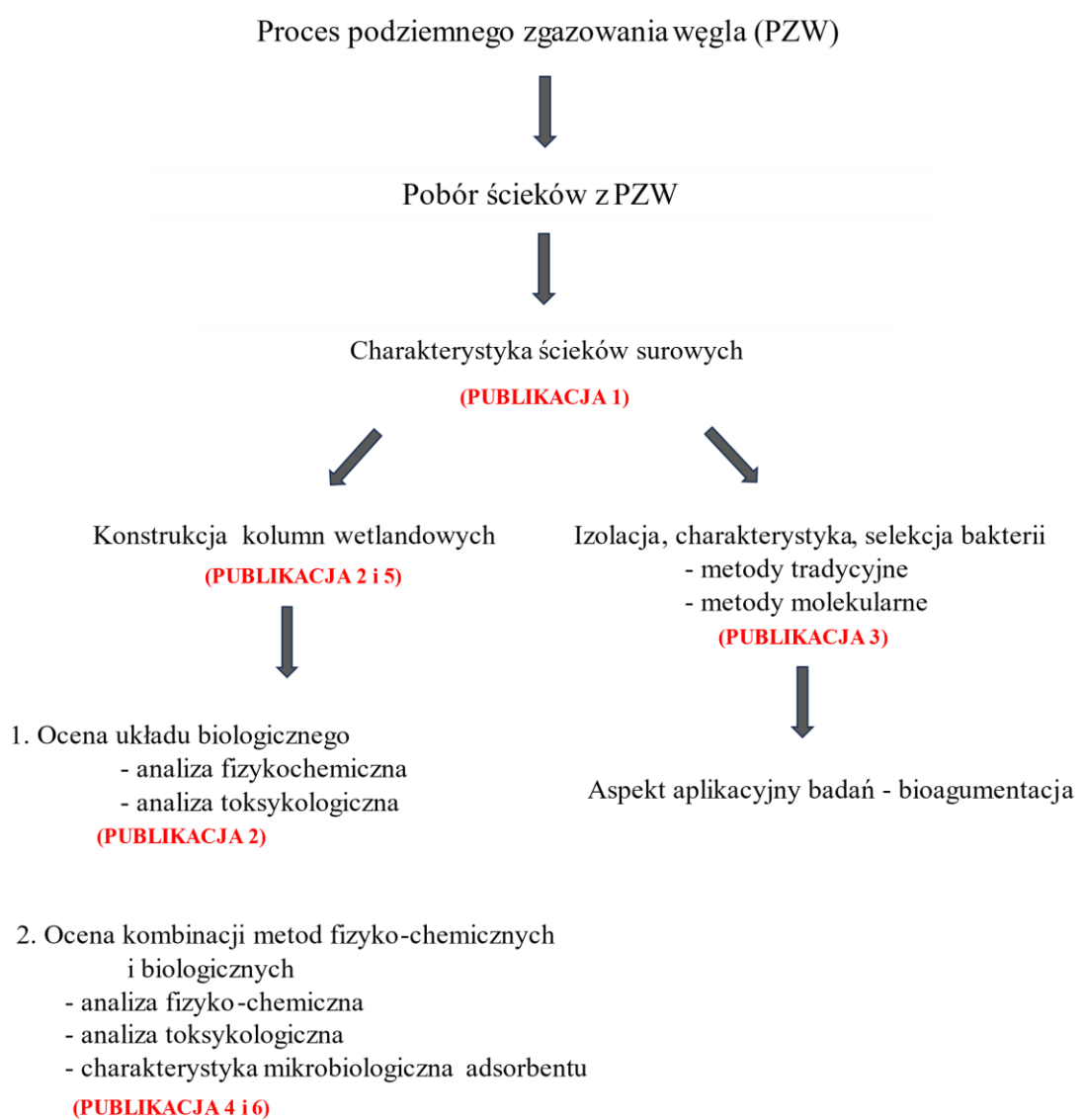
W tabeli 1 zaprezentowano opis technik badawczych wykorzystanych podczas realizacji pracy doktorskiej w powiązaniu z zakresem wykonanych badań.

Tabela 1. Wykaz technik badawczych wykorzystanych podczas realizacji pracy doktorskiej

Technika	Zakres badań
Spektrometria mas sprzężona z plazmą wzbudzaną indukcyjnie (ICP-MS, ang. <i>inductively coupled plasma – mass spectrometry</i>)	Analiza metali i metaloidów zgodnie z normą PN-EN ISO 11885:2009
Spektroskopia absorpcji atomowej zimnej pary (CV-AAS ang. <i>cold-vapour atomic absorption spectrometry</i>)	Oznaczanie śladowych ilości rtęci zgodnie z normą PN-EN ISO 12846:2012+Ap1:2016-07; US EPA 7473
Wysokosprawna chromatografia cieczowa z detektorem fluorescencyjnym (HPLC-FLD ang. <i>High-performance liquid chromatography</i>)	Analiza WWA zgodnie z normą PN-EN ISO 179993:2005
Chromatografia gazowa sprzężona ze spektrometrią mas (HS-GC-MS ang. <i>Headspace analysis with gas chromatography and mass detection</i>)	Analiza BTEX zgodnie z normą PN-ISO 11423-1:2002
Spektrofotometria/konduktometria/chromatografia jonowymienna (IC, ang. <i>Ion chromatography</i>)	Analiza ilościowa podstawowych parametrów fizykochemicznych, nieorganicznych i organicznych zgodnie z normami: PN-ISO 9280:2002, PN-EN ISO 10304-1:2009, PN-EN ISO 111732:2007, PN-EN 12260:2004, PN-EN 1899-2:2002, PN-EN ISO 5815-1:2019-12
Tensiometria	Pomiar napięcia powierzchniowego przy użyciu tensiometru SIGMA T702 (Attension, Finland) metodą pierścieniową Du Nöuy zgodnie z procedurą producenta
Aerobowa biodegradacja w bioreaktorze	19L bioreaktor New Brunswick BIOFLO 415. Bioreaktor wyposażony jest w niezbędną aparaturę kontrolno-pomiarową (czujniki pH, tlenu rozpuszczonego, temperatury, ciśnienia i poziomu piany)

Technika	Zakres badań
Fenotypowanie szczepów bakterii	Wykorzystanie metody BIOLOG™ (Biolog Inc., Hayward, USA) wykorzystując płytki PMs i Eco
Test toksyczności Microtox®	Ocena toksyczności ścieków zgodnie z normą PN-EN ISO 11348-3
Izolacja DNA genomowego z bakterii	Analiza przeprowadzona za pomocą zestawu komercyjnego (Roche) zgodnie z procedurą producenta
Izolacja DNA metagenomowego	Analiza przeprowadzona za pomocą zestawu komercyjnego PowerWater® DNA Isolation Kit (MoBio Laboratories Inc., CA, United States) zgodnie z procedurą producenta
Identyfikacja gatunkowa szczepów bakterii	Sekwencjonowanie w technologii Sanger i analiza genomu bakterii na podstawie sekwencji 16S rDNA. Analiza obejmuje wykonanie reakcji PCR matrycy do sekwencjonowania, oczyszczenie matrycy, sekwencjonowanie obu nici DNA genu 16S rDNA, analiza sekwencji (złożenie w consensus i porównanie z bazą danych GeneBanku)
Meta-barkodowanie (ang. <i>meta-barcoding</i>)	Przygotowanie bibliotek metodą dwustopniowego PCR, sekwencjonowanie z wykorzystaniem urządzenia MiSeq firmy Illumina w trybie sparowanych końców w dwóch odczytach, podstawowa analiza bioinformatyczna obejmowała filtrowanie odczytów, klastrowanie ASV. oraz klasyfikację odczytów do poziomu gatunku przeprowadzonej pakietem oprogramowania QIIME 2 z oparciem na bazie sekwencji referencyjnych Silva 138
Analiza statystyczna	Analiza statystyczna otrzymanych wyników z wykorzystaniem oprogramowania Statistica (Soft, Polska)

Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych



Rysunek 1. Schemat badań realizowanych w ramach pracy doktorskiej

**SYNTETYCZNE OMÓWIENIE WYNIKÓW BADAŃ
PRZEDSTAWIONYCH W CYKLU PUBLIKACJI WCHODZĄCYCH
W SKŁAD ROZPRAWY DOKTORSKIEJ**

VII. Syntetyczne omówienie wyników badań przedstawionych w cyklu publikacji wchodzących w skład rozprawy doktorskiej

Cykl tematycznie spójnych publikacji otwiera praca pt.: “Are wetlands as an integrated bioremediation system applicable for the treatment of wastewater from underground coal gasification processes?” (Borgulat J., Ponikiewska K., **Jałowiecki Ł.**, , Strugała-Wilczek A., Płaza G. *Energies* 2022,15, 4419. IF2024= 3; MEiN =140). Artykuł jest przeglądem publikacji naukowych opisujących eksperymenty procesu podziemnego zgazowania węgla (PZW) przeprowadzone w Polsce, przez zespół naukowców z Głównego Instytutu Górniczego, źródła powstawania ścieków z PZW i ich charakterystykę. Ścieki te są przykładem ścieków przemysłowych o niezwykle złożonej matrycy, zawierających duże ilości zanieczyszczeń organicznych i nieorganicznych. W publikacji, szczególną uwagę poświęcono biologicznym metodą oczyszczania jako przykład skutecznych i efektywnych metod w usuwaniu antropogenicznych związków z zanieczyszczonych środowisk wodnych. Większość naukowców i badaczy uważa, że żadna pojedyncza metoda biologiczna nie może w dostatecznym stopniu oczyścić zanieczyszczonego środowiska, ponieważ większość zanieczyszczeń występuje w skomplikowanych mieszaninach różnych związków chemicznych i o różnym stężeniu. W takich przypadkach skuteczna strategia oczyszczania powinna obejmować wdrożenie dwóch lub więcej metod biologicznych, np. bioremediacji i fitoremediacji.

Obecnie integracja biologicznych metod oczyszczania jest bardzo popularna i procesy hybrydowe (synergiczne), takie jak układy wetlandowe są uważane za holistyczne i zintegrowane podejście do oczyszczania środowisk wodnych. W tych układach wykorzystywane są procesy fizyczne, chemiczne i biologiczne występujące w naturze, takie jak sorpcja, strącanie, transpiracja, sedimentacja, aktywności roślin: fitostabilizacja i fitoekstrakcja, parowanie oraz aktywność biodegradacyjna związana z licznymi zespołami mikroorganizmów. W literaturze opisano wiele przykładów wykorzystania układów wetlandowych, jako naturalnych i inżynierskich rozwiązań (ekoinżynierskich rozwiązań), do oczyszczania ścieków komunalnych. Szczegółowa analiza dostępnych danych literaturowych wykazała, że rozwiązania te są również wykorzystywane do oczyszczania niektórych ścieków przemysłowych, odcieków i wód opadowych. Natomiast, analiza dostępnej literatury naukowej wykazała, że układy wetlandowe rzadko były stosowane do oczyszczania wód poprocesowych z PZW. W związku z powyższym, układ kolumn wetlandowych wykorzystano do badań

eksperymentalnych dotyczących biologicznego oczyszczania ścieków poprocesowych z PZW i opisano w niniejszej rozprawie doktorskiej.

Pierwszym etapem badań prezentowanej pracy doktorskiej była analiza fizykochemiczna, mikrobiologiczna i toksykologiczna 3 wód poprocesowych, oznaczonych jako WP1, WP2 i WP3 pochodzących z 3 procesów podziemnego zgazowania węgla, oznaczonych jako PZW1, PZW2 i PZW3. Procesy podziemnego zgazowania węgla przeprowadzono w instalacji eksperymentalnej w kopalni doświadczalnej „Barbara” należącej do Głównego Instytutu Górnictwa, w warunkach *ex situ*. Procesy te różniły się rodzajami zastosowanego węgla, jeden pochodził z kopalni „Piast-Ziemowit”, a drugi z kopalni „Wesoła”, oraz czynnikami zgazowania, t.j. powietrze nasycone tlenem i czysty tlen. W przypadku procesu zgazowania węgla z kopalni „Wesoła” zastosowano powietrze (PZW 3). Podczas gdy, węgiel z kopalni „Piast-Ziemowit” gazyfikowano zarówno tlenem (PZW2), jak i powietrzem (PZW1).

Analizowane ścieki charakteryzowały się wysokimi stężeniami związków organicznych typu BTEX, WWA i fenole oraz wysoką toksycznością. Ścieki należały do V klasy toksyczności określonej na podstawie wskaźnika TU (ang. Toxicity Unit). Ścieki z różnych procesów posiadały podobny skład chemiczny, natomiast stężenia poszczególnych związków różniły się znacznie. Analiza mikrobiologiczna wykazała również znaczne zróżnicowanie w ściekach. Charakterystyka metagenomu ścieków surowych wykazała, że w ściekach WP1 i WP3 dominowały *Proteobacteria*, odpowiednio, 84.64% and 77.92%. Podczas gdy, w ściekach W2 dominowały *Firmicutes* o udziale 66.85%. Wśród *Proteobacteria* dominowały *Gammaproteobacteria* i *Alphaproteobacteria*. Natomiast *Bacilli* i *Actinobacteria* były dominujące wśród *Firmicutes*. Począwszy od poziomu rzędu zaobserwowano znaczące różnice w liczebności taksonomicznej między ściekami WP1 i WP3. Jak wykazały analizy, procent niezidentyfikowanych gatunków zarówno w ściekach WP1 i WP3, jaki i w ścieku WP2 był wysoki i wahał się pomiędzy 86,3% w WP2 a 96% w WP3. W ścieku WP1 stwierdzono 93,35% niezidentyfikowanych bakterii. Ilość zidentyfikowanych bakterii była znacznie mniejsza (od 4,11 % do 13.73%) i większość z nich należała do grupy niehodowlanej, np. bakterie z rodzaju *Nevskia* stanowiły 15,82% zidentyfikowanych bakterii w ściekach WP1. Inne niehodowlane gatunki należały *Burkholderiales* (14.26%) i *Sphingobium* (13.2%). Natomiast wśród hodowlanych dominowały bakterie z rodzaju *Penibacillus* (26.51%), *Bacillus* (25.34%) i *Actinobacteria* (15.12%). Wśród nich kluczowymi gatunkami były: *Paenibacillus glucanolyticus*, *Paenibacillus favisporus*, *Bacillus horneckiae* i *Kocuria* spp. Wymienione powyżej bakterie posiadają specyficzne właściwości metaboliczne, które ułatwiają im

Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych

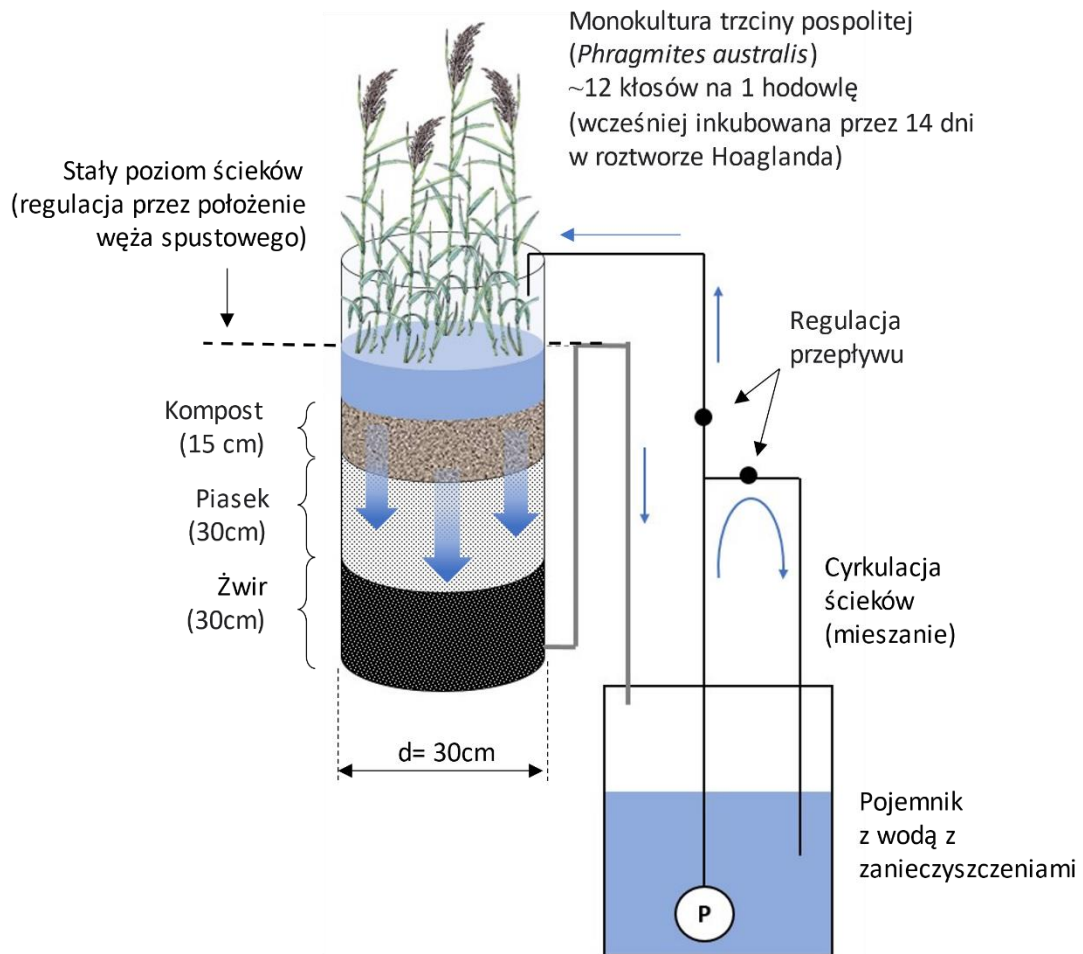
przetrawianie w ekstremalnych środowiskach oraz mogą być wykorzystywane w różnych procesach biotechnologicznych.

Zróżnicowanie w stężeniach zanieczyszczeń takich jak fenol, BTEX i WWA istotnie wpłynęło na różnice w składzie mikrobiologicznym ścieków surowych z PZW. Dodatkowo, jak wykazały analizy statystyczne, istotnym parametrem wpływającym na zróżnicowanie mikrobiologiczne miały parametry techniczne procesu podziemnego zgazowania węgla, takie jak czynniki zgazowania. W procesie PZW2 czynnikiem tym był czysty tlen, natomiast w procesach PZW1 i PZW3 było powietrze nasyczone tlenem.

Po raz pierwszy tak kompleksowo scharakteryzowano wody poprocesowe z PZW uwzględniając parametry fizyko-chemiczne, toksykologiczne oraz mikrobiologiczne, i przedstawiono w następnej publikacji wchodzącej w skład cyklu publikacji składającej się na rozprawę doktorską (**Jałowiecki Ł.**, Borgulat J., Strugała-Wilczek A., Jastrzębski J., Matejczyk M., Płaza G. Insights into bacterial diversity in industrial post-processing water from underground coal gasification (UCG) process. Archives of Environmental Protection, 2024, 50; IF = 1,5; MNiE = 100).

Na podstawie otrzymanych wyników oraz wcześniej przeprowadzonej analizy literatury podjęto dalsze badania zmierzające do opracowania i wybudowania układu kolumn wetlandowych z pionowym przepływem, który następnie wykorzystano do biologicznego oczyszczania 3 rodzajów wód poprocesowych, co było następnym, istotnym celem pracy doświadczalnej składającej się na niniejszą rozprawę doktorską (**Jałowiecki Ł.**, Strugała-Wilczek A., Ponikiewska K., Borgulat J., Płaza G., Stańczyk K. 2024. Constructed wetland as a green remediation technology for the treatment of wastewater from underground coal gasification proces. PLoS ONE 19(3): e0300485. IF=2,9; MEiN =100).

Na Rysunku 2 przedstawiono opracowany schemat kolumny wetlandowej wykorzystanej do oczyszczania ścieków poprocesowych.



Rysunek 2. Schemat systemu hydrofitowego do oczyszczania ścieków z zastosowaniem monokultury trzciny pospolitej (*Phragmites australis*)

Podczas prowadzenia procesu oczyszczania w kolumnach wetlandowych z pionowym przepływem zaobserwowano znaczną redukcję stężenia związków organicznych typu BTEX, WWA, fenole. Dla tych związków, już po 14 dniach prowadzenia procesu, otrzymano procent redukcji ponad 95%. Zaobserwowano również redukcję stężenia dla cyjanków i niektórych metali (Zn, Cr, Cd, Cu, Ni, Pb i Ti). Przy czym dla Cd, Cu i Ni odnotowano wysoką skuteczność redukcji sięgającą odpowiednio, 99,3%, 99,5% i 86,3%.

Równocześnie z analizami fizyko-chemicznymi prowadzono badania zmian toksyczności ścieków oczyszczanych. Po 14 dniach prowadzenia eksperymentu, toksyczność ścieków oczyszczanych znacznie spadła. Redukcja toksyczności wynosiła 74% i 77% odpowiednio, dla ścieków WP1 i WP2. Podczas gdy, dla ścieków oczyszczanych WP3 redukcja toksyczności wynosiła 99%. W czasie prowadzenia eksperymentu toksyczność oczyszczanych ścieków sukcesywnie malała, i po zakończeniu eksperymentu, oczyszczone ścieki WP1 i WP3 należały do I klasy toksyczności (nie toksyczne) o wartości TU równej odpowiednio, 0,9 i 0,7.

Natomiast, ściek WP2 należał do II klasy toksyczności (niska toksyczność) o wartości TU równiej 1,1. Podsumowując otrzymane wyniki, należy wskazać, że podczas oczyszczania ścieków w kolumnach wetlandowych toksyczność ich spadła z klasy V do klasy I i II. Spadek toksyczności związany był z biodegradacją toksycznych zanieczyszczeń zawartych w ściekach surowych do mniej toksycznych produktów końcowych lub produktów pośrednich procesu biodegradacji. Otrzymane wyniki toksyczności korelowały z niektórymi związkami organicznymi, np. BTEX, WWA i nieorganicznymi, np. cyjankami i związkami azotowymi.

Biorąc pod uwagę złożony skład zanieczyszczeń i obecność skomplikowanych strukturalnie związków chemicznych w wodach poprocesowych, opracowany schemat kolumn wetlandowych w połączeniu z wysoką aktywnością mikroorganizmów i licznymi procesami fizykochemicznymi okazał się bardzo dobrym rozwiązaniem do oczyszczania. Ponadto, odpowiednio opracowane i dobrane parametry techniczne pracy systemu wetlandowego były również niezbędne do osiągnięcia zadowalających wskaźników usuwania zanieczyszczeń organicznych i nieorganicznych. Uzyskane w badaniu wyniki wskazują na potencjał kolumn wetlandowych do oczyszczania zanieczyszczonych wód poprocesowych, zarówno związkami organicznymi jak i nieorganicznymi, jak również innych ścieków przemysłowych o podobnym charakterze, np. ścieków koksowniczych lub odcieków, wód opadowych.

Przeprowadzone badania pozwalają przypuszczać, że pomimo znacznej zawartości zanieczyszczeń, ścieki z PZW nadają się do biologicznego oczyszczania. Uzyskane wyniki pokazują, że technologia oczyszczania z zastosowaniem wetlandów może być stosowana również do oczyszczania innych, silnie zanieczyszczonych ścieków przemysłowych.

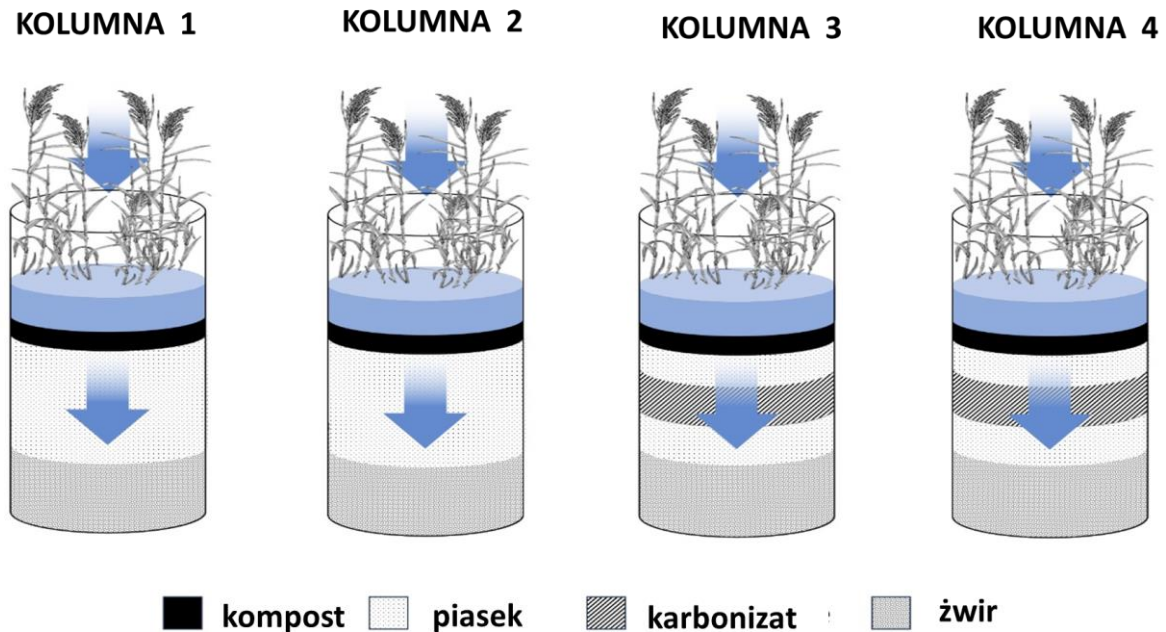
W celu skutecznej oceny technologii remediacji, istotne jest połączenia tradycyjnych parametrów fizykochemicznych z testami ekotoksyczności, co zapewni bezpieczeństwa środowiska i zminimalizowanie zagrożeń. Zastosowany system wetlandowy ma potencjał oczyszczenia wód poprocesowych do takiego stopnia, że mogą być one ponownie wykorzystane w postaci użytecznej wody z recyklingu.

Kolejnym etapem realizacji pracy doktorskiej był skrining bakterii wyizolowanych z zanieczyszczonych wód poprocesowych, w kierunku oceny ich zdolności do degradacji fenolu i potencjalnego zastosowania w bioagumentacji. W tym celu, z wód poprocesowych wyizolowano 100 szczepów bakteryjnych metodami hodowlanymi, z których zidentyfikowano 50%. Wśród zidentyfikowanych szczepów, najwięcej było tych należących do rodzaju *Bacillus*, *Paenibacillus* i *Oceanobacillus*. W wyniku przeprowadzonych badań wybrano trzy szczepy bakterii, które charakteryzowały się największym potencjałem degradacyjnym i posiadały

specyficzne właściwości biochemiczne, tj.: *Paenibacillus pasadensis* SAFN-007, *Paenibacillus humicus* Au34, and *Staphylococcus warneri* DK131. Otrzymane wyniki przedstawiono w następnej publikacji wchodzącej w skład rozprawy doktorskiej (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. J. Ecol. Eng. 25(2), 62-71. IF =1,3; MEiN=100). Wyżej wymienione szczepy charakteryzowały się wysoką zdolnością do rozkładu fenolu, ponad 90% przy stężeniu fenolu 565 mg/dm³. Do charakterystyki biochemicznej i metabolicznej szczepów wykorzystano płytki Eko oraz mikromacierze fenotypowe PM (ang. phenotypic microarrays) systemu Biolog, które są szeroko stosowane w pracach nad pozyskiwaniem szczepów o pożądanych cechach fenotypowych wykorzystywanych, np. w procesach biotechnologicznych. Spośród metabolitów węgla (PM1 i PM2): 74%, 67,4% oraz 94,2% zostało wykorzystanych odpowiednio przez, *Paenibacillus pasadenensis* SAFN-007, *Paenibacillus humicus* Au34 i *Staphylococcus warneri* DK131. Wśród źródeł węgla zidentyfikowano substraty przemian fenolowych, takie jak: N-acetylo-D-glukozamina, kwas bursztynowy, kwas octowy, gamma-lakton kwasu D-galaktonowego, kwas bromobursztynowy, bursztynian monometylu, pirogronian metylu.

Następnie, wybrane szczepy bakteryjne wykorzystano w procesie bioaugmentacji do oczyszczania ścieków koksowniczych w skali półtechnicznej, tj. w 19L bioreaktorze BIOFLO 415. W trakcie trwania badań, stwierdzono redukcję fenolu na poziomie 47%. Natomiast, toksyczność ścieków spadła z klasy V do IV. Powyższe badania miały charakter aplikacyjny i potwierdziły, że wyizolowane szczepy bakteryjne mogą być wykorzystane w procesie bioaugmentacji do oczyszczania środowisk zanieczyszczonych fenolem (Jałowiecki Ł., Borgulat J., Glaser M., Marchlewicz A., Płaza G. Zastosowanie bioaugmentacji w oczyszczaniu ścieków koksowniczych: badania bioreaktorowe. VIII Ogólnopolskie Sympozjum Mikrobiologiczne, Metagenomy różnych środowisk, 17-18.06.2024. Lublin. <https://doi.org/10.5281/zenodo.13959170>).

Ostatnim etapem pracy doktorskiej było zbudowanie i przetestowanie układów hybrydowych stanowiących kombinacje metod fizyko-chemicznych i biologicznych, tj.: adsorpcji, elektrokoagulacji i kolumny wetlandowej do oczyszczania ścieków przemysłowych z PZW. Opracowane układy hybrydowe przedstawiono na Rysunku 3. Celem tych badań było porównanie skuteczności oczyszczania ścieków z PZW przy użyciu metod fizykochemicznych i biologicznych.



Rysunek 3. Schemat hybrydowych kolumn filtracyjnych (Kolumna 1–4)

wykorzystywanych w badaniach nad oczyszczaniem ścieków z zastosowaniem monokultury trzciny pospolitej (*Phragmites australis*)

Proces elektrokoagulacji przeprowadzono w Instytucie Technologii Energetycznych i Przetwórstwa Paliw w Zabrze. W badaniach, jako adsorbent wykorzystano karbonizat - rodzaj odpadu pochodzącego z procesu PZW. Wyniki uzyskane na tym etapie badań przedstawiono w kolejnej publikacji wchodzącej w skład rozprawy doktorskiej (Strugała-Wilczek A., **Jałowiecki Ł.**, Szul M., Borgulat J., Płaza G., Stańczyk K. 2024. A hybrid system based on the combination of adsorption, electrocoagulation, and wetland treatment for the effective remediation of industrial wastewater from underground coal gasification (UCG). J. Environ. Managem. 371, 123180. IF2024= 8; MEiN =200).

W badaniach zaproponowano i przetestowano cztery różne konfiguracje systemów hybrydowych, tj.: (1) kolumna wetlandowa, (2) elektrokoagulacja/kolumna wetlandowa, (3) adsorbent/kolumna wetlandowa oraz (4) elektrokoagulacja/adsorbent/kolumna wetlandowa. Kluczowym elementem tego badania była kompleksowa analiza ścieków uwzględniająca parametry fizykochemiczne, w tym metale i pierwiastki śladowe, indeks fenolowy, siarczki, cyjanki, całkowity węgiel organiczny, chemiczne zapotrzebowanie tlenu (ChZT), biologiczne zapotrzebowania na tlen (BZT), BTEX, WWA oraz badania toksyczności. Zarówno elektrokoagulacja, jak i kolumna wetlandowa okazały się skuteczne w usuwaniu związków organicznych typu WWA, chociaż kolumna wetlandowa okazał się bardziej skuteczna

w przypadku BTEX. Jak wskazują wyniki, oczyszczanie wstępne za pośrednictwem elektrokoagulacji nie jest konieczne do późniejszego stosowania kolumn wetlandowych. Prowadzenie oczyszczania przez 45 dni okazało się wystarczającym czasem do właściwego przebiegu procesu. Dłuższy czas oczyszczania wód poprocesowych nie miał pozytywnego wpływu na niektóre parametry fizykochemiczne. Co więcej, stwierdzono niekorzystny wpływ w przypadku, np: ChZT, OWO i niektórych metali: Fe, Mn, As, Cr, Zn, Al, Co i Ti. Dlatego, nie tylko wybór odpowiedniej metody, ale także optymalizacja czasu oczyszczania wydaje się być kluczowa dla skutecznego usuwania zanieczyszczeń ze ścieków. Porównując skuteczność usuwania zanieczyszczeń w samej kolumnie wetlandowej i kolumnie wetlandowej z adsorbentem nie stwierdzono istotnych różnic pomiędzy tymi układami. Dodanie adsorbentu do systemu biologicznego zwiększyło tylko nieznacznie wydajność procesu oczyszczania.

Podsumowując wyniki tego etapu badań, stwierdzono, że metoda biologicznego oczyszczania wód poprocesowych z wykorzystaniem kolumn wetlandowych okazała się skuteczną i efektywną metodą, tak jak w przypadku poprzednich badań. Do tego sukcesu przyczyniły się odpowiednio zaprojektowana budowa kolumny wetlandowej i odpowiednio dobrane parametry techniczne jej funkcjonowania.

Wyniki badań w skali laboratoryjnej mogą być podstawą do zaprojektowania systemu oczyszczania na dużą skalę, który będzie skuteczny, opłacalny i przyjazny dla środowiska.

Jednym z celów tych badań było również scharakteryzowanie tworzącego się na powierzchni adsorbentu biofilmu, co przedstawiono w następnej publikacji wchodzącej w skład rozprawy doktorskiej (Jałowiecki Ł., Borgulat J., Strugała-Wilczek A., Stańczyk K., Jastrzębski J., Babis W., Płaza G. A look at biofilm on the coal waste-derived adsorbent enhanced wetland system (IF2025= 2,7; MEiN =100). Poznanie struktury biofilmu miało istotne znaczenie w charakterystyce zespołów bakterii biorących udział w procesie usuwania zanieczyszczeń z wód poprocesowych w systemie hybrydowym. Wykorzystanie karbonizatu jako adsorbenta stanowi wartość dodaną prowadzonych badań o podwójnych korzyściach, tj. wykorzystanie odpadu oraz oczyszczenie zanieczyszczonych wód poprocesowych z podziemnego zgazowania węgla. Takie podejście do badań jest zgodne z zasadą 4R (Reduce, Reuse, Recycle i Recovery) oraz celami gospodarki obiegu zamkniętego.

Analiza metagenomiczna biofilmu wykazała niewielką bioróżnorodność. Stwierdzono większy udział taksonów unikalnych lub o niskiej liczebności. Dwie rodziny, *Pseudomonadaceae* i *Bacillaceae* dominowały w strukturze biofilmu adsorbenta, odpowiednio o ilości, 92% i 7%. W rodzinie *Pseudomonadaceae* dominującym gatunkiem był *Pseudomonas*

putida. Natomiast, rodzina *Bacillaceae* wykazywała znaczne zróżnicowanie. Dominującymi gatunkami były: *Bacillus circulans*, *Bacillus australimaris*, *Paenibacillus ihbetae*, *Bacillus simplex*, *Bacillus butanolivorans* *Paenibacillus favisporus*.

Karbonizat jako adsorbent stanowił specyficzne, ekstremalne siedlisko dla wzrostu, kolonizacji i aktywności bakterii oraz jako źródło do poszukiwań mikroorganizmów o potencjalnych zastosowaniach biotechnologicznych. Mikroorganizmy takich siedlisk posiadają bogaty i bardzo często unikalny potencjał biochemiczny/metabolityczny dzięki czemu mogą przeprowadzać szereg procesów biotechnologicznych, m.in. w przemyśle węglowym, od produkcji po remediację terenów zanieczyszczonych. Zgodnie z danymi literaturowymi, najpopularniejsze bakterie występujące w środowiskach węglowych i węglowodnorodnych należą do grupy *Gammaproteobacteria* i są przede wszystkim bakterie z rodzaju *Arthrobacter* spp., *Sinomonas* spp. i *Bacillus* spp., które opisane w literaturze jako potencjalne biomarkery środowisk pokopalnianych

PODSUMOWANIE I WNIOSKI

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VIII. Podsumowanie i wnioski

Przegląd danych literaturowych pozwolił stwierdzić, że procesy hybrydowe (synergiczne), takie jak układy wetlandowe są obecnie uważane za holistyczne i zintegrowane podejście do oczyszczania środowisk wodnych. Układy wetlandowe oparte są na procesach zachodzących w przyrodzie. W literaturze opisano wiele przykładów wykorzystania układów wetlandowych zarówno w warunkach naturalnych, jaki i laboratoryjnych rozwiązań do oczyszczania ścieków komunalnych oraz niektórych ścieków przemysłowych, odcieków i wód opadowych. Natomiast, analiza dostępnej literatury naukowej wykazała, że układy wetlandowe rzadko były stosowane do oczyszczania wód poprocesowych z PZW.

W niniejszej pracy po raz pierwszy w sposób kompleksowy scharakteryzowano wody poprocesowe z PZW uwzględniając parametry fizyko-chemiczne, toksykologiczne oraz mikrobiologiczne. Zróżnicowanie w stężeniach zanieczyszczeń takich jak fenol, BTEX i WWA istotnie wpłynęło na różnice w składzie mikrobiologicznym ścieków surowych z PZW. Dodatkowo, jak wykazały analizy statystyczne, istotnym parametrem wpływającym na zróżnicowanie mikrobiologiczne miały parametry techniczne procesu podziemnego zgazowania węgla, takie jak czynniki zgazowania. W procesie PZW2 czynnikiem tym był czysty tlen, natomiast w procesach PZW1 i PZW3 było powietrze nasycone tlenem.

Badania pozwoliły także stwierdzić, że surowe ścieki z procesu PZW są źródłem szczepów bakterii o specyficznych właściwościach, co umożliwia wykorzystanie ich w procesach biotechnologicznych i bioremediacyjnych. W pracy wyizolowane i scharakteryzowane szczepy bakteryjne wykorzystano w procesie bioaugmentacji do oczyszczania ścieków koksowniczych.

Przeprowadzone badania wykazały możliwość zastosowania kolumn wetlandowych do oczyszczania wód poprocesowych z PZW. Biorąc pod uwagę złożony skład zanieczyszczeń i obecność skomplikowanych strukturalnie związków chemicznych w wodach poprocesowych, opracowany schemat kolumn wetlandowych w połączeniu z wysoką aktywnością mikroorganizmów i licznymi procesami fizykochemicznymi okazał się bardzo dobrym rozwiązaniem do oczyszczania. Ponadto, odpowiednio opracowane i dobrane parametry techniczne pracy systemu wetlandowego okazały się niezbędne do osiągnięcia zadowalających wskaźników usuwania zanieczyszczeń organicznych i nieorganicznych. Uzyskane w badaniu wyniki wskazują na potencjał kolumn wetlandowych do oczyszczania zanieczyszczonych wód

Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych

poprocesowych, zarówno związkami organicznymi jak i nieorganicznymi, jak również innych ścieków przemysłowych, np. ścieki koksownicze lub odcieki, wody opadowe.

Również, wyniki uzyskane z badań związanych z połączenia w różnych kombinacjach metod fizyko-chemicznych (elektrokoagulacji i adsorpcji) oraz kolumn wetlandowych potwierdziły, że kolumny wetlanowe mają duży potencjał w usuwaniu zarówno zanieczyszczeń organicznych, jak i nieorganicznych.

Badania te również wykazały, że karbonizat jako odpad z procesu PZW, a zastosowany jako adsorbent stanowił specyficzne, ekstremalne siedlisko dla wzrostu, kolonizacji i aktywności specyficznych bakterii oraz źródło do poszukiwań mikroorganizmów o potencjalnych zastosowaniach biotechnologicznych.

Podsumowując, przeprowadzone badania pozwalają przypuszczać, że pomimo znacznej zawartości zanieczyszczeń, ścieki z PZW nadają się do biologicznego oczyszczania. Uzyskane wyniki pokazują, że technologia oczyszczania z zastosowaniem wetlandów może być wykorzystana do oczyszczania również innych, silnie zanieczyszczonych ścieków przemysłowych, stąd efektem badań jest pozytywna weryfikacja hipotezy badawczej, że możliwe jest oczyszczenie ścieków z podziemnego zgazowania węgla (PZW) metodami biologicznymi.

Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych

STRESZCZENIE

IX. Streszczenie w języku polskim

Podziemne zgazowanie węgla (PZW) jest nowatorską technologią umożliwiającą przekształcanie węgla w gaz syntezowy bezpośrednio w złożu, co minimalizuje konieczność tradycyjnego wydobycia oraz ogranicza negatywny wpływ na środowisko i zdrowie pracowników. Proces ten generuje jednak ścieki zawierające złożone zanieczyszczenia organiczne, takie jak BTEX, wielopierścieniowe węglowodory aromatyczne (WWA) i fenole, a także nieorganiczne, w tym metale ciężkie. Ze względu na ich toksyczność i zmienność składu, efektywne oczyszczanie tych ścieków stanowi istotne wyzwanie technologiczne.

Celem niniejszej pracy było opracowanie metod oczyszczania ścieków pochodzących z PZW poprzez zastosowanie systemów wetlandowych oraz technologii hybrydowych integrujących procesy fizykochemiczne i biologiczne. Przeprowadzone analizy fizykochemiczne, mikrobiologiczne i toksykologiczne ścieków z PZW, pochodzących z różnych eksperymentów w warunkach *ex situ*, wykazały wysokie stężenia toksycznych związków organicznych i metali ciężkich oraz znaczną zmienność składu mikrobiologicznego w zależności od parametrów procesu, takich jak rodzaj zgazowanego węgla i zastosowany czynnik zgazowania. Wody poprocesowe sklasyfikowano do V klasy toksyczności (wysoko toksyczne), co podkreśla konieczność zastosowania zaawansowanych metod oczyszczania.

W pierwszym etapie badań skonstruowano kolumny wetlandowe z pionowym przepływem, wykorzystując rośliny hydrofitowe (tj. trzcinę pospolitą) oraz naturalnie występujące mikroorganizmy. Po 14 dniach procesu oczyszczania odnotowano znaczącą redukcję głównych zanieczyszczeń organicznych - ponad 95% BTEX, WWA i fenoli. Jednocześnie toksyczność ścieków uległa zmniejszeniu o 74 – 99%, co pozwoliło zaklasyfikować oczyszczone ścieki do I klasy toksyczności, oznaczającej brak szkodliwości dla środowiska. Wyniki te potwierdzają, że systemy wetlandowe stanowią efektywne i ekologiczne rozwiązanie umożliwiające usunięcie zarówno zanieczyszczeń organicznych, jak i nieorganicznych z wód poprocesowych. W kolejnym etapie pracy zbadano potencjał wzbogacenia systemu wetlandowego poprzez integrację technologii hybrydowych, takich jak elektrokoagulacja i adsorpcja z wykorzystaniem karbonizatu (odpadu powstałego w procesie PZW). Układy wykazały, że takie systemy skutecznie wspomagają usuwanie metali ciężkich oraz fenoli. Zarówno adsorpcja, jak i elektrokoagulacja okazały się efektywne w usuwaniu metali takich jak kadm, miedź i nikiel, osiągając poziomy redukcji do 99,5%. Systemy hybrydowe, zwłaszcza z zastosowaniem adsorbentów w kolumnach wetlandowych, wykazały większą elastyczność i wydajność w usuwaniu różnorodnych zanieczyszczeń w porównaniu

z samodzielnymi systemami biologicznymi. Przeprowadzone badania potwierdziły, że kolumny wetlandowe są efektywną metodą oczyszczania ścieków z PZW, przyczyniając się do biodegradacji toksycznych związków poprzez naturalne procesy biologiczne i fizykochemiczne. Uzyskane wyniki świadczą, że systemy wetlandowe oraz układy hybrydowe można zastosować w skali przemysłowej do oczyszczania różnorodnych typów ścieków, w tym pochodzących z PZW, koksowni, odcieków ze składowisk oraz wód opadowych. Dodatkowo, badania dotyczące charakterystyki mikrobiologicznej tych systemów umożliwiły identyfikację szczepów bakterii, takich jak *Paenibacillus*, *Bacillus* i *Pseudomonas*, wykazujących zdolność do rozkładu fenolu. Stwierdzono również, że biofilm formujący się na powierzchni karbonizatu zwiększa wydajność biodegradacji, a zastosowanie wyizolowanych bakterii w procesie bioaugmentacji dodatkowo poprawia efektywność oczyszczania. Wnioski płynące z przeprowadzonych badań wskazują na duży potencjał wykorzystania technologii wetlandowych oraz systemów hybrydowych w usuwaniu toksycznych związków ze ścieków przemysłowych. Systemy te wpisują się w założenia gospodarki o obiegu zamkniętym i zrównoważonego rozwoju, a ich adaptacyjność do różnych warunków i typów ścieków czyni je kluczowym elementem w transformacji przemysłu energetycznego w kierunku bardziej ekologicznych technologii.

X. Streszczenie w języku angielskim

Underground Coal Gasification (UCG) is an innovative technology that converts coal directly into syngas on site, minimizing the need for conventional mining and reducing the negative impact on the environment and occupational health. However, this process produces wastewater containing complex organic pollutants such as BTEX, polycyclic aromatic hydrocarbons (PAHs) and phenols, as well as inorganic pollutants including heavy metals. Due to their toxicity and the varying composition of the wastewater, the effective treatment of such wastewater poses a major technological challenge.

The aim of this study was to develop sustainable methods for treating wastewater from UCG by using wetland systems and hybrid technologies that integrate physico-chemical and biological processes. Physico-chemical, microbiological and toxicological analyses were performed on wastewater from different ex-situ experiments of UCG. The analyses revealed high concentrations of toxic organic compounds and heavy metals as well as a considerable variability in composition depending on the process parameters, such as the type of gasified coal and the gasification agent used. These pollutants were classified in the fifth toxicity class, thus emphasizing the need for advanced treatment methods.

In the first phase of the research, vertical flow columns were constructed with hydrophytes (e.g. *Phragmites australis*) and naturally occurring microorganisms. After 14 days of treatment, a significant reduction of the main organic pollutants was observed — over 95% of BTEX, PAHs and phenols were removed. At the same time, the toxicity of the wastewater was reduced by 74–99%, so that the treated wastewater could be classified as non-toxic (toxicity class I), which means that it does not harm the environment. These results confirm that wetland systems are an effective and environmentally friendly solution for the removal of organic and inorganic pollutants from UCG wastewater.

In the next phase of the study, the potential of improving wetland systems by integrating hybrid technologies, such as electrocoagulation and adsorption, was investigated. These systems showed effective removal of heavy metals and phenols. Both adsorption and electrocoagulation achieved heavy metal removal rates of up to 99.5% for cadmium, copper and nickel. Hybrid systems, especially those using adsorbents in wetland columns, showed greater flexibility and efficiency in removing various pollutants compared to stand-alone biological systems.

The study confirmed that wetland columns are an effective method for treating UCG wastewater, facilitating the biodegradation of toxic compounds through natural biological and

plant remediation processes. The results suggest that industrial-scale wetland systems and hybrid technologies can be used to treat various types of industrial wastewater, including wastewater from natural gas incinerators, coking plants and landfill leachate. In addition, studies on the microbiological activity of these systems have identified bacterial strains such as *Paenibacillus*, *Bacillus* and *Pseudomonas* that can degrade phenol. It has also been found that the formation of a biofilm on the surface of adsorbents increases the efficiency of biodegradation, while the use of isolated bacteria in bioaugmentation further improves treatment performance.

The conclusions from this study highlight the significant potential of wetland technologies and hybrid systems for the removal of toxic compounds from industrial wastewater. These systems are in line with the principles of circular economy and sustainable development. Their adaptability to different conditions and wastewater types makes them a key element in the energy industry's transition to more environmentally friendly technologies.

DOROBK NAUKOWY

XI. Dorobek naukowy

Sumaryczny dorobek naukowy

- Publikacje: 34; sumaryczny IF: 97,422; liczba cytowań: 408; h indeks – 9 (web of science - 21.01.2025), punkty MNiSW:1839
- Doniesienia Konferencyjne: 31
- Szkolenia i kursy: 5

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2. **Jałowiecki Ł.**, Borgulat J., Glaser M., Marchlewicz A., Płaza G. (2024) Zastosowanie bioaugmentacji w oczyszczaniu ścieków koksowniczych- badania bioreaktorowe. VIII Ogólnopolskie Sympozjum Mikrobiologiczne "Metagenomy różnych środowisk", Lublin, 17-18.06.2024 r.
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4. **Jałowiecki, Ł.**, Borgulat, J., Strugała-Wilczek, A., & Płaza, G. (2023) Charakterystyka metagenomu bakteryjnego wód poprocesowych z podziemnego zgazowania węgla. VII Ogólnopolskie Sympozjum Mikrobiologiczne „Metagenomy różnych środowisk”, Lublin, 20-21.06.2023 r.
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Udział w konferencjach zagranicznych

1. **Jałowiecki Ł.**, Nowrotek M., Harnisz M., Płaza G. (2019) Toxicity analysis of wastewater from full-scale municipal wastewater treatment plants at different treatment stages using Microtox® assay. 11th Eastern European Young Water Professionals Conference. 1-5 October 2019, Prague, Czech Republic.
2. Nowrotek M., Ciesielski S., **Jałowiecki Ł.**, Harnisz M., Korzeniewska E., Płaza G. (2019) Metagenomic analysis as a tool to reveal the changes in the structural biodiversity in sludge and wastewater samples taken from full-scale municipal wastewater treatment plants. 11th Eastern European Young Water Professionals Conference. 1-5 October 2019, Prague, Czech Republic.
3. Nowrotek M., **Jałowiecki Ł.**, Wasilkowski D., Płaza G. (2019) Antibiotic resistance profiling of *Aeromonas caviae* strains isolated from wastewater. International Conference on Geosciences – GEOLINKS, 26-29.03.2019, Athens, Grecja.
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6. **Jałowiecki Ł.**, Chojniak J., Płaza G., Dorgeloh E., Ejhed H. (2016) Antibiotic susceptibility of bacteria isolated from onsite wastewater treatment facilities. 16th International Multidisciplinary Scientific GeoConference SGEM 2016. 30 June-6 July, Albena, Bułgaria.

7. Płaza G., **Jałowiecki Ł.**, Chojniak J., Dorgeloh E., Hegedusowa B., Ejhed H., Magner J. (2015) Application of Phenotypic Microarrays to characterize the Multi- antibiotic resistance bacteria isolated from onsite wastewater treatment technologies, Florence Conference on Phenotype MicroArray Analysis of Microbial and Mammalian Cells, 10-12.09.2015, Florencja, Włochy.

Patenty

Płaza G., Biedroń I., **Ł. Jałowiecki**, J. Chojniak-Gronek + zespół z GiG-u i Haldex-u. Patent nr P.425072 [WIPO ST 10/C PL425072] pt.: „Biospoiwo i sposób jego wytwarzania”

Szkolenia/Staże naukowe/Warsztaty

1. 5-6.11.2022 Szkolenie Wprowadzenie do metagenomiki - analiza danych metagenomicznych oraz badaniem mikrobiomu. Szkolenie online organizowane przez data2biology.
2. 6-10.07.2015. 11th Poznań, Summer School of Bioinformatics Topic: “Modern approaches to RNA analyses RNA-Seq, microRNAs, lncRNAs, RNA structures”.
3. 27-29.05.2015. ukończenie praktycznego szkolenia oceny toksyczności. Szkolenie organizowane w Lublinie przez Tigret.
4. 19.11.2015. Udział w warsztatach „Projektowanie i optymalizacja procesów hodowlanych w bioreaktorach”, Poznański Park Naukowo- Technologiczny.
5. 5-8.04.2016. staż naukowy w Regional Centre of Advanced Technologies and Materials, Department of Experimental Physics and Physical Chemistry, faculty of Science, Palacký University in Olomouc, Olomouc, Czech Republic.

Kierowanie projektami krajowymi

1. „Identyfikacja genów antybiotykooporności i wirulencji w genomie *Aeromonas caviae*” temat statutowy(2019) – MEiSW.
2. „Badania ekotoksyczności gruntów” (2019), zlecenie rynkowe.
3. „Badanie ekotoksyczności z wykorzystaniem bakterii luminescencyjnych próbek odpadów przemysłowych” (2019), zlecenie rynkowe.
4. Badanie ekotoksyczności z wykorzystaniem bakterii luminescencyjnych próbek odpadów komunalnych i przemysłowych” (2018) zlecenie rynkowe.
5. „Identyfikacja genów antybiotykooporności w wybranych szczepach bakterii izolowanych z oczyszczalni ścieków”(2020), temat statutowy - MEiSW.
6. „Wpływ procesu liofilizacji na aktywność wybranych szczepów bakterii: System Biolog PM” (2021) temat statutowy - MEiSW

7. „Badania biobójczego wpływu lamp UVC w technologii LED na różne szczepy bakterii” (2021), zlecenie rynkowe.
8. „Szczepy wspomagające wzrost roślin jako producenci biosurfaktantów” (2022) temat statutowy - MEiSW.

Udział w projektach krajowych i międzynarodowych

1. “Coal- and bio-based water remediation strategies for underground coal gasification”. 2021-2024 - Research and Innovation Framework Programme; RFCS. Projekt z Funduszu Węgla i Stali, nr 101033964 oraz z Ministerstwa Nauki i Szkolnictwa Wyższego nr 5198/FBWiS/2021/2 i 5211/FBWiS/2021/2 (wykonawca).
2. „Metagenomiczny profil genów antybiotykooporności i genetycznych elementów mobilnych w ściekach komunalnej oczyszczalni ścieków”. NCN, konkurs Harmonia 9, (2018-2022) nr projektu 2017/26/M/NZ9/00071 (wykonawca).
3. „Wpływ nanocząstek srebra (Ag-NPs) syntetyzowanych biologicznie na aktywność metaboliczną komórek ssaczych” NCN, konkurs Preludium 10, (2017-2019), nr. projektu 2015/19/N/NZ9/01621 (wykonawca).
4. “Optimization of small wastewater treatment facilities” (akronim: OPITREAT; BONUS call 2012: Innovation; 2.4 Eco-technological approaches). nr projektu BONUS-INNO-2012-01/2014 (wykonawca).
5. “Phytoremediation driven energy crops production on heavy metal degraded areas as local energy carrier” (akronim: Phyto2Energy; FP7-PEOPLE-2013-IAPP) (2014-2018) nr projektu: 610797 (wykonawca).
6. „Wpływ biosurfaktantów produkowanych przez bakterie z rodzaju *Bacillus* na proces biosyntezy nanocząstek srebra (Ag-NPs) i ich właściwości” NCN, konkurs OPUS 5 2013/09/B/NZ9/01759.
7. “Antibiotic resistance genes (ARG) prevalence in a sewage treatment plant and its effluent-receiving water in Portugal and Poland: comparison research” projekt dwustronnej współpracy w ramach Programu Wymiany Osobowej na lata 2017-2018, MNiSW (wykonawca).

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XII. Literatura uzupełniająca

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7. Edgar, T. F., Humenick, M. J., Kaiser, W. R., & Charbeneau, R. J. (1981). Environmental effects of *in situ* gasification of Texas lignite. Project Summary. US EPA; Texas University
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Insights into bacterial diversity in industrial post-processing water from underground coal gasification (UCG) process

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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 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/

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-NO ₃ (mg L ⁻¹)	Spectrophotometric / ion chromatography (IC)	0.11	0.12	<0.1
N-NO ₂ (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.

<i>Preprocessed sequences</i>	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
<i>dada2 filtered sequences</i>			
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 merged	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; acenaphthalene (46%), pyrene (14%), and phenanthrene (10%) in W2; and naphthalene (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 O₂ L⁻¹ and from 397 to 1130 mg O₂ L⁻¹, respectively (Pankiewicz- Sperka et al.

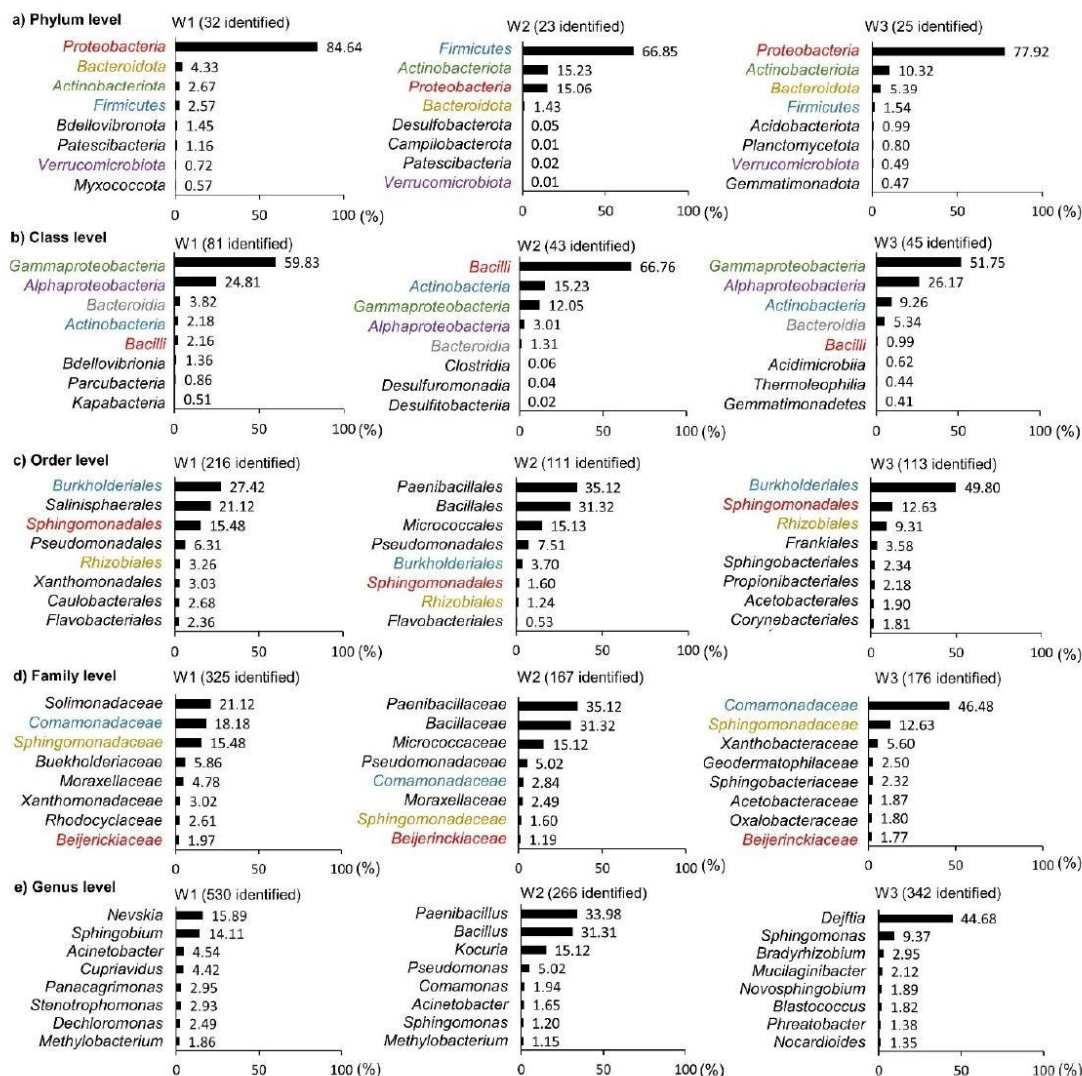


Figure 1. Taxonomic hierarchy and relative abundance of bacteria in investigated post-processing wastewaters (top of 8 most common taxa from each wastewater).

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 *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 *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 pasadenensis* SAFN-007, *Paenibacillus 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 glucanolyticus* 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.

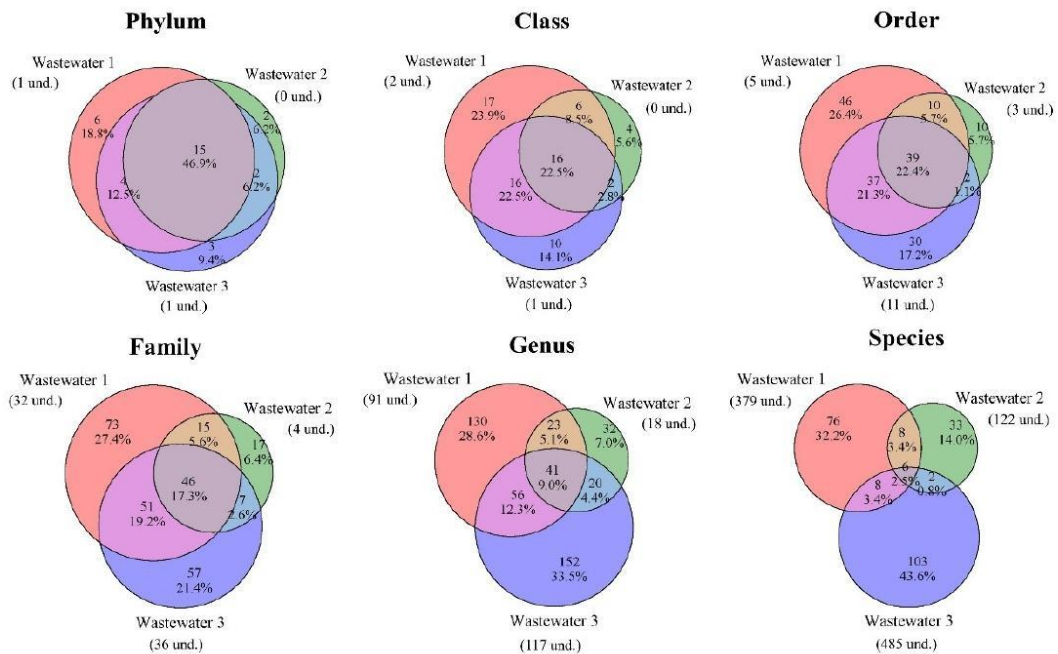


Figure 3. Venn diagrams showing the proportion of common bacterial taxa at different taxa levels in all analysed wastewaters. The number of unidentified reads that were not classified at the respective taxonomic level is shown in brackets.

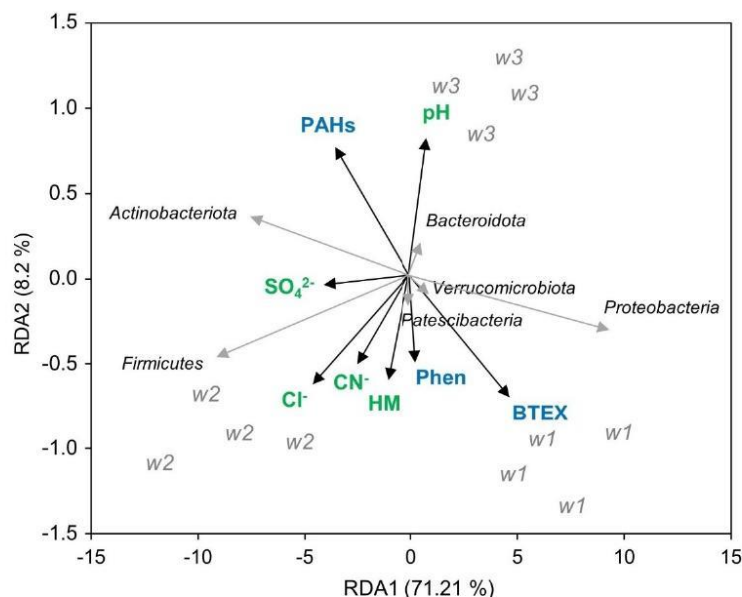


Figure 2. The redundancy analysis (RDA) order diagrams showing the relationships between the bacterial phylum (the taxons with the highest abundance which are found in each wastewater) and selected wastewater characteristics (green labels indicate inorganic substances, blue – organic compounds). Arrows 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 pasadenensis* SAFN-007, *Paenibacillus cineris* cu1-7, *Paenibacillus 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 ligninolytic 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.

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Conflicts of Interest

The authors declare no conflict of interest.

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Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych

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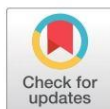
RESEARCH ARTICLE

Constructed wetland as a green remediation technology for the treatment of wastewater from underground coal gasification process

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Abstract

The wastewater from underground coal gasification (UCG) process has extremely complex composition and high concentrations of toxic and refractory compounds including phenolics, aliphatic and aromatic hydrocarbons, ammonia, cyanides, hazardous metals and metalloids. So, the development of biological processes for treating UCG wastewater poses a serious challenge in the sustainable coal industry. The aim of the study was to develop an innovative and efficient wetland construction technology suitable for a treatment of UCG wastewater using available and low-cost media. During the bioremediation process the toxicity of the raw wastewater decreased significantly between 74%–99%. The toxicity units (TU) ranged from values corresponding to very high acute toxic for raw wastewater to non-toxic for effluents from wetland columns after 60 days of the experiment. The toxicity results correlated with the decrease of some organic and inorganic compounds such as phenols, aromatic hydrocarbons, cyanides, metals and ammonia observed during the bioremediation process. The removal percentage of organic compounds like BTEX, PAHs and phenol was around 99% just after 14 days of treatment. A similar removal rate was indicated for cyanide and metals (Zn, Cr, Cd and Pb). Concluded, in order to effectively assess remediation technologies, it is desirable to consider combination of physicochemical parameters with ecotoxicity measurements. The present findings show that wetland remediation technology can be used to clean-up the heavily contaminated waters from the UCG process. Wetland technology as a nature-based solution has the potential to turn coal gasification wastewater into usable recycled water. It is economically and environmentally alternative treatment method.

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Introduction

Although the underground coal gasification (UCG) process generates a smaller amount of wastewater than traditional coal mining, it however the wastewater contains a high concentration of environmentally hazardous compounds [1, 2]. The release of numerous organic and

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inorganic contaminants from coal tars and ashes produced is connected with heterogeneous and homogenous reactions during different stages of UCG process such as pyrolysis, reduction, oxidation [3, 4]. Waste water stream from the UCG process is an important source of environmental pollution disrupts ecological sustainability and causes human health risk. It contains extremely complex high-concentration aromatic hazardous, toxic and refractory compounds including phenolics, polycyclic aromatic hydrocarbons (PAHs), nitrogen heterocyclic compounds (NHCs) and long chain n-alkanes., potentially hazardous and harmful to the environment [5–7].

Nowadays, UCG wastewater treatment has been considered as a emerging challenge to the sustainable development of coal industry. The extensive effort has been devoted to developing various cost-effective and environmentally friendly processes to removal of hazardous and refractory organic compounds from the wastewater. So far, integrated engineering systems including biological and chemical advanced treatment system as holistic approach have been considered to clean up of UCG wastewater [2, 8–10]. The development of an appropriate treatment method to remove pollutants from UCG wastewater is extremely important for the successful application of UCG technology in energy transformation.

As described in the literature, a lot of industrial wastewater has been treated by biological processes, among which the constructed wetlands (CWs) as nature-based treatment technology are good solutions. The proven advantages of CWs as green and sustainable technology has been discussed by many researchers and resulted in numerous applications [11–20]. Literature analysis showed that so far, CWs have not been applied to the treatment of wastewater from the coal industry.

In this context, the aim of this work was to develop an innovative and efficient wetland construction technology suitable for the treatment of wastewater from underground coal gasification process using available and low-cost media, which allowed to deepen the knowledge about working conditions and possible applications of CWs. In our study the ability of vertical flow constructed wetland to treat UCG wastewater was tested.

Materials and methods

UCG experiments and production of UCG post-processing wastewater

Three separate experimental simulations of UCG were carried out in a large-scale *ex-situ* installation located in the Barbara Experimental Mine in Mikołów, a part of the laboratory facilities of the Center for Clean Coal Technologies in GIG Research Institute (Katowice, Poland).

Two Polish coals from “Piast-Ziemowit” mine and “Wesoła” mine were gasified. Before gasification, raw coals were subjected to analysis in the accredited laboratory in GIG Research Institute (Katowice, Poland). A short summary of the basic properties of coals is presented in Table 1. Both raw coals have a relatively high content of volatile matter (approx. 30%). “Piast-Ziemowit” coal sample has a higher content of moisture and ash in comparison to the “Wesoła” coal. Coal from the “Wesoła” mine contains about two times less moisture and a lower content of ash, is more coalified and has a greater calorific value. Due to their properties, both coals can be classified as medium-rank bituminous coals [21, 22]. The raw coals were subjected to gasification using oxygen-enriched air (OEA) as a gasifying agent (Experiment 1 and Experiment 3). Additionally, the “Piast-Ziemowit” coal was gasified in the oxygen atmosphere (Experiment 2). Characteristics of the UCG experiments are presented in Table 2. Detailed information on the UCG simulations was described by Wiatowski et al. [21].

Table 1. Comparison of some parameters of coals used in the experiments.

Parameters (units)	Coal	
	"Piast-Ziemowit"	"Wesola"
Moisture W ^a (%)	7.47	3.49
Ash A ^a (%)	7.64	2.15
Volatile matter content V ^a (%)	30.49	30.12
Lower heating value Q ^a (kJ kg ⁻¹)	26,103	31,543
Total sulphur S _t ^a (%)	0.99	0.21
Carbon C ^a (%)	68.62	82.01
Hydrogen H ^a (%)	4.30	5.18
Nitrogen N ^a (%)	1.08	2.24
Oxygen O ^a (%)	10.20	4.83

^aAnalytical state<https://doi.org/10.1371/journal.pone.0300485.t001>

UCG wastewater sampling

Three post-process wastewater average samples were generated as a result of three different coal gasification experiments differing in the coal rank and gasifying agent. Wastewater collected after every completion of each UCG process were produced in the water scrubber and represents the average sample of wastewater for a given gasification experiment. The addition of water to the scrubber prevented clogging the process gas discharge pipes. The actual volume of wastewater generated in each of conducted UCG processes was obtained by subtracting the water that has been injected to the water scrubber during the experiment with the flow rate 14 kg h⁻¹.

The total amount of raw wastewater generated as a result of coal gasification process was collected in a plastic tank with a capacity of 1 m³ (Mauser type) and after mixing and pouring into smaller containers, the samples were transported to the laboratory. Subsequently,

Table 2. General assumptions and characteristics of conducted UCG Experiments 1–3 (Wiatowski et al., [21]).

Parameters (units)	Experiment no. 1	Experiment no. 2	Experiment no. 3
Coal origin	"Piast-Ziemowit" mine (Poland)		"Wesola" 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 gasified (kg)	140.9	323.9	165.3
Wastewater produced* (kg)	234 (1018)	364 (1372)	189 (1197)
Wastewater production rate (kg/h)	4.18	5.01	2.63
Wastewater outflow (kg/kg gasified coal)	1.66	1.12	1.14

* the real quantity of post-process water obtained from coal gasification after subtracting the volume of the water added to the scrubber; the total amount of water before correction is given in brackets.

<https://doi.org/10.1371/journal.pone.0300485.t002>

wastewater samples were filtered through a 0.45 µm pore diameter membrane filter under vacuum to remove coal tar and other undissolved residues before laboratory analysis. All raw wastewater samples were characterized by an unpleasant odour and a certain amount of fine suspension. The samples remained slightly coloured after filtration. All filtrates were stored at 4°C until analysis. The raw wastewater from each of the three UCG experiments was biologically treated with constructed wetland column set. The effluents from the constructed wetlands were collected after 14 and 60 days for physico-chemical analysis. The toxicity analysis of effluents was performed after 14, 30, 45, 60, 75 and 150 days.

Wetland column set up

Laboratory-scale vertical subsurface flow constructed wetlands (VSF CWs) were set up in columns made of poly-methyl methacrylate polymer (PMMA). A total of four columns, one control and three experimental columns were set up. In experimental columns, the raw post-processing wastewater from the UCG processes was used.

The raw wastewater from UCG Experiment No. 1 was used in the constructed wetland marked No. 1 (CW1). Similarly, In the case of the raw wastewater from UCG experiments Nos. 2 and 3 was used to set up constructed wetlands Nos. 2 (CW2) and 3 (CW3).

The columns were filled with sand and gravel, a filling typically used in reed-bed wastewater treatment plants and the top layer of the column was green compost, whose task was to provide nutrients to the system. Eight cuttings of common reed (*Phragmites australis*) with a stem height of about 30 cm were planted in each column. The systems were stabilized with tap water for 2 weeks while maintaining the same flow parameters as when the systems were fed with UCG wastewater. The plants grew under natural light conditions. At the beginning of the experiment, the tanks placed next to a given experimental column contained 70 liters of wastewater. The raw wastewater passed through the column and returned to the tank by recirculation, where it was constantly mixed by the pump. Fig 1 shows an illustrative diagram of the experimental set-up. Technical details of the laboratory-scale constructed wetlands including column effluent retention time and water storage capacity of the bed are provided in Table 3.

Physicochemical analysis

Optimally selected techniques and analytical methods used for the analysis of post-process UCG wastewater and effluents from the wetland columns are presented in detail in S1 Table. The analytical methods were selected appropriately according to the expected high content of impurities in the UCG polluted wastewater. The key instrumental equipment used for the analysis was IC analyzer Dionex ICS-5000 from Thermo Fisher Scientific (determination of inorganic anions); ICP-OES analyzer Optima 5300 DV from Perkin Elmer (determination of metals and metalloids); HS-GC-MS analyzer from Agilent Technologies (BTEX analysis); HPLC analyzer 1200 series with FLD detector from Agilent Technologies (PAHs analysis). All physico-chemical wastewater analysis were conducted in the accredited laboratories of GIG Research Institute in accordance with the currently applicable standards.

Toxicity evaluation

Toxicity assessment of raw wastewater samples and effluents from wetland columns was conducted with the Microtox® test recommended by ISO Standard 11348:1998. The test was carried out in the Microtox M500 toxicity analyzer according to the standard procedure [23, 24] with some modifications. In the test commercially available lyophilised bacteria *Vibrio fischeri* (NRRL-B 11177) was applied. The determination of the acute toxicity was performed by a two-step analysis. At first, in the initial screening test the toxicity was determined on non-

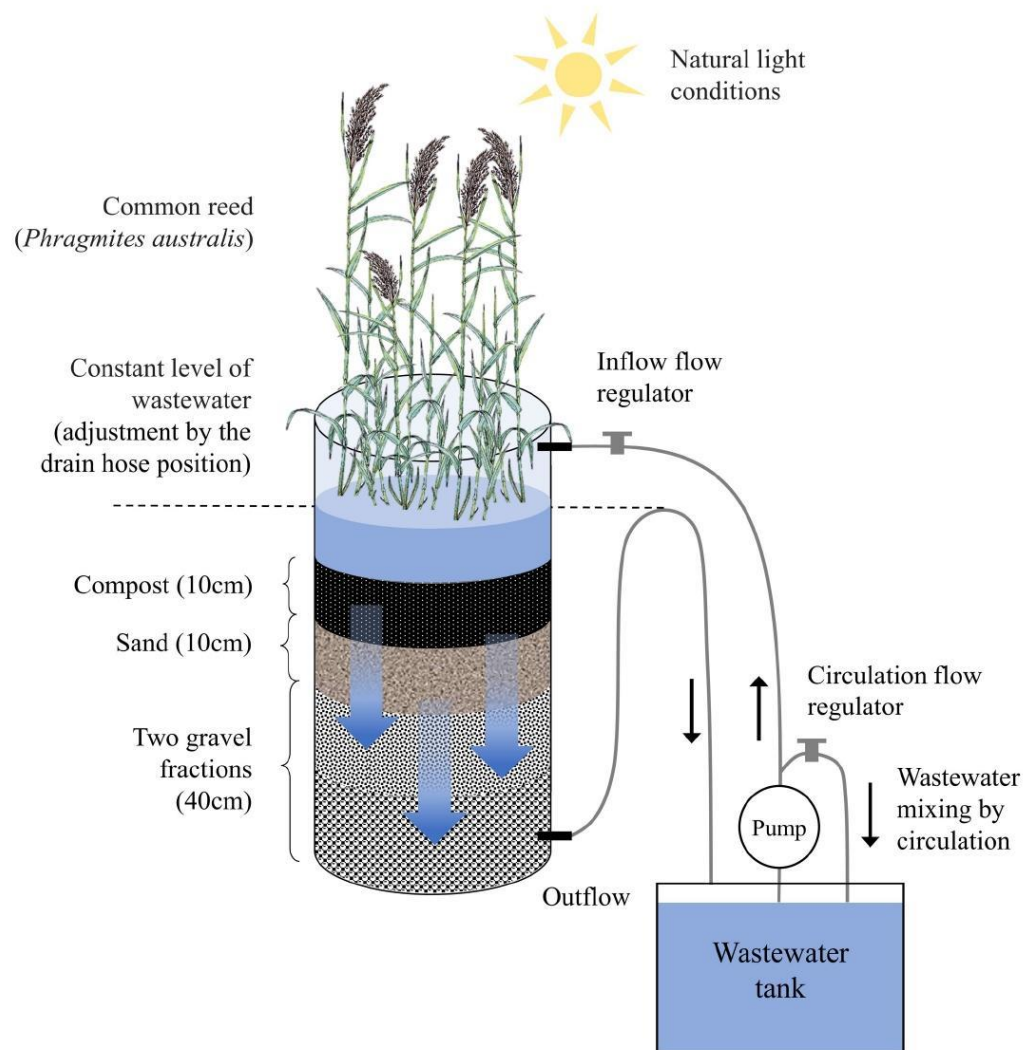


Fig 1. Scheme of the vertical subsurface flow constructed wetlands (VSCWs) used in the bioremediation experiment.

<https://doi.org/10.1371/journal.pone.0300485.g001>

diluted samples. In a second step, toxicity was assessed on sample solutions diluted with 2% NaCl to achieve more than a 50% effect over the initial screening. The luminescence inhibition after 15 min was taken as the endpoint. The EC50 values (concentration causing 50% reduction in the bioluminescence of the bacteria) and the corresponding 95% confidence intervals

Table 3. Technical and working parameters of laboratory scale vertical-subsurface flow constructed wetlands (VSF CWs).

Column parameters ^a		Values
Diameter		0.30 m
Height		0.80 m
Column area		633.5 cm ²
Filling layers	(1) green compost	0.10 m
	(2) sand <1.0 mm	0.10 m
	(3) gravel fraction 5.0–8.0cm	0.20 m
	(4) gravel fraction 3.0–5.0cm	0.20 m
Volume		38 L
Maximum water volume		11.3 L
Water storage capacity of the bed		0.8 L
Average flow through the column (Q) ^b		5.65 L d ⁻¹
Hydraulic load		30 cm d ⁻¹
Hydraulic retention time (HRT)		2 day

^aThe columns were made of translucent Plexiglas. For this reason, each column was wrapped with aluminium foil from the outside to prevent algal blooms;

^bFlow parameters were monitored at least once a day

<https://doi.org/10.1371/journal.pone.0300485.t003>

were computed using the MicrotoxOmni[®] Software. Then, the toxicity unit (TU) as 1/ EC50 was calculated. Finally, obtained results were ranked based on TU values into one of five classes described by Persoone et al.[25]. Each sample was run in triplicates assay. Toxicity tests were evaluated in the microbiological laboratory of the Institute for Ecology of Industrial Areas (Katowice, Poland).

Statistical and computational analysis

In order to show the changes in the physico-chemical parameters of wastewater after the UCG processes and their effects on toxicity, the PCA analysis was carried out on the basis of a correlation matrix. The range of continuous output variables has been standardised so that each contributes equally to the analysis. All calculations were performed using Dell Statistica (data analysis software), version 13.

For selected post-process wastewater contaminants, the degree of wastewater treatment ($\eta_{14,60}$, %) was calculated, defined as the ratio of the amount of pollutants retained as a result of wastewater treatment to the amount of pollutants contained in raw wastewater. The percent reduction was calculated after 14 and 60 days of contact of wastewater with constructed wetlands according to the equation:

$$\eta_{14,60}(\%) = \frac{c_e - c_0}{c_0} \cdot 100 \quad (1)$$

where:

c_0 —the concentration or index of pollutants in the raw wastewater (mg L⁻¹),

c_e —the concentration or index of pollutants in the wastewater after 14 or 60 days of contact with constructed wetland (mg L⁻¹).

Results and discussion

Characterization of UCG raw wastewater samples

The real quantities of post-process water obtained from coal gasification experiments were 234 kg, 364 kg and 189 kg in Experiment 1, Experiment 2 and Experiment 3, respectively (Table 2). The total amount of produced UCG wastewater was higher in the case of coal gasification under oxygen (experiment No. 2) (Table 2). A higher amount of coal gasified in the process with oxygen in comparison to oxygen-enriched air (OEA) results in relatively lower amount of water per unit mass of coal (Table 2). The wastewater production rate was higher in the case of gasification of „Piaś-Ziemowit” coal (experiments No. 1 and No. 2). This can be connected with significantly higher moisture content (8.50% vs 3.73%, respectively), and together with unreacted steam, water evaporation or hydrogen combustion may result in the relatively high wastewater outflow in the Experiment No. 1 (with OEA as the gasifying agent) in reference to the coal consumption.

Table 4 presents changes in physicochemical parameters in UCG wastewater during the treatment by the constructed wetlands after 14 days and 60 days. In accordance with the Best Available Techniques (BAT) recommendations for coke wastewater, which are similar to UCG wastewater in terms of composition, the pollutant concentrations in coking wastewater after treatment and before discharging it to water or land, should not exceed the following levels for selected parameters: COD < 220 mg L⁻¹, BOD₅ < 20 mg L⁻¹, free sulfides < 0.1 mg L⁻¹; thiocyanides < 4 mg L⁻¹; free cyanides < 0.1 mg L⁻¹, polycyclic aromatic hydrocarbons (PAHs) < 0.05 mg L⁻¹, phenols < 0.5 mg L⁻¹, total nitrogen (sum of ammonia nitrogen, nitrate nitrogen, and nitrite nitrogen) < 15–50 mg L⁻¹ [25].

In analyzed samples, pH ranged from 1.8 to 7.0 (Table 4). The wastewater originated from the gasification of „Piaś-Ziemowit” coal were strongly acidic, which may be related to the relatively high sulfur content in the raw coal (five times more compared to „Wesola” coal). A significant increase in the pH of the raw water sample after experiments 1 and 2 (from 2.6 to 6.3 and from 1.8 to 6.4, respectively for raw wastewater 1 and raw wastewater 2) may be related to the leaching of potential impurities in the form of calcium and magnesium carbonates present in the quartz gravel filling the column. On the basis of a blank test, it was verified that in the case of contact of gravel with a neutral solution, the pH does not change (the blank test was carried out on tap water; the initial pH of the solution was 6.7, after 14 days it was 6.8, and after 30 days it slightly increased to 6.9). In the acid blank, the starting pH was 2.4 and increased to 5.9 after only 5 hours, then slowly increased to 6.4 (after 14 days) and 6.5 (after 30 days). Additional analysis carried out confirmed the increase in Ca and Mg concentrations in the tested samples over time (Table 4).

The raw wastewater after Experiment No. 2 had high conductivity. Total nitrogen consisted mainly of ammonia and remained constant in all three wastewater samples. Total cyanide content was found in all samples, with the highest concentration 42 mg L⁻¹ determined in the raw wastewater 2, which deserves special attention due to the strongly acidic nature of the sample. The raw wastewater 1 and 2 contained significantly more COD, BOD, TOC and phenol index than the sample number 3 with moderate content of organic substances. Significant differences between the wastewater from coal gasification „Piaś-Ziemowit” and „Wesola” were also present in the TOC content (330–350 mg L⁻¹ and 130 mg L⁻¹, respectively). These differences may indicate a significant impact of the type and composition of the gasified coal on the composition of wastewater generated in the process, especially the concentrations of organic parameters. The relationship between the values of general organic pollutant indicators (such as BOD, COD, TOC) determines the susceptibility of wastewater to biological treatment plant processes

Table 4. The changes of the parameters of wastewater during the bioremediation process (mean values, n = 3). Parameters that are exceeded in relation to the normative values given in BAT document (2012) are marked in bold.

Parameters (units)	CW1			CW2			CW3		
	day 0 ^{a)}	day 14	day 60	day 0 ^{a)}	day 14	day 60	day 0 ^{a)}	day 14	day 60
pH	2.6	5.2	6.3	1.8	5.1	6.4	7.0	7.7	7.1
Conductivity (mS cm ⁻¹)	2610	1860	2220	8640	2830	3300	1530	1480	1850
Redox (mV)	263	186	274	382	175	259	112	211	254
Ammonia NH ₄ (mg L ⁻¹)	180	140	42	160	110	98	190	130	<0.02
N-NO ₃ (mg L ⁻¹)	0.11	<0.10	36	0.12	<0.10	<0.10	<0.1	<0.1	58
N-NO ₂ (mg L ⁻¹)	<0.006	0.0079	11	<0.002	<0.002	0.011	0.016	0.033	0.21
Total nitrogen (mg L ⁻¹)	150	120	83	120	90	82	150	100	63
BOD (mgO ₂ L ⁻¹)	360	260	7	550	320	9	170	14	28
COD (mgO ₂ L ⁻¹)	1130	660	120	1060	660	72	397	143	129
TOC (mgC L ⁻¹)	330	220	44	350	210	26	130	53	46
Chlorides (mg L ⁻¹)	516	450	483	1120	961	1080	269	228	312
Sulfates (mg L ⁻¹)	154	216	276	106	78.8	<10	130	198	276
Nitrates (mg L ⁻¹)	0.50	<0.50	160	0.53	<0.50	<0.50	<0.5	<0.5	260
Nitrites (mg L ⁻¹)	<0.006	0.026	35	<0.006	<0.006	0.036	0.051	0.11	0.70
Total cyanides (mg L ⁻¹)	6.7	2.8	0.065	42	0.16	<0.002	5.1	0.064	0.070
Phenol index (mg L ⁻¹)	94	60	0.017	165	68	0.0091	41	0.014	0.0042
Total phosphorus (mg L ⁻¹)	0.12	0.24	0.29	0.10	0.22	<0.065	<0.065	2.43	0.57
Sulfides (mg L ⁻¹)	<0.02	0.023	<0.02	0.25	0.054	<0.02	<0.02	<0.02	<0.02
Fe (mg L ⁻¹)	3.64	2.42	0.11	22.7	29.8	0.22	0.12	1.22	0.15
Mn (mg L ⁻¹)	0.14	1.27	1.45	0.39	2.41	1.77	0.053	0.23	0.22
Sb (mg L ⁻¹)	0.24	0.16	0.10	<0.05	<0.05	<0.05	0.2	<0.05	<0.05
As (mg L ⁻¹)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
B (mg L ⁻¹)	0.28	0.70	0.48	1.75	1.77	1.49	0.26	0.30	0.44
Cr (mg L ⁻¹)	0.28	0.077	<0.005	4.83	0.12	0.0058	<0.005	<0.005	<0.005
Zn (mg L ⁻¹)	13.4	4.2	0.40	4.65	1.19	<0.05	0.92	0.11	<0.05
Al (mg L ⁻¹)	1.6	0.81	<0.10	3.02	2.58	0.17	0.13	<0.1	<0.1
Ca (mg L ⁻¹)	2.12	93.6	294	1.34	319	397	0.86	63.6	286
Cd (mg L ⁻¹)	0.0078	0.003	<0.001	0.0084	<0.001	<0.001	<0.001	<0.001	<0.001
Co (mg L ⁻¹)	0.0059	0.066	0.048	0.071	0.11	0.0052	<0.005	0.032	0.033
Cu (mg L ⁻¹)	0.013	0.099	<0.01	<0.01	<0.05	<0.01	0.026	<0.01	0.011
Mg (mg L ⁻¹)	0.45	8.19	18.3	0.39	21.0	25.6	0.16	7.01	22.1
Mo (mg L ⁻¹)	0.0063	<0.05	<0.005	<0.005	<0.05	<0.005	0.011	0.014	0.0065
Ni (mg L ⁻¹)	0.40	0.22	0.034	2.21	0.58	0.044	0.32	0.10	0.084
Pb (mg L ⁻¹)	1.25	0.31	<0.02	3.06	<0.05	<0.02	<0.02	<0.02	<0.02
Hg (mg L ⁻¹)	<0.0005	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Se (mg L ⁻¹)	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05	<0.05
Ti (mg L ⁻¹)	0.023	0.021	<0.005	0.069	0.005	0.005	<0.005	0.0071	<0.005
PAHs (μg L ⁻¹)	188.26	45	0.68	173.1	9.5	5.5	2057	12	0.214
BTEX (μg L ⁻¹)	1357	745	<0.8	732	194	<0.4	604	5.32	1.40

^{a)} Raw UCG wastewater used in a bioremediation process with constructed wetlands (CW)<https://doi.org/10.1371/journal.pone.0300485.t004>

(Fig 2) and is only an estimate, however can be important in terms of research on effective wastewater treatment using constructed wetlands.

The most significant relationship is attributed to the COD/BOD ratio, which can be an indicator of the degradation of organic matter and is constant for a specific type of wastewater. It is

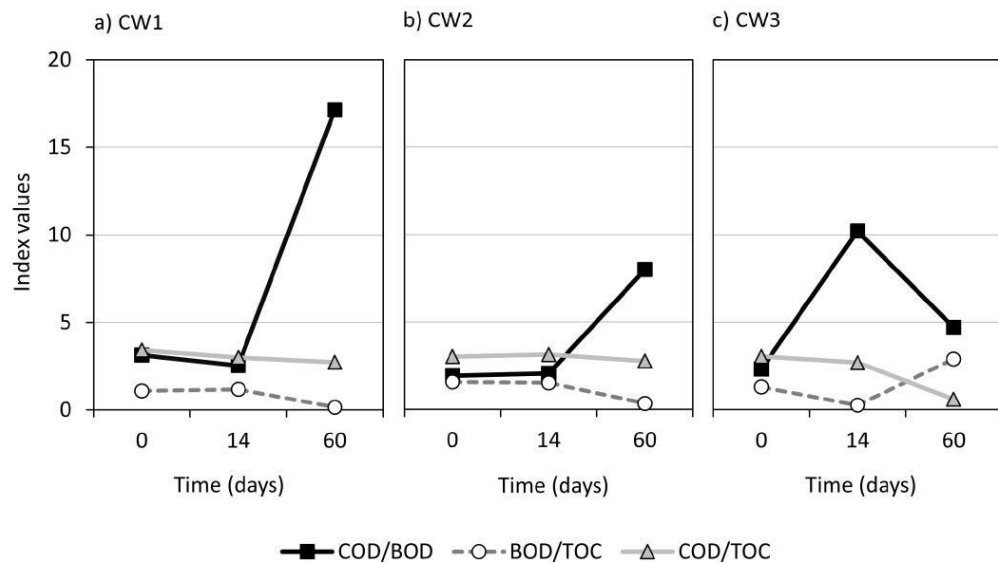


Fig 2. Change in the quotients of organic pollutant indicators in wastewater samples during bioremediation processes using CWs.

<https://doi.org/10.1371/journal.pone.0300485.g002>

assumed that organic pollutants present in wastewater are biodegradable if this ratio is in the range of 1.5 to 2.5 [26, 27]. Above the value of 2.5, the decomposition of pollutants is slow and/or the wastewater contains organic substances that are hardly or not biodegradable, which is common in industrial wastewater. Among the tested UCG wastewater, the highest COD/BOD ratio occur for the wastewaters 1 and 2 after 60-days and for the wastewater 3 after 14-days of bioremediation (17.1, 8.0 and 10.2, respectively). The BOD/TOC ratio in the tested samples was below the typical range for raw wastewater (1.4–2.1) [26] confirming the content of hardly degradable pollutants. The COD/TOC quotient showed little variation and ranged from 2.70 to 3.42. According to Lee and Nikraz [27], the characteristic range of 2.5/3.0–4.0 is adopted for raw domestic wastewater. The presence of Hg was not observed in any sample, which is probably related to its high volatility and high UCG temperature. Process temperature for the experiment No. 2 with pure oxygen ranged from 1200°C to 1500°C and was higher than for the tests with OEA (the highest temperatures for experiments No. 1 and 3 were up to 1200°C). Toxic metals (Cd, Pb, Cr) were detected in wastewater after both gasification experiments of “Piast-Ziemowit” coal. The concentrations of other metals and metalloids were relatively low for all wastewater. Differences in the determination limits obtained for some parameters (e.g. Hg and N-NO₂) result from the presence of interfering substances and the need for test sample dilution.

The characteristic feature of wastewater from industrial processes was the high content of BTEX and polyaromatic hydrocarbons (PAHs). The highest efficiency of CWs in removing pollutants was observed in the case of organic compounds (phenol index, PAHs, BTEX), especially in the case of water purification from the “Wesoła” coal gasification process. After bioremediation with CWs, the content of cyanides and metals (Fe, Cr, Zn, Ni, Pb and Ti) also decreased. M-Ridha et al. [28] used pilot scale horizontal subsurface flow constructed wetlands

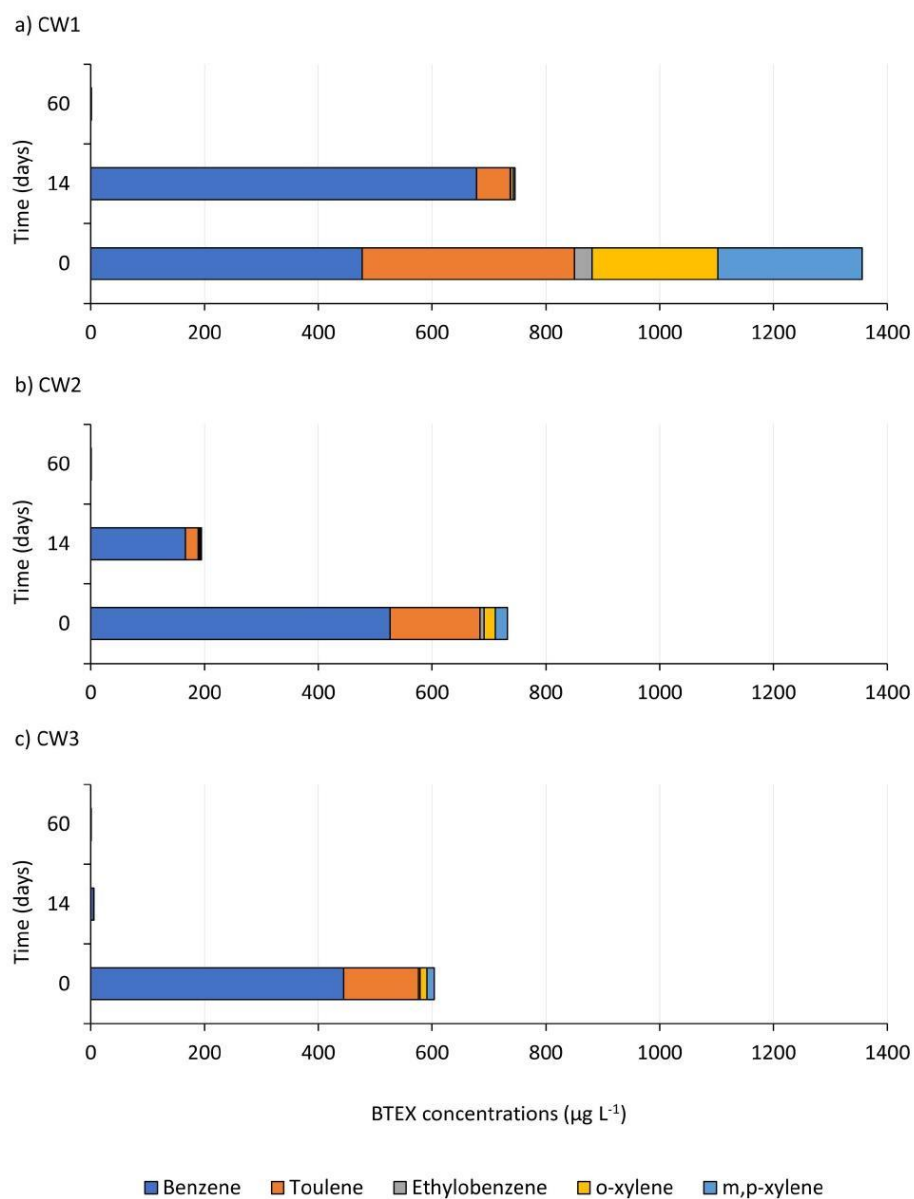


Fig 3. Changes in the individual BTEX compounds over the time of bioremediation.

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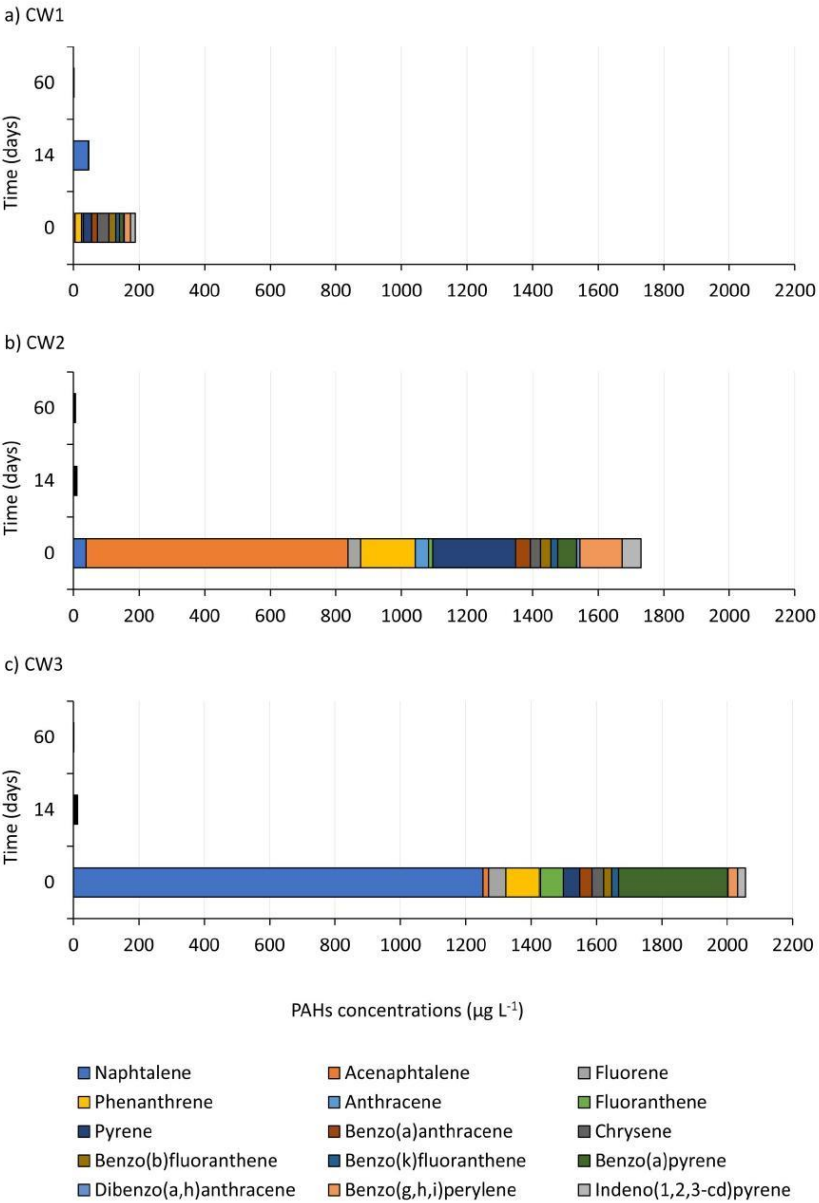


Fig 4. Changes in the individual PAHs compounds over the time of bioremediation.

<https://doi.org/10.1371/journal.pone.0300485.g004>

Table 5. The degree (η_{14} / η_{60}) of wastewater treatment from UCG processes after 14 and 60 days of bioremediation using CWs (mean values). A negative sign indicates a decrease in the content of the substance or measured parameter in question compared to the initial value measured at the beginning of the experiment. The parameters that were not detected in the tested samples and for which it was impossible to calculate the degree of wastewater treatment are marked with gray cells and the highest values above 80% are marked in bold.

Parameter	CW1		CW2		CW3	
	η_{14} (%)	η_{60} (%)	η_{14} (%)	η_{60} (%)	η_{14} (%)	η_{60} (%)
NH ₄	-22.2	-76.7	-31.3	-38.8	-31.6	-100.0
N-NO ₃	-9.1	>32,000	-16.7	-16.7	0.0	>57,000
N-NO ₂	31.7	>183,000	0.0	450.0	106.3	1212
Total nitrogen	-20.0	-44.7	-25.0	-31.7	-33.3	-58.0
BOD	-27.8	-98.1	-41.8	-98.4	-91.8	-83.5
COD	-41.6	-89.4	-37.7	-93.2	-64.0	-67.5
TOC	-33.3	-86.7	-40.0	-92.6	-59.2	-64.6
Chloride	-12.8	-6.4	-14.2	-3.6	-15.2	16.0
Sulfate	40.3	79.2	-25.7	-90.6	52.3	112.0
Nitrate	0.0	>31,000	-5.7	-5.7	0.0	>51,000
Nitrite	333.3	>583,000	0.0	500.0	115.7	1272
Total cyanide	-58.2	-99.0	-99.6	-100.0	-98.7	-98.6
Phenol index	-36.2	-100.0	-58.8	-100.0	-100.0	-100.0
Total phosphorus	100.0	141.7	120.0	-35.0	>3,000	776.9
Sulfide	15.1	0.0	78.4	92.0		
Fe	-33.5	-97.0	31.3	-99.0	916.7	25.0
Mn	807.1	935.7	517.9	353.8	334.0	315.1
Sb	-33.3	-58.3			-75.0	-75.0
As						
B	150.0	71.4	1.1	-14.9	15.4	69.2
Cr	-72.5	-98.2	-97.5	-99.9		
Zn	-68.7	-97.0	-74.4	-98.9	-88.0	-94.6
Al	-49.4	-93.8	-14.6	-94.4	-23.1	-23.1
Cd	-61.5	-87.2	-88.1	-88.1		
Co	>1,000	713.6	54.9	-92.7	540.0	560.0
Cu	661.5	-23.1	400.0	0.0	-61.5	-57.7
Mo	693.7	-20.6	900.0	0.0	27.3	-40.9
Ni	-45.0	-91.5	-73.8	-98.0	-68.8	-73.7
Pb	-75.2	-98.4	-98.4	-99.3		
Hg						
Se						
Ti	-8.7	-78.3	-92.8	-92.8	42.0	0.0
PAHs	-76.1	-99.6	-99.5	-99.7	-99.4	-100.0
BTEX	-45.1	-99.9	-73.5	-99.9	-99.1	-99.8

<https://doi.org/10.1371/journal.pone.0300485.t005>

In our study vertical flow constructed wetland to treat UCG wastewater was used to clean up the UCG post-processing wastewater. The research carried out in this article allowed to add another type of sewage to the above list. A high pollutant removal rate was obtained in the described wetland system. The removal percentage of organic compounds like BTEX, PAHs and phenol were 99% after 14 days of the treatment. A similar removal rate was indicated for cyanide and metals (Zn, Cr, Cd, Pb). The higher pollutant removal rate from our study were consistent to the results obtained by Saeed et al. [13] and Saeed & Khan [41]. While, Waly et al. [20] reported that the removal efficiency of PAHs in secondary treatment

of traditional wastewater treatment plants (WWTPs) was lower, and ranged between 45%–82%.

Considering the complex composition of pollutants and the presence of recalcitrant organics in UCG post-processing wastewater, the combination of different types of materials and compost used in CWs increased removal rates due to high activities of plant root-associated microbiota with the combination of physicochemical processes. Also, the appropriate structure and properly selected operation parameters of CWs system were essential for achieving satisfactory removal rates of organic and inorganic pollutants during the experiment. Obtained in the study results indicate the potential of CWs to treat polluted and poorly biodegradable UCG post-processing wastewater.

Evaluation of toxicity during the bioremediation process

The changes of toxicity as a function of bioremediation in wetland columns were determined over 150 days. The results are presented in Table 6. The toxicity indicator evaluated as Toxicity Unit (TU) was high for all three raw wastewater obtained from the UCG processes. The values of TU were 373, 405 and 732 for 1, 2 and 3 raw wastewater, respectively, and wastewater were classified to the fifth toxicity class (very high acute toxicity) according to classification proposed by Persoon et al. [25]. After 14 days of running the experiment, the toxicity of the wetland column effluents decreased significantly. The reduction of toxicity was 74% and 77% for raw wastewater 1 and 2, respectively, while, 99% reduction of toxicity was noted for raw wastewater 3. During the rest of the experiment, the toxicity of all wetland column effluents decreased, and at the end of the experiment the 1 and 3 effluent belonged to the first class of toxicity (no toxicity) with the TU values 0.9 and 0.7, respectively. After the end of the experiment, the effluent 2 belonged to the second class of toxicity (low toxicity) with the TU value of 1.1. Our results showed the toxicity of the wastewater samples ranged from very high acute toxicity (raw wastewater) to non-toxic (effluents from wetland columns) during the bioremediation process. The observed decrease was due to the conversion of the toxic compounds to less or non-toxic intermediates and by-products during experiment time. The obtained results correlated with some organic compounds such PAHs, BTEX, phenols and inorganic compound such as ammonia and cyanides (Fig 5). The mechanisms involved in pollutant removal in CWs is classified into biotic processes such as biodegradation, biofilm, root and plant uptake and physicochemical processes. Nevertheless, CWs are highly complex systems usually consisting of several layers such as compost, sand, gravel, soil that represent specific

Table 6. Changes of effluents toxicity during the bioremediation experiment (mean $\pm$ SD, n = 3).

Time (days)	0	14	30	45	60	75	150
CW1							
EC50	0.00	1.02 $\pm$ 0.015	0.83 $\pm$ 0.028	27.98 $\pm$ 3.51	36.91 $\pm$ 2.60	33.60 $\pm$ 1.25	86.00 $\pm$ 2.12
TU	373.6 $\pm$ 22.5	98.39 $\pm$ 1.56	79.1 $\pm$ 0.78	3.572 $\pm$ 0.37	2.7 $\pm$ 0.21	2.97 $\pm$ 0.09	1.1 $\pm$ 0.21
Toxicity class	V	IV	IV	III	III	III	II
CW2							
EC50	0.00	1.09 $\pm$ 0.01	0.94 $\pm$ 0.01	5.82 $\pm$ 0.51	47.72 $\pm$ 4.5	86.76 $\pm$ 2.11	104 $\pm$ 3.21
TU	405.4 $\pm$ 8.76	91.74 $\pm$ 0.56	89.7 $\pm$ 0.28	17.19 $\pm$ 1.73	2.09 $\pm$ 0.22	1.15 $\pm$ 0.25	0.9 $\pm$ 0.03
Toxicity class	V	IV	IV	IV	III	II	I
CW3							
EC50	0.00	30.96 $\pm$ 0.45	86.76 $\pm$ 9.4	93 $\pm$ 5.65	109 $\pm$ 5.65	106 $\pm$ 0.00	112 $\pm$ 2.12
TU	732 $\pm$ 15.67	3.2 $\pm$ 0.05	1.15 $\pm$ 0.15	1.28 $\pm$ 0.07	0.8 $\pm$ 0.07	1.0 $\pm$ 0.00	0.7 $\pm$ 0.07
Toxicity class	V	III	II	II	I	I	I

<https://doi.org/10.1371/journal.pone.0300485.t006>

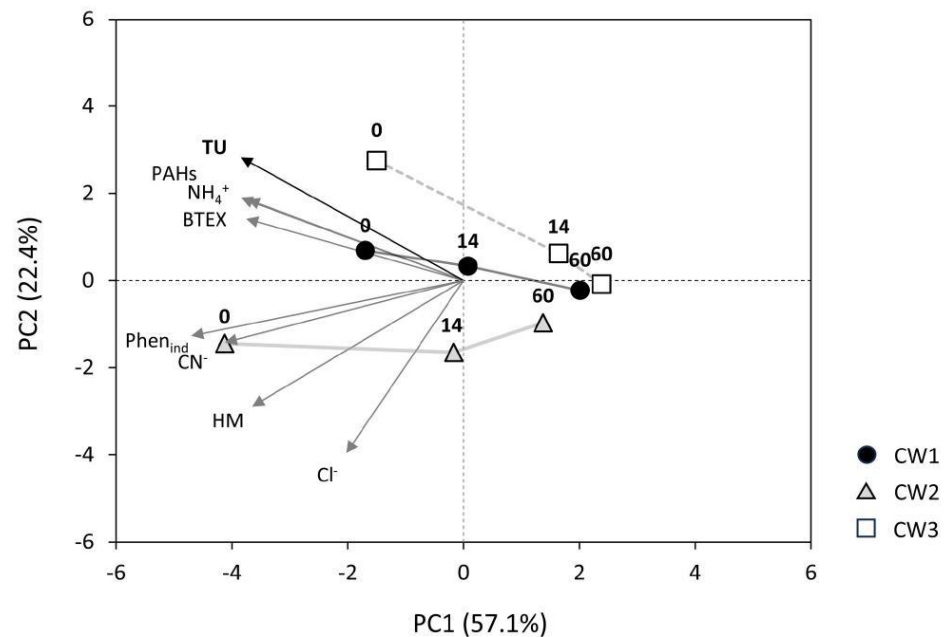


Fig 5. Dependence between selected physicochemical parameters and toxicity over bioremediation time evaluated by PCA analysis. Numbers above icons indicate day of measurement. Abbreviations: BTEX- volatile aromatic hydrocarbons, CN⁻-cyanides, HM- heavy metals including metalloids (As and Sb), PAHs- polycyclic aromatic hydrocarbons, Phen_{ind}- phenol index, TU—toxicity unit.

<https://doi.org/10.1371/journal.pone.0300485.g005>

environments and microenvironments with different physicochemical and microbiological conditions involved in the removal of pollutants.

The evaluation of the remediation effect of natural and constructed wetland systems has been monitored by using only physicochemical parameters. However, several authors have emphasized the importance of including bioassays to monitor the effect of wetland remediation technologies and they introduced different toxicity tests to their research [42–47]. The Microtox test was found to be one of the most sensitive and to have a better ability to evaluate the toxicity of treated and nontreated wastewater samples [44]. In our study, the Microtox test has proven to be a useful complement to chemical analysis in terms of evaluating the course of wetland remediation technologies. As recommended by scientists, the ecotoxicity bioassays could be used as supplementary tools for monitoring the effectiveness of wetland remediation technologies.

CWs as nature-based processes remove pollutants from the water, avoiding the use of chemical products and the input of high amounts of external energy [33, 48, 49]. One of the positive characteristics of CWs is their work under controlled conditions such as the well-defined composition of media, plant types, flow patterns, controlled hydraulic pathways and retention time. Additional advantages of CWs include site selection, flexibility in sizing, and low operational and maintenance costs [32]. In the constructed wetlands the removal of pollutants is carried out by different physical, chemical, and biological processes like sedimentation,

filtration, precipitation, ion exchange, adsorption, microbial processes for instance, nitrification, denitrification, sulphate reduction, carbon metabolization, and plant uptake. The activities of the processes are dependent on the interaction between plants, microorganisms, wastewater characteristics, media used and operation conditions [33, 50]. Traditionally CWs have been used for urban wastewater, stormwater runoff and domestic wastewater treatment, but in the last two decades, the applications for industrial wastewater purification increased due to the development of the technology and the extended research in this field.

Conclusions

The vertical flow constructed wetlands with high activity of plant root-associated microbiota and a combination of physicochemical processes turned out to be a very good technology to clean up the post-processing wastewater from the underground gasification process. Also, the appropriate design and operating parameters of the CW system were essential for achieving a satisfactory pollutants removal rate during the experiment. Such results indicate the potential of CWs for the treatment of highly contaminated and poorly biodegradable industrial wastewater, such as UCG wastewater or coking wastewater. The conducted study allows to assume that despite the significant content of impurities, the UCG wastewater is suitable for biological treatment. The present findings show that wetland remediation technology can be used to clean-up the heavily contaminated waters from the UCG process. In environmental monitoring, in order to effectively assess remediation technologies, it is desirable to consider combining the use of traditional physicochemical parameters with ecotoxicity tests. To assess the toxicity of treated waters biological tests can be carried out together with physicochemical analyzes to ensure environmental safety and minimize ecotoxicological issues. Wetland technology has the potential to turn coal gasification wastewater into usable recycled water.

To understand the removal of pollutants by CWs, further research is needed, including balancing the process and examining the role of plants-microbes interactions in wastewater treatment.

Supporting information

S1 Checklist. Author formatting checklist.
(DOCX)

S1 Table. Methods used for the physicochemical analysis of UCG wastewater samples.
(DOCX)

Author Contributions

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Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych

Searching of phenol-degrading bacteria in raw wastewater from underground coal gasification process as suitable candidates in bioaugmentation approach.

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Searching of Phenol-Degrading Bacteria in Raw Wastewater from Underground Coal Gasification Process as Suitable Candidates in Bioaugmentation Approach

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ABSTRACT

The aim of the conducted study was to isolate, identify and characterize suitable bacterial strains from UCG wastewater as potential candidates for the bioaugmentation approach. For this purpose, the straightforward cultivation procedure and unique biochemical selection were employed to gain insights into the specific properties of bacteria. From the 100 strains isolated from UCG wastewater, three (*Paenibacillus pasadensis* SAFN-007, *Paenibacillus humicus* Au34, and *Staphylococcus warneri* DK131) demonstrated the capacity to degrade phenol and specific biochemical properties. Phenol degradation reached more than 90% for the above-mentioned strains, while the average phenol removal rate for other selected strains was 82.9%, ranging from 66.1% to 90%. The bacterial strains belong to multi-enzyme producers and constitute a possible source of potential technologically important enzymes. Phenotypic microarray plates were used to characterise the metabolic properties of the strains. It was found that 74%, 67.4% and 94.2% of the carbon metabolites tested were utilised by *Paenibacillus pasadensis* SAFN-007, *Paenibacillus humicus* Au34 and *Staphylococcus warneri* DK131, respectively. Among C sources, the strains have the capability to metabolize some substrates appearing in phenol pathways, such as: N-acetyl-D-glucosamine, succinic acid, α -hydroxy-glutaric acid- γ -lactone, bromosuccinic acid, mono-methyl succinate, methyl-pyruvate, p-hydroxy-phenyl acetic acid, m-hydroxyphenylacetic acid, L-galactonic acid- γ -lactone, D-galactonic acid- γ -lactone, phenylethylamine. Bacteria show different levels of tolerance to pH and osmolality, and they can thrive in different habitats. Another characteristic of these strains is their high resistance to many antibiotics (multi-resistant bacteria). These properties allow the use of the isolated bacterial strains as good candidates for bioremediation of phenol-contaminated environments. The wastewater from the underground coal gasification process is an example of a good extreme environment for the isolation of unique bacteria with specific metabolic properties.

Keywords: post-processing wastewater, underground coal gasification (UCG) process, biodegradation, phenol, *Paenibacillus* sp., *Staphylococcus warneri*, phenotypic microarrays.

INTRODUCTION

One of the key sources of environmental pollution is industrial wastewater. The discharge of industrial wastewater containing the complex of various types of inorganic and organic

pollutants into various water sources disrupts ecological sustainability and poses a risk to human health (Riggio et al., 2018; Vymazal, 2021). The wastewater from the underground coal gasification process (UCG process) and from the coke industry are examples of heavily

contaminated sources of environmental pollution. UCG is considered to be a clean green technology in which gas is obtained directly from a coal deposit in situ, without the need for coal extraction. Numerous organic and inorganic contaminants are formed during different process stages and after process termination. Then, the UCG process carries some environmental risks, mainly related to the raw post-processing wastewater. Underground gasification produces a higher total amount of polluted wastewater (Smolinski et al., 2012). The main contaminants in UCG wastewater are organic compounds, such as: BTEX, benzene, PAHs, phenols and inorganic substances, such as: heavy metals, ammonia and cyanides (Kapusta et al., 2011; Borgulat et al. 2022). The average values of some parameters in UCG wastewater, such as COD, BOD, TOC and phenol index were noted as very high, e.g.: $850 \text{ mgO}_2 \cdot \text{L}^{-1}$, $360 \text{ mgO}_2 \cdot \text{L}^{-1}$, $270 \text{ mgC} \cdot \text{L}^{-1}$, and $100 \text{ mg} \cdot \text{L}^{-1}$, respectively. In the case of BTEX, the concentration ranged from 600 to $1360 \mu\text{g} \cdot \text{L}^{-1}$ and benzene was dominant. The average concentration of PAHs was $1300 \mu\text{g} \cdot \text{L}^{-1}$ (from 190 to $2060 \mu\text{g} \cdot \text{L}^{-1}$) (Wiatowski et al., 2023). UCG wastewater, despite its negative impact on the environment, can be considered as a source for the isolation of microorganisms with specific and useful catabolic properties.

The bioaugmentation strategy in environmental remediation technologies is one of the routes to apply suitable microbes. Bioaugmentation is known as the introduction of microbial strains or microbial consortia with new metabolic properties into the polluted environment. It is a promising but controversial approach used mainly for degrading recalcitrant compounds (Thompson et al., 2005). The basic idea of bioremediation is the multiplication of catabolically relevant microbes that are responsible for accelerating the efficiency of remediation processes. However, the success of bioaugmentation methods depends on the identification and isolation of suitable microbial strains as well as their subsequent survival and activity in the foreign environment. Thompson et al. (2005) discussed the bioaugmentation as one of the remediation strategies used in situ. Future prospects and ways to improve the selection process for isolating effective microbial strains for bioaugmentation were also discussed in this paper. The authors concluded that the initial selection of strains was based on a single key criterion, e.g. degradability, while the potential ability of the strains to replicate and be active at the sites of use was not

considered. Then, the challenge of selecting the process of strains for bioaugmentation and the recent technical advances used in the selection methods of strains were discussed and summarized.

The bacteria considered for bioaugmentation should be able to degrade the respective pollutant even in the presence of potentially inhibitory pollutants, survive and compete in the environment after their inoculation and be compatible with autochthonous communities (Singer et al. 2005; Herrero & Stuckey, 2015). Bioaugmentation has been successfully used in the remediation of sites contaminated with aromatic compounds (Adams et al., 2015; Villaverde et al., 2019). The specific bioaugmentation research was carried out in different countries as described by Adams et al. (2015). The aim of the conducted study was to isolate, identify and characterize the selected bacterial strains from UCG wastewater as potential candidates for bioaugmentation approach in phenol-contaminated environments. For this purpose, the straightforward cultivation procedure and unique biochemical selection were employed to gain insights into the specific properties of bacteria. The isolates can be considered as a “tool” in the bioaugmentation approach for the remediation of phenol-polluted environments.

MATERIALS AND METHODS

Sampling of raw post-processing wastewater

The post-process wastewater samples were generated at the Central Mining Institute as a result of coal gasification experiments described by Wiatowski et al. (2023). The total amount of raw wastewater samples was collected in a plastic tank with a capacity of 1 m^3 (Mauser type) under sterile conditions. After mixing and decanting into smaller sterile containers, the raw wastewater samples were taken to the laboratory and used for microbiological analysis.

Isolation of bacteria from UCG post-processing water

Plating and serial dilution methods were used to isolate the culturable and aerobic bacteria. Aerobic bacteria were incubated on an SMA medium (Standard Methods Agar, BioMérieux) containing $100 \text{ mg cycloheximide/L}$ at 30°C for 24–48 h. To obtain pure bacterial cultures by separating the

individual cells from each other, the individual bacterial colonies were transferred to Luria-Bertani solid medium with a pH of 7.0 containing 20.0 g·L⁻¹ agar, 10.0 g·L⁻¹ g·L asen peptone, 5.0 g·L⁻¹ yeast extract and 5.0 g L⁻¹ NaCl, using the strip plate technique. Then, the single bacterial isolates were transferred into a liquid LB medium, and the liquid cultures were incubated with constant shaking (100 rpm) at 30°C for 24 - 48 h. The strains are deposited at -80°C in a Luria-Bertani medium supplemented with 20% (v/v) glycerol in the Institute for Ecology of Industrial Areas (IETU) collection.

Identification of isolated bacterial strains

The genomic DNA from 100 bacteria was isolated with a Roche kit, according to the manufacturer's instructions. DNA concentration, yield and purity were assessed using absorbance ($A_{260/280}$, $A_{260/280}$), optical density and agarose gel electrophoresis. DNAs are stored at -20°C at IETU. Then, the DNA samples were sent to Genomed SA (Poland) for sequencing. The isolates were identified by 16S rRNA gene sequencing. The universal bacteria primers, 8F and 1492R were used to amplify region V3-V4 of 16S rRNA genes in accordance with the conditions established by Genomed SA (Poland). For each sequencing reaction, 3 µl of BigDye™ Terminator v 3.1 Ready Reaction Mix, 1 µl of BigDye™ Terminator v1.1 & v 3.1 5× sequencing buffer, 5 pmol of appropriate primer and 50–250 ng of DNA template were mixed in a final volume of 10 µl. Cycle sequencing was performed in 100 µl PCR tubes. Incubation at 96°C for 1 minute as the first denaturation step was followed by 25 cycles of 96°C for 10 seconds, 54°C for 5 seconds and 60°C for 4 minutes incubation. Prior to purification, the reaction mix was incubated at 4°C. The purified reaction products were separated by electrophoresis on the 3730XL DNA analyzer according to the producer (ThermoFisher) instructions. The sequences were compared and analyzed in NCBI's BLAST

program. Taxonomic identification was done by comparison with the National Centre for Biotechnology Information (NCBI) database using the BLASTN algorithm.

The 16S rRNA sequences of the strains, characterized by the greatest biodegradation of phenol, were deposited in the NCBI GenBank database with accession numbers (Table 1). The PhyML software (Approximate Likelihood-Ratio Test: SH-like) available at *phylogeny.fr* website was used to generate the phylogenetic tree (Dereeper et al., 2008). The Gblocks program was used to eliminate poorly aligned positions and divergent regions.

Biochemical characterization of bacteria

The following properties of bacterial strains were evaluated: phenol biodegradation on a laboratory scale, biosurfactant production, enzyme activities, and biochemical and metabolic properties by phenotypic microarrays (PMs plates, Biolog).

Phenol biodegradation

To evaluate phenol biodegradation, bacterial strains were grown aerobically in 100 mL liquid LB medium supplemented with various concentrations of phenol at 30°C with constant shaking (100 rpm). During a 10-day acclimatization period, phenol was continuously added to each culture every 48 hours. The degradation of phenol was measured after 24 hours in the liquid cultures to which a phenol concentration of 6.0 mM was added as the initial concentration. The amount of phenol in the culture was measured colorimetrically with p-nitroaniline.

Biosurfactant production

Bacterial cultures were grown in LB medium on an orbital shaker (100 rpm, 30°C, 24 hours) and then diluted with LB medium to achieve OD= 0.8 ($\lambda= 600$ nm). After 72 hours, the cultures were

Table 1. Phylogenetic affiliations of selected bacteria

Accession number	NCBI affiliation	Similarity (%)	Phylum, class, order, family, genus
OQ996958	<i>Paenibacillus humicus</i> Au34 (MW534854.1)	99.6	Firmicutes, Bacilli, Paenibacillales, Paenibacillaceae, Paenibacillus
OQ996960	<i>Paenibacillus pasadenensis</i> SAFN-007 (NR_042757.1)	100.0	Firmicutes, Bacilli, Paenibacillales, Paenibacillaceae, Paenibacillus
OQ997235	<i>Staphylococcus warneri</i> DK131 (MT642942.1)	100.0	Firmicutes, Bacilli, Staphylococcales, Staphylococcaceae, Staphylococcus

centrifuged (12,300 RCF, 10 min). The resulting supernatants were used to evaluate the production of biosurfactants. Indirect methods, e.g. blood agar, oil-spreading test and drop-collapse method as described by Plaza (2014) were used. The surface tension assessment was used as a direct method to evaluate biosurfactant production. The supernatant samples from bacterial cultures were measured for ST using a Du Nöuy ring with a tensiometer SIGMA T702 (Attension, Finland) according to the supplier's instruction.

Measurements of each sample were taken three times at room temperature. Clean water was used to calibrate the device and as a reference measurement. The average of the measurements was given as the final SF result. The average values of surface tension were 72.01 ± 0.23 and 68.34 ± 0.86 for water and LB medium, respectively. An average standard deviation was calculated.

Enzymatic activities

First, the isolated bacterial strains were cultured on LB liquid medium ($t = 72$ h, $T = 30^\circ\text{C}$, 90 rpm). The bacterial suspension was then applied to the previously prepared differentiation media. After incubation (incubation time varied depending on the medium used, $T = 30^\circ\text{C}$), the ability of the selected strains to produce a specific group of enzymes was assessed (Table 2).

Phenotypic characterization by biolog phenotype microarrays (PMs)

An appropriate amount of liquid culture of the test strain (LB medium, $T = 30^\circ\text{C}$, $t = 48$ h, 90 rpm) was added to the Biolog IF-0 a solution (5:1) diluted with water so that the final optical density (OD) of the obtained suspension of microorganisms was 42% (the turbidity

of the suspension was determined with a turbidimeter). Subsequently, the previously prepared solutions according to the BIOLOG™ method (Biolog Inc., Hayward, USA) were added to the suspension so that a transmittance of 85% was obtained. The detailed methodology of the prepared solutions is described, among others, in the paper by Jałowiecki et al. (2020). Subsequently, the solutions together with the suspension of microorganisms were applied to PM plates using a multichannel pipette, 100 μl per well. The plates were incubated for 7 days in an OmniLog incubator at the same temperature as the culture (30°C). The growth of the microorganisms was read automatically every 30 minutes. Plates PM1-5 and PM9-PM13 were used in this study. The results are presented in the form of a circular chart (Fig. 2). The different parts of the diagram were marked according to the types of compounds present on the plates: (PM1-PM2) carbon sources, nitrogen sources (PM3), phosphorus and sulfur sources (PM4), nitrogen sources (PM5). The PM plates from 9 to 13 including tolerance/sensitivity to different osmolytes (PM9), pH conditions (PM10) and other chemicals i.e. antibiotics and chemicals (PM11-13) were studied.

RESULTS AND DISCUSSION

Around 100 bacterial strains were isolated and 100 DNA samples were sequenced. However, only 50% of the isolates were identified with a probability of 95–100%. The rest of the isolates were not identified because of the quality of the DNA samples or because they did not exist in the NCBI database. Among the identified bacteria, *Paenibacillus* and *Bacillus* species were dominant in UCG post-processing waters.

Table 2. Substrates used to assess enzymatic activity

Medium, incubation time, temperature	Enzymes activity	A positive result indicating the ability to produce the quoted enzymes
Urea Agar Base (UAB), 48 hrs., 30°C	Urease (ability to hydrolyse urea)	Change medium colour from pale yellow to violet
Nutrient agar with starch (1%), 48 hrs., 30°C	Amylase	A clear halo appeared around the colonies after the flooding the plate with 1% Lugol's liquid
Milk agar (SMA), 48 hrs., 30°C	Protease	Clear zones around the bacterial strains confirmed the proteolytic activity of the strains
Carboxymethyl cellulose agar (CMC), 7 days, 30°C	Celulase	Yellow color around the colonies after the flooding the plate with 1% Lugol's liquid
MHI medium (Li et al. (2011), 3 to 5 days, 30°C	Inulinase	A clear halo appeared around the colonies after the flooding the plate with 1% Lugol's liquid

The most numerous bacterial strains were selected for the biodegradation of phenol, e.g.: *Paenibacillus glucanolyticus* P69, *Bacillus altitudinis* VMFR48, *Bacillus nealsoni* LE3, *Bacillus pseudomycoloides* S2015-2C, *Bacillus sp.* CMAA 1185, *Bacillus stratosphericus* TR4, *Margalitia shackletoni* LMG-18435, *Oceanobacillus picturae* B5, *Paenibacillus cineris* BB, *Paenibacillus favisporus* U3, *Paenibacillus humicus* Au34, *Paenibacillus humicus* Sp29, *Paenibacillus pasadenensis* SAFN-007, *Paenibacillus cineris* cu1-7, *Paenibacillus cellulositrophicus* KACC 16577 and *Staphylococcus warneri* DK131. Figure 1 shows the phylogenetic tree of the above strains, which was constructed on the basis of 16S rRNA sequences.

On the basis of their ability to survive the increasing concentration of phenol, *Pseudomonas humicus* Au34, *Paenibacillus pasadenensis* SAFN-007 and *Staphylococcus warneri* DK131 were selected for further study. The selected strains were also characterized by the highest phenol degradation rate, amounting to more than 94% (Table 3). The average phenol degradation rate of the other selected strains was 82.9% and ranged from 66.1% to 90%. Many researchers examined and isolated phenol-degrading microorganisms. Microbes, such as *Pseudomonas sp.*, *Alcaligenes sp.*, *Ewingella americana*, *Bacillus sp.*, *Acinetobacter sp.*, *Streptomyces sp.*, *Achromobacter sp.*,

Coriarius versicolor, *Ralstonia sp.*, *Fusarium sp.*, *Phanerocheate chrysosporium*, having the ability to decompose various aromatic compounds including phenol, were used in several remediation technologies (Anhu et al., 2017; Tian et al., 2017; Alshabib & Onaizi, 2019; Filipowicz & Cieślinski, 2020; Shebl et al., 2022; Bibi et al., 2023). Screening bacteria with degradation capabilities is of great importance in the clean-up of contaminated environments using the bioaugmentation strategy. The properties and important role of bacteria of the genus *Paenibacillus* in sustainable farming and biotechnological processes in industry were described by Grade et al. (2016). The selected strains also belong to the multi-enzyme producers and represent a potential source of technologically important enzymes; for example, in the enzymatic pre-treatment of organic recalcitrant waste (biomasses), wastewater prior to its anaerobic/aerobic digestion or in the biodegradation of organic pollutants such as antibiotics, dyes and petroleum hydrocarbons. The enzymatic activity of the selected strains is listed in Table 3.

Enzymes are a crucial pillar of industrial biotechnology and can be used in a wide range of bioindustrial sectors, for example in the production of biofuels, washing detergents, food and feed production and in the preparation of bio-based chemicals, enhanced biological treatment

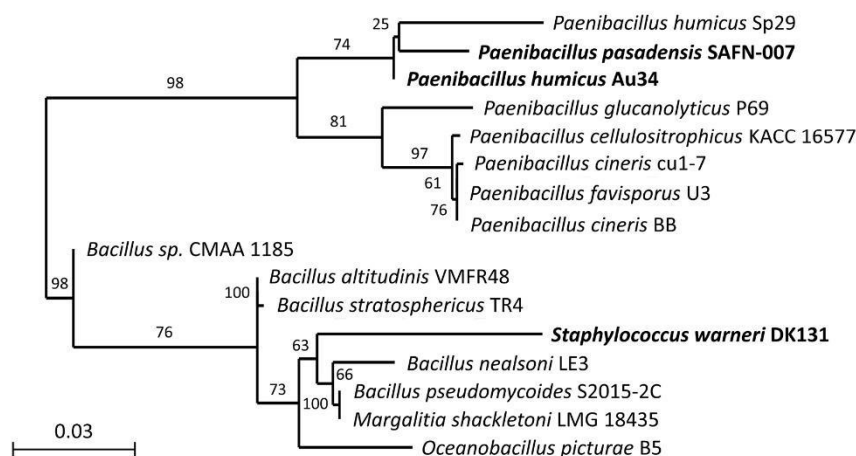


Figure 1. Maximum likelihood tree based on the 16S rRNA gene. The diagram shows, among other things, the relationships between the two selected *Paenibacillus* strains (*Paenibacillus pasadenensis* SAFN-007 & *Paenibacillus humicus* Au34) and *Staphylococcus warneri* DK131 – strains isolated from UCG post-processing waters and characterised by the highest phenol biodegradation. The representation of the branches is the result of neighbour-joining (support values are given in %, number of bootstraps 500)

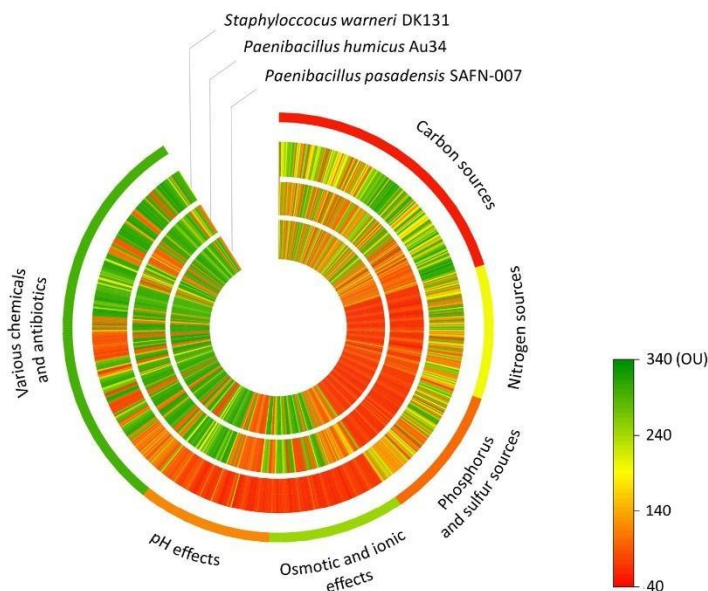
Table 3. Parameters indicating phenol biodegradation degree, biosurfactants production and enzymes activities

Measurements		<i>Paenibacillus humicus</i> Au34	<i>Paenibacillus pasadenensis</i> SAFN-007	<i>Staphylococcus warneri</i> DK131
Biosurfactants production	Surface tension (mN/m) ^a	52.14 ± 0.60	55.01 ± 0.59	52.25 ± 0.76
	Blood agar	-	-	-
	Oil spreading test	-	-	-
	Emulsification index (%)	0.0	0.0	0.0
Enzymatic activity ^b	Ureases	-	-	-
	Proteases	+	+	+
	Cellulases	+	+	+
	Inulinases	+	+	+
	Amylases	-	+	-
Phenol biodegradation Removal rate (%) ^c		93.3 ± 5.1	94.8 ± 6.2	92.8 ± 4.8

Note: ^a Control measurement (H₂O) – 56.73 mN/m, ^b Clearing zones: (–) lack of clearing zone, (+) clearing zone occurred, ^c Initial phenol concentration (C₀) in culture – 6.0 mM·L⁻¹

of biomass. The broad variability of enzyme applications is presented in 28 technological processes saving raw material energy and/or chemicals obtained by the implementation of enzymatic processing (Jałowiecki et al., 2017; Cabrera Blamey, 2018; Bhandari et al., 2021; Mesbah, 2022). Many authors support the hypothesis that enzyme technology is a promising means of moving toward cleaner industrial production and

offers great potential for bioeconomy development. Industries are looking for new microbial strains in order to produce different enzymes to fulfil the current requirements of bio-based industry. The global enzyme market was valued at \$5.8 billion in 2021 and is projected to reach \$10.2 billion by 2031, growing by 6% from 2022 to 2031 (Mesbah, 2022). The discovery of new microbial enzymes through extensive and persistent

**Figure 2.** Changes in phenotypic diversity of investigated bacterial strains evaluated by PM plates

screening is an open and simple route for biosynthetic processes and, consequently, new ways of solving environmental problems are developed. The need for discovering new isolates with better enzymatic properties is now growing.

Biolog microplates were used to characterize the phenotypic properties of three strains that were the potential candidates for the bioaugmentation strategy. Figure 2 shows the metabolic profiles of the three strains using phenotype microarray plates. The results obtained showed that C-sources were the most used substrates. The following percentages of the carbon metabolites (PM1 and PM2): 74%, 67.4% and 94.2% were utilized by *Paenibacillus pasadenensis* SAFN-007, *Paenibacillus humicus* Au34, and *Staphylococcus warneri* DK131, respectively. Among C sources, the strains have the capability to metabolize some substrates appearing in phenol pathways like N-acetyl-D-glucosamine, succinic acid, D-galactonic acid- γ -lactone,

L-galactonic, acid- γ -lactone, α -hydroxy-glutaric, acid- γ -lactone, bromosuccinic acid, mono-methyl succinate, methyl-pyruvate, phenylethylamine m-hydroxy phenyl, acetic acid. *Staphylococcus warneri* DK131 metabolized 92% of 190 substrates belonging to nitrogen sources and phosphorus/sulfur sources. These substrates were metabolized with very low percentages, ranging from 2% to 4% by *Paenibacillus pasadenensis* SAFN-007 and *Paenibacillus humicus* Au34. Bacteria have the ability to live in different habitats as well as show varying tolerance to the pH and osmolality of the environment. Therefore, the influence of pH and different osmolytes on the aerobic growth of bacteria was also assessed using 9-10 PM plates (data not presented). The strains tested grew in a wide pH range (4.5–9.5), especially in the presence of various amino acids. Growing *Paenibacillus pasadenensis* SAFN-007 and *Paenibacillus humicus* Au34 cells were shown to cause progressive changes in pH

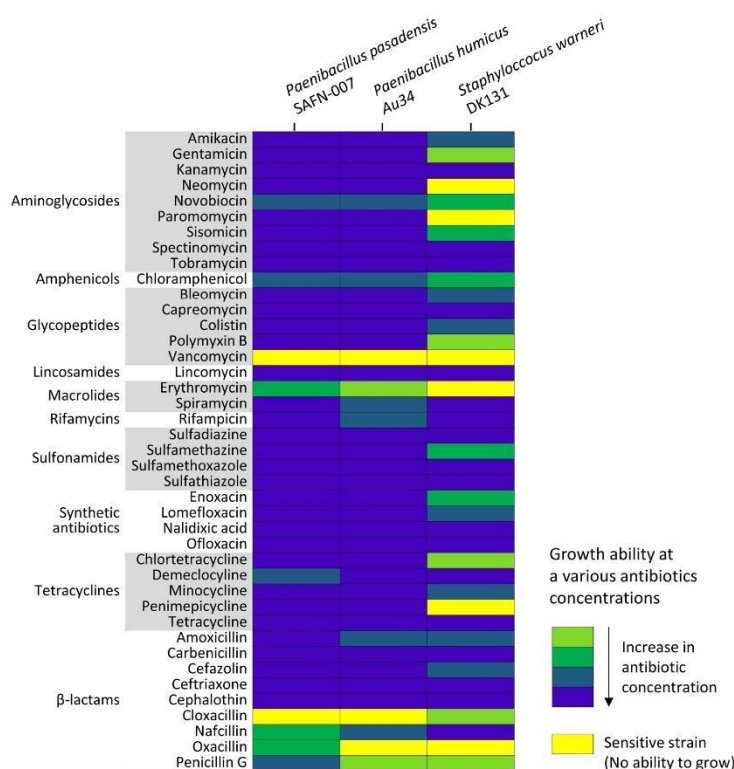


Figure 3. Heat map of antibiotic resistance of *Paenibacillus pasadenensis* SAFN-007, *Paenibacillus humicus* Au34 and *Staphylococcus warneri* DK131 evaluated by microarrays plates (PM9-PM11)

variations as well as different osmolytes and their level. The behavior of both strains under the tested conditions was similar. Their growth curves are composed of the following phases: the lag phase, the exponential phase and the stationary phase. In the case of *Staphylococcus warneri* DK131, the growth curve showed a quite different pattern compared to *Paenibacillus* species. The growth curve was linear, at a constant level under varying conditions. The pH and osmolytes did not change the bacterial growth and the values of OmniLog units (OU) were in a similar range. These data could be useful for the selection and optimization of bacteria in many biotechnological applications such as the production of chemicals and in bioremediation technologies that predict the growth of bacteria in different ecological niches.

Using the PM11-PM13 plates, the three bacterial strains were tested for their sensitivity to 41 different antibiotics in four different concentrations (Figure 3). The producer of the PM plates (Biolog Inc.) does not specify the concentrations of the substances in its plates. The magnitude of the concentration increases in the four PM wells is also not known for the individual substances. The lack of information on the working range of the antibiotic concentrations makes it difficult to properly assess the sensitivity/resistance of the bacteria. The tested strains showed growth in the presence of examined antibiotics. In the case of *Paenibacillus* sp., both strains were resistant to most antibiotics belonging to various chemical classes in four concentrations. They showed full growth in all four wells for 30 from 41 antibiotics (73%) (Figure 3). In the case of vancomycin, erythromycin, cloxacillin, nafcillin, oxacillin and penicillin G no growth was observed. *Staphylococcus warneri* DK131 showed greater fluctuation in antibiotic resistance. It was sensitive to medium and high concentrations of antibiotics, showing tolerance to their lowest concentrations. Its growth was observed in all four concentrations of 18 antibiotics and amounted to 44%. The strains tested could be called multidrug-resistant bacteria, because they are resistant to several antibiotics from different chemical classes. The PM approach has been used previously as an additional tool to show variations in the antibiotic susceptibility of environmental bacteria (Jałowiecki et al., 2017). The literature discusses the use of microarrays as a tool to assess mutant phenotypes or to study phenotypic changes under different environmental stressors (Rodrigues et al., 2011; Greetham, 2014;

Blumenstein et al., 2015; Dunkley et al., 2019). Moreover, the PM technology was used to identify the microorganisms and perform their characterization with selectable traits (Orro et al., 2015).

CONCLUSIONS

From the 100 strains isolated from UCG wastewater, three (*Paenibacillus pasadenensis* SAFN-007, *Paenibacillus humicus* Au34, and *Staphylococcus warneri* DK131) demonstrated their capacity to degrade phenol. Their properties were evaluated by traditional microbiological methods and phenotype microarray plates. The obtained results show that *Paenibacillus pasadenensis* SAFN-007, *Paenibacillus humicus* Au34, and *Staphylococcus warneri* DK131 can be used as suitable candidates in industrial and environmental biotechnology. UCG wastewater and other similar extreme environments are good sources for the isolation of specific microbes. The data obtained could be useful for the selection of bacteria in biotechnological applications as well as in bioremediation technologies by predicting the growth of bacteria in different ecological niches.

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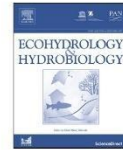
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A look at biofilm on the coal waste-derived adsorbent enhanced wetland system

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ABSTRACT

This paper addresses an integrated constructed wetland system designed to treat raw wastewater from the process of underground coal gasification. To enhance the performance of the vertical flow constructed wetland technology, UCG-derived char was integrated as an adsorbent. The main objective of the study was to characterize the biofilm structure from UCG-derived adsorbent. This is the preliminary research to characterize the biofilm layer of waste adsorbent used in the wetland column. Findings indicated that the treatment system was able to improve post-processing wastewater chemical composition. During the 45-days experiment wetland with the adsorbent achieved over 90 % efficiency in removing pollutants from treated UCG post-process water. This study demonstrated that UCG-derived adsorbent can be used as a carrier to effectively trap microbes in contaminated environmental systems, facilitating the assessment and study of hydrocarbon-degrading microorganisms. The biofilm on UCG adsorbent was dominated by microbial taxa from *Proteobacteria* and *Firmicutes*. Community analysis of UCG char-associated microorganisms revealed taxa related to hydrocarbon-degrading treatments, including *Pseudomonas* and *Bacillus*, which may facilitate hydrocarbon degradation and bio-surfactant production.

1. Introduction

Wastewater from the underground coal gasification (UCG) is an example of a source of potentially hazardous, harmful and refractory contaminants (Kapusta and Stańczyk, 2015). Application of an appropriate treatment method of pollutants removal from UCG wastewater is critical to the implementation of this technology into the coal industry.

Recently, constructed wetlands (CWs) has been widely applied to clean up the industrial wastewater (Dutta et al., 2021). Constructed wetlands are artificial engineered systems that simulate the natural wetlands which are referred as bridge-builders between biodiversity conservation and development. The proven advantages of CWs as a green and sustainable technology have been discussed by many researchers and resulted in numerous applications (Saeed and Khan, 2019; Waly et al., 2022). The constructed wetlands as nature-based treatment technology have good remediation effectiveness, simple operation, cost advantages, and benefits for ecosystem services.

According to the cited literature, wetlands systems have been suggested for an efficient acid mine drainage (AMD) treatment (Nguyen et al., 2019; Pat-Espadas et al., 2018; Sheridan et al., 2018). Pat-Espadas et al. (2018) have reviewed the current advances, applications, advantages and disadvantages in applying the CW technology for AMD treatment. In this review the symbiosis between plants and rhizospheric bacteria constitutes the main factor during bioremediation process of ADM in CW systems. Nguyen et al. (2019) described the study in which the removal of arsenic and heavy metals from mining wastewater by the combination of adsorption, using modified iron-ore drainage sludge, and horizontal-subsurface-flow constructed wetland was applied.

However, there is a gap in the context of microbiological research in the world literature covering both water from coal processing by underground gasification, as well as solid materials in the form of post-process UCG chars. Wastewater from UCG is an example of a dangerous source of potentially hazardous, harmful and refractory contaminants, e.g. PAHs, BTEX, phenols (Kapusta and Stańczyk, 2015).

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Table 1

Elemental and textural analysis of UCG char used as an adsorbent (Wiatowski et al., 2023).

Moisture (wt.%)	Ash (wt.%)	C (wt.%)	H (wt.%)	N (wt.%)	S (wt.%)	V_T (cm ³ /g)	S_{BET} (m ² /g)	V_{DR,N_2} (cm ³ /g)
0.80	6.24	90.81	0.77	1.42	0.21	0.005	9	0.004

Table 2

Toxicity of UCG-derived adsorbent measured by Microtox system (mean values; n=3).

Parameters	Time	
	5 min	15 min
EC50	0.2032	0.2251
TU	492.1	444.3
Toxicity class	V	V

The preliminary microbiological characterization of potentially hazardous post-process wastewater from UCG have been described by Jałowiecki et al. (2024a).

Adsorption can be considered as a cost-effective method when using adsorbents that require little processing or they are industrial wastes or by-products (Deng et al., 2021; Gao et al., 2021; Kasprzyk, 2019; Wu et al., 2022; Susantil et al., 2023; Rana et al., 2023). The use of UCG-derived char as an adsorbent can be considered as an environmental-friendly technique with double benefits, as it allows to treat both solid wastes and contaminated water, approaching the ideal goal of 4'R (Reduce, Reuse, Recycle and Recovery) and the closed-loop economy.

The present study aimed to evaluate the potential of using a hybrid model consisting of UCG waste-derived adsorbent and CW system in the treatment of highly polluted UCG post-processing wastewater. The main objective of the study was to characterize the microbial composition of the adsorbent's biofilm. This is the preliminary research to characterize the biofilm layer of the adsorbent used in the wetland system. Understanding the structure of the biofilm layer may be useful in interpreting the process of pollutant removal by UCG-adsorbent integrated with the wetland system.

2. Materials and methods

2.1. Description of UCG char used as an adsorbent in the experiment

The UCG post-process char sample was obtained after the completion of the underground gasification of Polish bituminous coal sample using oxygen as a gasifying agent. The more detailed information about the UCG simulation carried out in a large-scale *ex-situ* installation located in the Barbara Experimental Mine (Mikolów, Poland) and the coal characteristics can be found in the paper of Wiatowski et al. (2023).

The UCG process is characterized by two main stages: short-term pyrolysis of coal and gasification of the char (Zhang et al., 2021). As the temperature increases, carbonaceous matter decompose and gaseous substances are released, and the coal mass residues remains in the form of chars or ashes (Palarski, 2010). The short-term pyrolysis of coal has a significant impact on the reactivity of the generated char. As a result of heating, the internal surface of coal undergoes transformation, which depend on the type of coal and the degree of its coalification. UCG char is characterized by a larger specific grain surface than coal, but it is less reactive. The removal of volatile parts from carbonaceous matter is accompanied by an increase in the specific surface of the char grain due to the formation of pores, and then a decrease in the grain surface due to shrinkage. As a result of thermal degradation, the elementary composition of the char changes. The content of carbon increases and the content of oxygen and hydrogen decreases, as well as the heteroatoms responsible for creating active sites on the surface of the material, i.e.



Fig. 1. UCG-derived char used as an adsorbent.

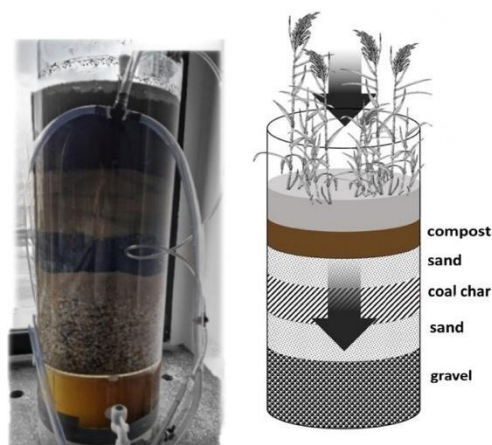


Fig. 2. Wetland column set up.

sulfur and nitrogen. In turn, with smaller char grain sizes and a rapid increase in temperature, numerous cracks are formed in the grains, which increase the concentration of active sites, resulting in greater reactivity of char (Smoliński et al., 2006). The physico-chemical and toxicity characteristics of UCG char are presented in Tables 1 and 2, respectively. The toxicity tests have shown that the adsorbent belongs to toxicity class 5, i.e. it is highly toxic. Fig. 1 presents structure of UCG char used as an adsorbent in CW.

V_T – total pore volume; S_{BET} – The Brunnauer–Emmet–Teller (BET) specific surface area; V_{DR,N_2} – N_2 micropore volume.

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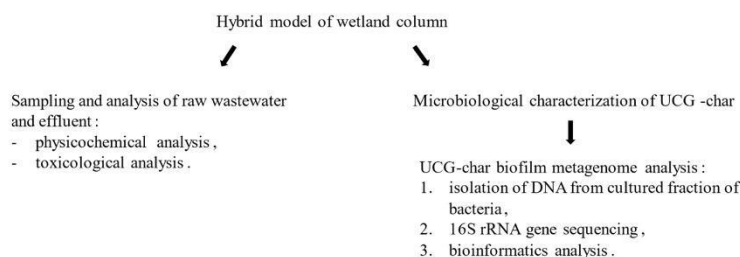


Fig. 3. Flow chart of methods used in the research.

2.2. Wetland column set up

Laboratory-scale vertical subsurface flow constructed wetland (VSFCW) was set up in poly-methyl methacrylate polymer (PMMA) column with dimensions of 1 m × 0.30 m × 0.30 m (W×L×D) and surface area of 0.064 m². The column was filled with the slight layer of mixed soil and green compost media from the top (0.10 m), then sand layer was established. The 0.10 m thick adsorbent layer amendment was located between two sand layers. The 0.40 m thick gravel layer was placed in the bottom of the column. The construction of column is presented in Fig. 2. The green compost used as a fertilizer was provided from the local composting unit. Constructed wetland was planted with the shoots of the common reed (*Phragmites australis*) which were transferred from the natural reed beds from the local plants company. *Phragmites australis* is commonly used in CW systems due to its unique properties such as cosmopolitan distribution, fast growth, and easy adaptable to different environments and substrates, like gravel, coke, zeolite, limestone, paddy and red soils (Ayaz et al., 2020). Laboratory-scale VSFCW was operated in Environmental Microbiology Unit of the Institute for Ecology of Industrial Areas.

After transplantation, clean water was loaded into the columns until the water level was 0.10 m higher than the compost surface in order to establish the plants growth for 2 weeks. At the end of the establishment period and acclimatization of plants, the constructed wetland columns were fed with UCG post-process wastewater. Storage tank with a capacity of 100 liters filled with wastewater were placed near the column. The raw wastewater passed through the column and returned to the tank by recirculation, where it was constantly mixed by the pump. Each cycle was operated with slow recirculation to enhance the mass transfer of substrate to the biofilm and to provide aeration. Detailed operating parameters of wetland column are presented by Jałowicki et al. (2024b).

The adsorbent layer (coal char) is marked with continuous diagonal lines.

2.3. UCG wastewater sampling

After the completion of UCG experiment the raw wastewater representing the average sample for a conducted gasification experiment was collected in a Mauser type tank with a capacity of 1 m³. The sample after mixing and pouring into smaller container was immediately transported to the laboratory. The raw wastewater from the UCG process was biologically treated with constructed wetland column set. The effluents from the constructed wetlands were collected after 14 and 45 days for physicochemical and toxicological analysis. The flow chart of analysis used in the research is presented in Fig. 3.

2.4. Physicochemical and toxicological analysis of effluents

All physicochemical wastewater analysis were conducted in the accredited laboratories of the Central Mining Institute - National

Research Institute in accordance with the currently applicable standards. For each parameter, samples were analyzed in duplicate.

Toxicity assessment of raw wastewater sample and effluents from wetland columns was conducted with the Microtox® test recommended by ISO Standard 11348:1998. The test was carried out in the Microtox M500 toxicity analyzer according to the standard procedure (Microtox Manual, 1992). The toxicity unit (TU) as 1/EC50 was calculated and the results were ranked based on TU values into one of five classes described by Persoone et al., 2003. Each sample was performed in triplicates assay. Toxicity tests were evaluated in the Institute for Ecology of Industrial Areas.

2.5. Microbial community analysis of adsorbent's biofilm by 16S rRNA gene sequencing

After 60 days of the system operation, some pieces of adsorbent were aseptically transferred to sterile tubes with sodium phosphate buffer, and vortexed for 15 min to detach the microbial cells from the carbonize. Microorganisms were collected by centrifuging at 14,000 rpm for 10 min, discarding the supernatant, and immediately proceeding with the extraction. For DNA extraction, a modified FastDNA™ SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) protocol was used. However, the samples yielded DNA concentrations too low to quantify. All samples had no quantifiable DNA recoveries to sequencing.

In this regard, metagenomic DNA (metDNA) was isolated from the culturable fraction of the bacteria. The adsorbent were aseptically transferred to sterile tubes with sodium phosphate buffer, and vortexed for 20 min at room temperature to detach the microbial cells from the UCG-derived char. 100 µl of bacterial suspension was placed on Luria-Bertani (LB) solid medium containing (L): 10 g – tryptone, 5 g – yeast extract, 10 g – NaCl, 15 g – agar, pH 7.0. The Petri dishes were incubated at 30 °C during 24 - 48 h. After incubation, 1 ml of physiological saline (0.90 % w/v of NaCl) was poured over grown bacterial colonies. The plates were incubated at 30 °C with constant mixing (70 rpm) for 30 min. The bacterial suspension was used to isolate metDNA of culturable bacteria fraction. Power Water DNA kit (MoBio Laboratories Inc., Carlsbad, CA, USA) was used to extract the total DNA according to the manufacturer's instruction. DNA extractions were done in triplicate, then the extracts were pooled to form a single mixed sample that was stored at -80 °C until subsequent molecular analyses. The quality and quantity of the genetic material was checked by microspectrophotometry (BioSpectrometer, Eppendorf). The library preparation and sequencing of metDNA samples were done by Genomed (Warsaw, Poland). Identification of cultured bacteria forming a biofilm was performed based on the V3-V4 hypervariable region of the 16S rRNA gene. The specific sequences of 341F and 785R primers were used for amplification and library preparation. PCR was carried out using Q5 Hot Start High-Fidelity 2x Master Mix. The reaction conditions were established in accordance with manufacturer's recommendations. Sequencing was performed using 2×300 bp paired-end technology on the Illumina MiSeq system with using Illumina's v. 3 kit. Automated preliminary data

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Table 3

Physico-chemical analysis of raw UCG wastewater (day 0) and leachates after 14 and 45 days of treatment with CW-adsorbent system.

Parameters	Unit	Time of treatment		
		Day 0	Day 14 th	Day 45 th
pH	-	7.1	7.8	7.7
conductivity	µS/cm	1540	1540	1430
ammonia NH ₄	mg/L	170	24	1.2
Total nitrogen	mg/L	150	80	15
Biochemical oxygen demand (BOD)	mgO ₂ /L	120	47	3.8
Chemical oxygen demand (COD)	mgO ₂ /L	303	98	105
Total organic carbon (TOC)	mgC/L	80	37	45
Chloride	mg/L	279	275	284
Sulfate	mg/L	54.7	123	116
Nitrate	mg/L	0.60	170	40
Nitrite	mg/L	<0.006	23	0.90
Total cyanide	mgCN/L	2.3	0.037	0.0040
Phenol index	mg/L	15	0.010	<0.001
Total phosphorus	mgP/L	<0.065	0.18	0.18
Sulfide	mg/L	0.022	<0.02	<0.02
Fe	mg/L	0.015	0.17	0.053
Mn	mg/L	0.36	0.18	0.21
Polycyclic aromatic hydrocarbons (PAHs, 15 compounds)	µg/L	1228	0.31	0.050
BTEX (incl. styren)	µg/L	3599	3.8	0.21
Toxicity EC ₅₀		0.00	0.65	91
TU (Toxicity Unit)		835	198	2.5
Toxicity class		V	V	III

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.

2.6. 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/>). The files are available at <https://www.ebi.ac.uk/ena/submit/webin/report/runs/ERP145122>.

Bioinformatics analysis providing classification of reads to species level was conducted with the QIIME 2 software (<https://doi.org/10.1038/s41587-019-0209-9>) based on the Silva 138 reference sequence database (<https://doi.org/10.1093/nar/gkm864>). The software was used for filtering out sequences containing errors created during the sequencing process (denoising), combining paired reads due to increase the accuracy of sequencing. This was performed in paired-end mode, so that at later stages of analysis the corresponding forward and reverse reads were merged, dereplication - merging of identical, unique sequences while maintaining the number of their occurrences, quality profile and chimera filtering.

3. Results and discussion

3.1. Characterization of leachates from the wetland column

Characteristics of the raw UCG wastewater and the changes of selected physicochemical parameters and toxicity during the treatment are shown in Table 3. Findings indicate that the treatment system was able to decrease the values of unwanted chemical parameters and achieve high removal performance. The reduction of the chemical parameters after 14 days of treatment ranged from 40-90 %. The toxicity of the raw wastewater also decreased significantly, from values corresponding

to V class of toxicity for raw wastewater to III class of toxicity for leachates after 45 days of treatment.

In the paper of Jałowicki et al. (2024a) vertical flow constructed wetland column was used to treat UCG wastewater obtained from three underground coal gasification processes using different kind of coal and gasification agents. The decrease of toxic compounds to less or non-toxic intermediates and by-products during experiment time was observed. Comparing the results described by Jałowicki et al. (2024a) with those obtained in this study, there are no apparent differences between both systems, i.e. wetland system without adsorbent and the hybrid wetland system described in this paper. They have similar removal efficiencies and have the potential to generate effluents due to the higher water quality and meet reuse standards. In this case, it was difficult to assess the effect of the UCG-derived char on the removal of organic and inorganic compounds and to evaluate the adsorbent amendment as an enhancer for CWs efficiency. The detailed characteristics of UCG wastewater and description of its treatment efficiency are also presented by Strugała-Wilczek et al. (2024). The authors evaluated removal efficiency of four different system configurations: wetland, electrocoagulation/wetland, adsorbent/wetland, and electrocoagulation/adsorbent/wetland. Each wetland experiment lasted 60 days. All tested systems were effective in removing both organic and inorganic contaminants.

The sorption characteristics of materials obtained from UCG cavity residues were investigated by Strugała-Wilczek et al. (2020). In the laboratory-scale tests sorption parameters for selected metals (Cd, Co, Cu, Pb) were determined for two different samples: subbituminous coal mined from the coal-bed used in underground coal gasification process and char formed during the UCG process. The post-UCG char efficiently removed metals and was found to have a better sorption capacity than the raw subbituminous coal due to the char's increased surface, as well as the thermal transformation caused by the gasification process and coal oxidation at high temperatures. The gasification characteristics such as temperature, gasification agents play a significant role in determining the adsorbent's structure (Yin et al., 2020). As presented by Yin et al. (2020), the adsorption amount, specific surface area and pore volume have shown a decreasing trend with the increase of coalification degree.

Previous studies have shown that low-cost adsorbent materials such as zeolite and biochar enhanced the efficiency of CWs (Gu et al., 2021; Gao et al., 2021; Lam et al., 2023; Li et al., 2024; Xu et al., 2021; Zaboon et al., 2022). The pilot CW studies with and without adsorbents showed that adsorbent addition significantly improved removal of ammonia, color, COD, and can promote wetland plant growth. CWs enhanced with adsorbent, significantly improved physicochemical parameters of effluents compared to CW without adsorbent material. The coupling adsorption and biological treatment increased the efficiency of leachates treatment to meet reuse or disposal requirements described in the literature and legal documents.

Wetland technology as a nature-based solution had the potential to turn post-process UCG wastewater into usable recycled water. Findings indicated that the wetland enhanced by adsorbent treatment system were able to improve the UCG raw wastewater characteristics. The wetland with UCG-derived adsorbent enhanced the efficiency of BOD, ammonia, and organic pollutants (phenol index, BTEX, PAHs) removal. However, the highest and similar efficiency of both CW systems (with and without UCG-derived adsorbent) in removing inorganic and organic pollutants was observed.

By integrating ecological processes with hydrological ones, sustainable water management systems, such as constructed wetlands and green infrastructure systems are examples of ecology research which allows for the optimization of water resource use in a way that minimizes human impact on the environment. Constructed wetland systems combine biological and hydrological processes to remove contaminants and contribute to the development of effective and eco-friendly wastewater treatment technologies.

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Table 4

Summary of the metagenomic sequencing and preprocessing results for adsorbent's biofilm samples ABK1 and ABK4.

Preprocessed sequences	ABK1	ABK4	Average
Raw reads	158 660	95 115	126 888
Raw bases (Mbp)	47.76	28.63	38.2
Trimmed reads	135 743	76 283	106 013
Trimmed bases (Mbp)	37.74	19.60	28.67
Trimmed reads (%)	85.59	80.20	83.55
Trimmed bases (%)	79.02	68.46	73.74
Average length (bp)	224	226	225
Max length (bp)	296	272	284
GC content (%)	52.5	51.5	52
<i>dada2</i> filtered sequences			
input sequences	135 743	76 283	106 013
filtered sequences	122 316	65 397	93 856.5
% of filtered	90.11	85.73	87.92
denoised	122 203	65 266	93 734.5
merged	122 080	64 625	93 352.5
% of merged	89.93	84.72	87.33
non-chimeric	115 852	59 931	87 891.5
% of input non-chimeric	85.35	78.56	81.95
ASVs	29	24	26.5

The obtained results provide the scientific basis for implementing nature-inspired solutions that can replace or complement traditional engineering technologies which are helpful in water resource management, nature protection, and climate change adaptation.

3.2. Microbial structure of adsorbent's biofilm

In this study, UCG-derived adsorbent was assessed for its ability to trap microorganisms in the working hybrid CW system. The microbial degradation of recalcitrant organic compounds is an important process that can contribute to the remediation of UCG raw wastewater. Due to the complex composition of raw wastewater, it is important to identify

the microbial communities that may be contributing to biodegradation processes, along with their abilities to metabolize different hydrocarbons in situ.

In Table 4 summary results from sequencing and preprocessing are presented. The average value of filtered sequences from the adsorbent samples was 93 856.5 which constitutes of 78 % raw reads. Similar microbial communities were captured in both adsorbent samples, although in varying relative abundances. The low average DNA recoveries were detected, and therefore metagenomic DNA was isolated from cultured fraction of microorganisms. Metagenomic analysis of biofilm showed little diversity and had a greater proportion of unique or low abundance taxa (Fig. 4 and Table 5). Two families, *Pseudomonadaceae* and *Bacillaceae*, were dominated in microbial communities of UCG-derived adsorbent with mean relative abundances of 92 % and 7 %, respectively. *Pseudomonas putida* dominated in the family *Pseudomonadaceae*. While, the great variety among the member of *Bacillaceae* was found. The dominant species were: *Bacillus circulans*, *Bacillus australimaris*, *Paenibacillus iibetiae*, *Bacillus simplex*, *Bacillus butanolivorans* and *Paenibacillus favisporus*.

Abbreviations: "d" – domain; "p" – phylum; "c" – class; "o" – order; "f" – family; "g" – genus

Sparse - taxa that made up less than 5 % of total classified reads
Unidentified category – bacteria unclassified at given taxonomic level or are poorly researched

The coal-derived environment provide a specific, extreme and suitable habitats for the microbial growth, colonization and activities associated with their complex, heterogeneous and carbonaceous structures. It can be a potential hotspot in the search of microbial strains with potential biotechnological applications. Native microbes possess rich functional potentials and can carry out a range of biotechnological processes, mainly in the coal industry, from production to remediation (Akimbekov et al., 2022). The most popular bacteria in coal-derived environments belong to the *Gammaproteobacteria* group. In addition, some bacterial genus like *Arthrobacter* spp., *Sinomonas* spp., and *Bacillus*

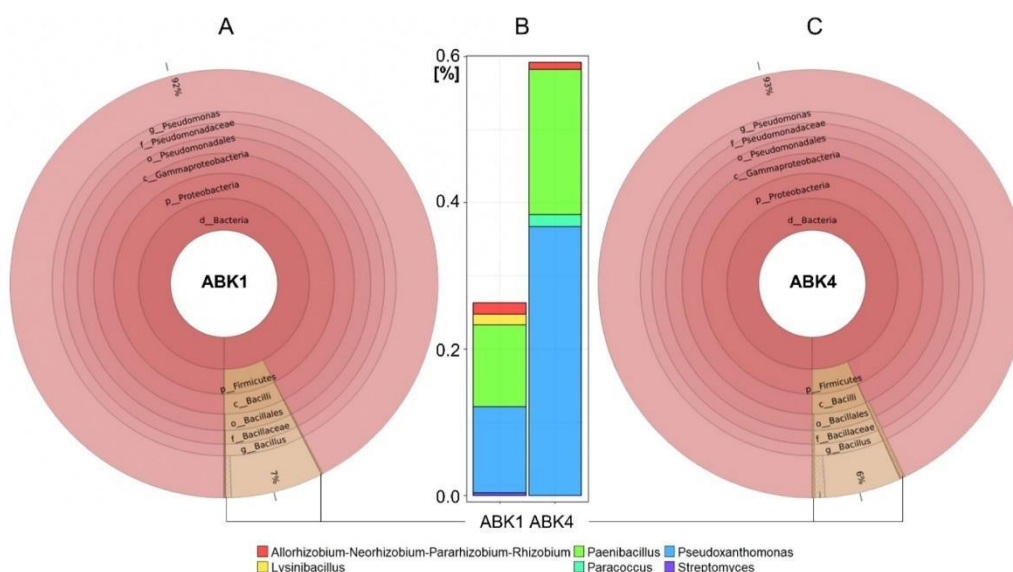


Fig. 4. The taxonomic classification on 6 levels of adsorbent's biofilm in samples named ABK1 and ABK4. The Krona plots (A and C) outline the overall proportion of the contribution of each taxonomic level from the analyzed samples: A – ABK1 and C – ABK4. The barplots B show the proportion of individual taxa at the genus level beyond *Bacillus* and *Pseudomonas*.

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Table 5

Classification rate of more abundant taxons of adsorbent's biofilm samples named ABK1 and ABK4.

ABK1	ABK4
Taxonomy abundance at Phylum level	
<i>Proteobacteria</i> (92.39 %)	<i>Proteobacteria</i> (93.12 %)
<i>Firmicutes</i> (7.609 %)	<i>Firmicutes</i> (6.88 %)
Sparse (0.004 %)	
(<i>Actinobacteriota</i>)	
Taxonomy abundance at Class level	
<i>Gammaproteobacteria</i> (92.37 %)	<i>Gammaproteobacteria</i> (93.09 %)
<i>Bacilli</i> (7.609 %)	<i>Bacilli</i> (6.88 %)
Sparse (0.0194 %)	Sparse (0.02723 %)
(<i>Alphaproteobacteria</i> , <i>Actinobacteria</i>)	(<i>Alphaproteobacteria</i>)
Taxonomy abundance at Order level	
<i>Pseudomonadales</i> (92.25 %)	<i>Pseudomonadales</i> (92.73 %)
<i>Bacillales</i> (7.497 %)	<i>Bacillales</i> (6.682 %)
Sparse (0.2483 %)	Sparse (0.592 %)
(<i>Xanthomonadales</i> , <i>Paenibacillales</i> , <i>Rhizobiales</i> , <i>Streptomycetales</i>)	(<i>Xanthomonadales</i> , <i>Paenibacillales</i> , <i>Rhodobacteriales</i> , <i>Rhizobiales</i>)
Taxonomy abundance at Family level	
<i>Pseudomonadaceae</i> (92.25 %)	<i>Pseudomonadaceae</i> (92.73 %)
<i>Bacillaceae</i> (7.482 %)	<i>Bacillaceae</i> (6.682 %)
Sparse (0.2631 %)	Sparse (0.592 %)
(<i>Xanthomonadaceae</i> , <i>Paenibacillaceae</i> , <i>Rhizobiaceae</i> , <i>Planococcaceae</i> , <i>Streptomycetaceae</i>)	(<i>Xanthomonadaceae</i> , <i>Paenibacillaceae</i> , <i>Rhodobacteraceae</i> , <i>Rhizobiaceae</i>)
Taxonomy abundance at Genus level	
<i>Pseudomonas</i> (92.24 %)	<i>Pseudomonas</i> (92.73 %)
<i>Bacillus</i> (7.482 %)	<i>Bacillus</i> (6.682 %)
Sparse (0.2631 %)	Sparse (0.592 %)
(<i>Pseudoxanthomonas</i> , <i>Paenibacillus</i> , <i>Rhizobium</i> , <i>Lysinibacillus</i> , <i>Streptomyces</i>)	(<i>Pseudoxanthomonas</i> , <i>Paenibacillus</i> , <i>Paracoccus</i> , <i>Rhizobium</i>)
Taxonomy abundance at Species level	
Unidentified (98.99 %)	Unidentified (83.69 %)
	<i>Pseudomonas putida</i> (15.11 %)
Sparse (1.198 %)	Sparse (1.012 %)
(<i>Bacillus circulans</i> , <i>Pseudomonas putida</i> , <i>Bacillus australis</i> , <i>Paenibacillus ibetiae</i> , <i>Bacillus simplex</i> , <i>Paenibacillus favisporus</i>)	(<i>Bacillus circulans</i> , <i>Paenibacillus ibetiae</i> , <i>Bacillus butanolivorans</i>)

spp. were described as a potential biomarker of coal mine spoils (Roy and Mukherjee, 2022). The indigenous and mostly novel bacteria isolated from the coal-derived environments have the great ability to offer an alternative clean remediation strategies for coal mining areas (Akimbekov et al., 2022). The application of various microorganisms depends on their metabolic and functional properties. Then, the future studies such as functional metagenomic screening, metaproteomic, metatranscriptomic and metabolomic could be undertaken. The current research provides preliminary characterization of microbes isolated from UCG-derived char adsorbent used in the wetland column.

4. Conclusions

The following conclusions can be formulated from the study:

- (1). Wetland system as a nature-based solution had the potential to turn coal gasification wastewater into usable recycled water. The hybrid model enhanced the removal efficiency of biological oxygen demand (BOD), ammonia (NH₃), and organic pollutants (phenol index, BTEX, PAHs).
- (2). The UCG-derived adsorbent can be a microbial carrier that promotes the assistance of specific microbes in remediation technologies for polluted environments.
- (3). The addition of waste adsorbent to biological systems may not necessarily be beneficial; rather, it may generate side effects for

specific bacteria that are important in environmental technologies.

- (4). The practical influence of adsorbent on constructed wetland efficiency is still unknown.
- (5). The integrated system ecotechnology composed of CW and UCG adsorbent was effective in reducing the pollutant from UCG wastewater. The concentrations of detected compounds during 45 days of the combined system operation were lower than the maximum allowable limits for industrial wastewater.
- (6). The results contribute to the implementation of integrated CW system as an effective post-treatment process for contaminants removal.
- (7). The promising results of the study allow for further research aimed at utilizing wetlands for purification of similar wastewater such as coke wastewater, leachates and stormwater.

CRediT authorship contribution statement

Łukasz Jałowiecki: Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Jacek Borgulat:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Aleksandra Strugała-Wilczek:** Writing – review & editing, Investigation. **Krzysztof Stańczyk:** Writing – review & editing, Supervision, Project administration, Funding acquisition. **Jan P. Jastrzebski:** Visualization, Validation, Software, Data curation. **Wiktor Babis:** Writing – review & editing, Visualization, Validation, Software, Data curation. **Grażyna Plaza:** Writing – review & editing, Writing – original draft, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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**PUBLIKACJE WCHODZĄCE W SKŁAD
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Review

Are Wetlands as an Integrated Bioremediation System Applicable for the Treatment of Wastewater from Underground Coal Gasification Processes?

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Abstract: Underground coal gasification (UCG) can be considered as one of the clean coal technologies. During the process, the gas of industrial value is produced, which can be used to produce heat and electricity, liquid fuels or can replace natural gas in chemistry. However, UCG does carry some environmental risks, mainly related to potential negative impacts on surface and groundwater. Wastewater and sludge from UCG contain significant amounts of aliphatic and aromatic hydrocarbons, phenols, ammonia, cyanides and hazardous metals such as arsenic. This complicated matrix containing high concentrations of hazardous pollutants is similar to wastewater from the coke industry and, similarly to them, requires complex mechanical, chemical and biological treatment. The focus of the review is to explain how the wetlands systems, described as one of bioremediation methods, work and whether these systems are suitable for removing organic and inorganic contaminants from heavily contaminated industrial wastewater, of which underground coal gasification wastewater is a particularly challenging example. Wetlands appear to be suitable systems for the treatment of UCG wastewater and can provide the benefits of nature-based solutions. This review explains the principles of constructed wetlands (CWs) and provides examples of industrial wastewater treated by various wetland systems along with their operating principles. In addition, the physicochemical characteristics of the wastewater from different coal gasifications under various conditions, obtained from UCG's own experiments, are presented.

Keywords: wetlands; bioremediation; bacteria; underground coal gasification (UCG); industrial wastewater

1. Introduction

Underground coal gasification (UCG) can be considered as one of the clean coal technologies that enables recovering valuable gas from coal in situ, even in the case of coal seams that are too deep, low grade or non-mineable with conventional methods. Produced gas can be applied in power generation, heat production or as a chemical feedstock. Years of development of UCG technology have shown that it is technically feasible and economically, socially and environmentally viable, i.e., due to its light surface footprint. The gasification process has come a long way from the first attempts in the former Soviet Union in the 1930s to complex plants with integrated combined cycle gasification or CO₂ capture and storage [1–5]. However, the UCG process in situ also carries some environmental risks, related mainly to the potential negative impact on surface and groundwater. Many heterogeneous and homogenous reactions that take place in the oxidation, reduction and pyrolysis zones developed during gasification affect the release of UCG-related contaminants from coal tars and ashes produced in the volatilisation process.

Conducting the process below the hydrostatic pressure prevents gas migration to the surrounding rock strata and can effectively control contaminants migration because the groundwater influx is towards the cavity, however, the main environmental risk is related to the removal of process condensates and drainage of the operating site during unloading to the surface for cleaning. Numerous organic and inorganic contaminants are formed both during different process stages and after process termination. Then, the UCG wastewater and sludge contain a significant amount of aliphatic and aromatic hydrocarbons, including benzene, polyaromatic hydrocarbons and phenols, but also ammonia, cyanides and hazardous metals [6,7].

Among various physical, physico-chemical and biological processes, biological treatments have been identified as suitable and nature-based methods for the clean-up of wastewater contaminated with various pollutants. Searching for novel approaches for using biological systems based on natural biochemical processes has been the main topic of research. A holistic approach to bioremediation has been developed, involving the activity of microorganisms and terrestrial and aquatic plant species in the biodegradation of hazardous compounds. Natural wetlands (NWs) and constructed wetlands (CWs) are the examples of low-cost, sustainable, easy-to-operate and ecofriendly options for the effective removal of various organic and inorganic compounds. They are designed as natural biofilters that simulate natural processes dependent on the interaction between media (such as gravel, pumice rock, soil), plants and associated microorganisms. In particular, microbes are considered to be a key player in the removal of pollutants from contaminated wastewater [8]. Knowledge of the wetland microbial communities is useful in monitoring the restoration and wastewater treatment processes. Wetland ecosystems are habitats for all kinds of microbes, aerobic, anaerobic, anoxic and facultative. Their role is also crucial in the functioning and growth of plant communities, and for maintaining the sustainability of wetland ecosystems.

The aim of this paper is to review the use and development of wetland systems and to identify the challenges and propose alternative methods for the biological treatment of industrial wastewater, including wastewater from UCG processes.

2. Bibliometric Analysis

The output for the review was collected from the bibliometric database Scopus on 8 June 2022. The search included only English-language articles and reviews from all available years. Since a search for the phrases “constructed wetlands” and “underground coal gasification” returned no results, it was conducted for scientific publications containing the phrases “constructed wetlands” and “industrial wastewater” in the title, abstract or keywords. The total number of publications found in the study area was 321. Detailed searching criteria are presented in Table 1.

Table 1. Criteria and results of filtering scientific publications in the Scopus database.

Criterion	Phrase	Number of Publications Identified
1 Title, abstract, keywords	“Constructed wetlands” and “Industrial wastewater”	603
2 Document type and language	Article, Review, English	462
3 Subject area	Environmental Science, Agriculture and Biological Sciences, Chemistry, Engineering, Chemical Engineering, Multidisciplinary, Immunology and Microbiology	321

The first publication on the subject, entitled “Constructed wetlands for wastewater treatment” by S.C. Reed [9] in Scopus dates from 1991. Over the three consecutive decades, there has been a marked increase in interest in the topic of wastewater treatment using wetlands (Figure 1). By mid-2022, 13 articles had already been published, confirming the relevance of the topic of this review [10].

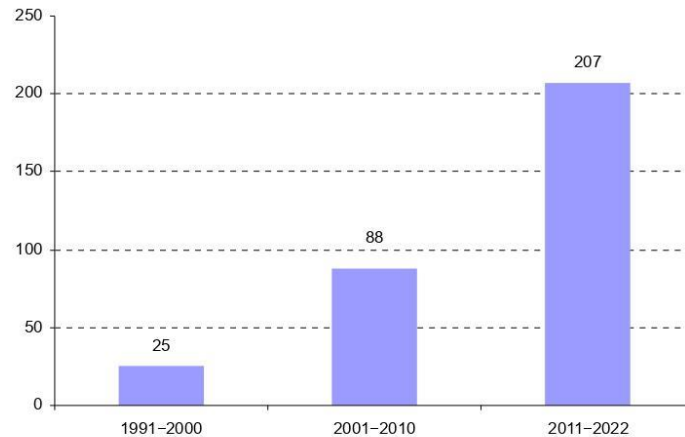


Figure 1. Number of publications in the study area between 1991 and 2022 (based on Scopus, 8 June 2022).

3. Underground Coal Gasification

3.1. Recent Worldwide Experiments

Underground coal gasification technology has been the subject of numerous pilot-scale studies and experiments in mining basins around the world for years. Large-scale UCG research programmes were initiated in the 1950s in the Soviet Union, in the 1970s and 1980s in the United States and in the 1980s, intensive research was launched in the People's Republic of China [4]. Currently, the development of UCG technology to enable its widespread and commercial application is underway almost all over the world (including China, India, UK, Slovakia, Poland) and some countries such as Australia, the UK, Canada, New Zealand and the USA are already introducing licensing rules in this area [11]. The summary of significant UCG in situ trials operated after the year 2000 is presented in Table 2.

Table 2. Worldwide significant UCG experiments in situ after year 2000 (from [4] with some modifications).

Country	UCG Site	Startup Year	Coal Type/Seam Depth and Average Thickness [m]	Gasifying Agent
Australia	Chinchilla G1	2000	subbituminous/132/10	air
	Chinchilla G3	2007	subbituminous/132/10	air
	Chinchilla G4	2009	subbituminous/132/10	air
	Chinchilla G5	2011	subbituminous/132/5.5	air, oxygen/steam
	Bloodwood Creek P1	2009	subbituminous/200/9	air, oxygen/steam
Canada	Bloodwood Creek P2	2011	subbituminous/200/9	air
	Swan Hills	2009–2011	high volatile bituminous/1400/4.5	oxygen/steam
China	Xinwen	2000	high volatile coal/100/1.8	air/steam
	Feichang	2001	bituminous/90/1.5	air
	Xiyang	2001	anthracite/190/6	air/steam

3.2. Experiments from Central Mining Institute (GIG)

In recent years, intensive experimental studies in the field of UCG using large-scale experimental simulations on artificial coal seams were conducted by the UCG research group from the Central Mining Institute (GIG) in Poland. The first trials of surface UCG experiments at GIG were designed and developed within the RFCS-funded project HUGE 1 (2007–2010). Simultaneously with the UCG trials in Poland, the EU-based Research Fund

for Coal and Steel (RFCS) programme provided funding to support further UCG research in Europe. The most important projects in the UCG area include, apart from the above-mentioned pioneering project HUGE 1 and HUGE 2 (2011–2014), the following projects: TOPS (2013–2016), COGAR (2013–2016), Coal2Gas (2015–2017), UCG&CO₂Storage (2009–2010), MEGAPlus (2018–2021) and the ongoing UCGWATERPlus.

After 2010, more advanced ex situ experimental units for UCG simulation were designed and built at GIG's Clean Coal Technology Centre, and the GIG team conducted more than a dozen underground gasifications of coals of different rank, from semi-anthracite to ortho-lignite, using various gasification agents and under different conditions (Table 3).

Table 3. General characteristics of the main UCG in situ and ex situ experiments conducted by the Central Mining Institute in years 2007–2021.

Origin of Coal	Type of Coal	Type of Experiment/ Installation Pressure *	Gasifying Agent	Experiment Duration [h]	Coal Gasified [kg]	Wastewater Produced [kg]	Wastewater Outflow [kg/kg Gasified Coal]	References
Experimental mine "Barbara" (I) (Poland)	subbituminous	in situ	oxygen/air	355	21,980	14,810	0.67	[12]
Experimental mine "Barbara" (II) (Poland)	subbituminous	in situ	oxygen/steam	142	5364	2960	0.55	[13]
Hard coal mine "Wieczorek" (Poland)	subbituminous	in situ	air/oxygen/CO ₂	60 days	230,500	d.n.a.	d.n.a.	[14]
Hard coal mine "Bobrek" (Poland)	bituminous	ex situ	oxygen	48	176.9	46.0	0.26	[15]
Hard coal mine "Ziemowit" (Poland)	subbituminous	ex situ	oxygen	48	164.2	130	0.79	[15]
Brown coal mine "Bełchatów" (Poland)	lignite	ex situ	oxygen	50	970.0	480	0.49	[16]
Hard coal mine "Bielszowice" (Poland)	bituminous	ex situ	oxygen/air/steam	73	145.0	79.6	0.55	[16]
Premogovnik Velenje (Slovenia)	meta-lignite	ex situ	oxygen	120	730.0	d.n.a.	d.n.a.	[17]
Premogovnik Velenje (Slovenia)	meta-lignite	ex situ/3.5 MPa	oxygen	72	591.0	d.n.a.	d.n.a.	[18,19]
Coal mine Oltenia (Romania)	ortho-lignite	ex situ	oxygen/steam	96	790.0	d.n.a.	d.n.a.	[17]
Coal mine Oltenia (Romania)	ortho-lignite	ex situ/1 MPa	oxygen	72	585.0	d.n.a.	d.n.a.	[18,19]
Hard coal mine "Piaś" (Poland)	subbituminous	ex situ	oxygen	72	2300	521	0.23	[20]
Coal mine "Six Feet" (UK)	semi-anthracite	ex situ/2 MPa	oxygen/steam	96	436.1	46.5	0.11	[21]
Coal mine "Six Feet" (UK)	semi-anthracite	ex situ/4 MPa	oxygen/steam	96	455.5	38.6	0.08	[21]
Hard coal mine "Wesola" (Poland)	bituminous	ex situ/2 MPa	oxygen/steam	96	504	67.3	0.13	[21]
Hard coal mine "Wesola" (Poland)	bituminous	ex situ/4 MPa	oxygen/steam	96	530.2	55.2	0.10	[21]

* no information means pressure close to atmospheric; d.n.a.—data not available.

3.3. Characterisation of Wastewater from Underground Coal Gasification Process: Experience from GIG

Wastewater from UCG is an example of industrial wastewater with an extremely complex matrix, containing large quantities of organic and inorganic pollutants, and therefore, the possibility of applying CWs to neutralise such wastewater appears to be important for investigating the possibility of biological treatment. Four main water sources in the process can be indicated: a. static water resources from the coal seam, b. dynamic resources (groundwater infiltrating from surface into the reactor environment), c. processing water—chemically bonded water present in minerals in the coal seam, d. water steam used as an addition to the gasifier and contain in the supply air of the UCG reactor [6]. The risk of groundwater contamination can be reduced by selecting an appropriate location and operation method of the pilot UCG plant. Wastewater generated during UCG has a similar composition to coking wastewaters [22], and requires complex mechanical, chemical and biological treatment [23] due to the high content of organic and inorganic impurities. According to Smoliński et al. [15], a higher total amount of wastewater (as well as higher coal consumption rate) is generated in the underground gasification of lignite than in the case of hard coal. This is most likely due to the high moisture content in the lignites.

UCG wastewater is produced mainly during the evaporation of coal, coal-pyrolysis, gas cooling and gas purification processes. The wet UCG gas consists of water vapour, originating principally from the evaporation of coal moisture, pyrogenic water and from unintended hydrogen combustion. The amount of wastewater arising from UCG ought to be more minor than from traditional mining, nonetheless, the water flux from a gas scrubber includes a high concentration of hazardous compounds, e.g., phenols, PAHs, monoaromatic compounds (BTEX) and heavy metals, for which the concentration should be continuously monitored to avoid any leakage into the environment [24].

Total amount of wastewater produced during UCG experiments conducted in GIG is presented in Table 3. It should be noted that the total volume of UCG wastewater produced during in situ trials was difficult to evaluate, e.g., in the experimental mine “Barbara”, approximately 500 kg of post-processing water per day was collected [25]. During ex situ experimental trials, the volume of wastewater obtained varied from 38.6 kg (gasification of semi-anthracite) to 521 kg (subbituminous coal “Piast”) and 480 kg (lignite “Bełchatów”). If bed water is most responsible for the amount of wastewater, then gasification of lignite should produce more waste than, e.g., anthracite, which has the lowest moisture content among all coal types. It should be emphasised that the quality and quantity of the UCG wastewater obtained differs depending on many complex aspects of operating conditions, such as UCG experimental installation and process parameters. The volume and composition of UCG wastewater solidly depends on the type and rank of gasified coal and its properties, the parameters of the process (temperature and pressure), the type of gasifying agent and applied gasification technology. The amount of physicochemical compounds depends mainly on the thermodynamic conditions (temperature) and mutual proportions of pyrolysis and oxidation zones [18]. Moreover, the concentration of pollutants in UCG wastewater vary significantly with the progress of the UCG process. The average amount of wastewater produced was about 0.30 kg per 1 kg of the gasified coal. The relatively high wastewater production during gasification of bituminous coal “Bielszowice” in reference to the coal consumption (0.55 kg wastewater per 1 kg of coal) can result from the unreacted steam, the evaporation of water or hydrogen combustion [16].

The average values of the parameters determined in the UCG wastewater originating from in situ and ex situ experiments are presented in Table 4. The analyses were performed in the laboratories of the Central Mining Institute in Katowice; some of the results have been published [12,14,16–19,21] and some are included in unpublished reports. As it can be concluded from Table 4, the values of measured parameters significantly differ for each UCG experiment. The physicochemical parameters as pH range from 4.9 to 7.8, COD_{Cr} from 48 to 5060 mg O_2/L and BOD_5 from 300 to 4373 mg O_2/L . The ammonia nitrogen concentration varies from 11 to 7800 mg N/L, which might be determined by the pH values, and the sulphates level is between 33 and 3220 mg/L. In all wastewaters, low concentration levels of cyanides from 0.5 to 5.7 mg/L were observed. Additionally, the concentrations of metals were relatively low for all wastewaters, except iron which differs from 0.02 to 650 mg/L. Pankiewicz-Sperka et al. [21] validated that the type of gasified coal has a significant influence on the concentration levels of organic parameters. Moreover, metal concentrations occurring in raw coals have no direct impact on the composition of UCG wastewater, but it is rather driven by organic contaminants derived from the tars produced during the UCG process [21].

Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych

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Table 4. Composition of the UCG post-processing wastewater from selected experiments conducted in the Central Mining Institute.

Parameter/ Compound *	Unit	Coal Type and Origin (Installation Pressure)											
		SB Barbara I (atm) [12]	SB Wieczorek (atm) [14]	B Bielszowice (atm) [16]	L Belchatów (atm) [16]	SA Six Feet (2 MPa) [21]	SA Six Feet (4 MPa) [21]	B Wesola (2 MPa) [21]	B Wesola (4 MPa) [21]	ML Velenje (atm) [17]	ML Velenje (3.5 MPa) [18,19]	OL Olefinia (atm) [17]	OL Olefinia (1 MPa) [18,19]
pH	-	6.3	7.3	7.8	5.4	6.4	5.2	5.3	4.9	7.3	6.0	7.7	5.1
Conductivity	µS/cm	14,425	57,400	19,200	8638.0	1228.4	253.38	942.00	1006.7	2478.0	1770.0	3155.0	5253.0
COD _{Cr}	mg/L O ₂	4308	5330	n.d.	n.d.	151.6	48.63	322.7	185.9	5060	691.0	2010	4177
BOD ₅	mg/L O ₂	2228	2840	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	4373	300.0	1048	2105
Ammonia nitrogen	mg/L N	1950	7800	3300	1225	160.1	11.68	96.41	95.74	280.0	189.0	463.0	778.0
Chlorides	mg/L	1660	18,000	n.d.	n.d.	11.15	11.68	29.18	45.94	n.d.	n.d.	n.d.	n.d.
Cyanides	mg/L	1.26	3.90	5.69	<0.5	1.11	1.43	1.70	0.87	1.31	0.70	1.01	3.00
Sulphates	mg/L	3220	980.0	651.0	1014	33.51	47.66	42.86	52.97	45.70	105.0	44.30	204.0
Mn	mg/L	4.91	n.d.	n.d.	n.d.	0.017	0.021	0.018	0.012	0.010	0.13	0.050	0.34
Fe	mg/L	650	n.d.	10.6	325	0.823	0.284	0.131	0.248	0.050	2.49	0.020	21.98
Sb	mg/L	<0.05	n.d.	n.d.	n.d.	0.036	0.12	0.064	0.013	0.030	0.030	0.030	0.070
As	mg/L	2.93	n.d.	n.d.	n.d.	0.036	<0.02	<0.01	<0.01	0.040	<0.005	0.21	0.16
B	mg/L	6.5	n.d.	3.1	0.18	0.072	0.056	0.13	0.25	0.21	0.58	0.040	0.48
Cr	mg/L	0.51	7.3	0.022	<0.005	0.013	0.012	0.010	0.006	<0.005	0.17	<0.005	1.8
Zn	mg/L	3.53	0.570	1.15	10.8	0.021	0.499	0.320	0.200	0.060	0.180	0.080	0.300
Al	mg/L	17.7	n.d.	n.d.	n.d.	0.031	0.046	0.029	0.023	0.060	0.220	<0.01	1.41
Cd	mg/L	<0.02	n.d.	n.d.	<0.002	<0.0005	0.001	<0.0005	<0.0005	<0.001	0.001	<0.001	0.002
Co	mg/L	0.031	n.d.	n.d.	n.d.	0.004	0.003	<0.003	<0.003	<0.005	0.010	<0.005	0.043
Cu	mg/L	<0.01	0.062	0.065	<0.01	0.005	0.010	0.009	0.002	0.010	<0.005	0.010	<0.005
Mo	mg/L	0.133	n.d.	0.140	<0.01	0.005	<0.005	0.026	<0.005	<0.01	0.020	<0.01	0.011
Ni	mg/L	0.243	1.16	0.029	<0.01	0.088	0.312	0.051	0.027	0.050	1.16	0.010	2.83
Pb	mg/L	0.044	0.035	<0.02	<0.005	<0.005	0.064	0.046	0.060	0.010	0.040	<0.01	0.28
Hg	mg/L	<0.005	n.d.	n.d.	n.d.	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	<0.0005	0.003
Se	mg/L	0.14	n.d.	n.d.	n.d.	0.016	0.017	0.036	0.027	0.040	<0.01	0.030	0.066
Ti	mg/L	0.52	n.d.	0.050	<0.005	<0.0005	0.001	0.001	<0.0005	<0.003	0.004	<0.003	0.055
Total phenol	mg/L	484	820 **	3090	247	29.7	2.14	49.5	29.2	733	17.0	246	201
TOC	mg/L	616.0	1500	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2400	167.0	882.5	1250
Total BTEX incl.	µg/L	55.80	414.0	790.0	106.0	5483	1497	2514	1354	1994	804.0	1784	1562
Benzene	µg/L	51.10	n.d.	504.0	96.00	4156	1341	2197	1059	1189	512.3	1190	1072
Toluene	µg/L	3.730	n.d.	140.0	7.000	n.d.	n.d.	n.d.	n.d.	356.3	175.2	277.0	236.3
Ethylbenzene	µg/L	0.700	n.d.	22.00	0.500	n.d.	n.d.	n.d.	n.d.	238.7	24.40	263.5	209.8
Xylene	µg/L	1.820	n.d.	124.0	2.500	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total PAH	µg/L	1912	399.0	1987	1066	1658	362.0	1090	477.2	n.d.	n.d.	n.d.	n.d.

n.d.—not determined; SB—subbituminous; B—bituminous; L—lignite; ML—meta-lignite; OL—ortho-lignite; SA—semi-anthracite. * In case of some parameters (e.g., metals), the limit of quantification of the method may be modified according to the presence of interfering substances. ** Total phenols volatile.

4. Bioremediation Process: Current Challenges and Trends

Bioremediation is a biological process using metabolic activities of living organisms, e.g., bacteria, yeast, fungi and plants, to destroy or neutralise to less hazardous organic and inorganic contaminants via complete mineralisation or co-metabolism. Compared to physico-chemical methods, generally, bioremediation technology is considered as less expensive, environmental friendly and more socially acceptable [26,27]. Most bioremediation systems are performed under aerobic conditions, but the systems under anaerobic conditions or aerobic–anaerobic conditions are also developed. In hybrid anaerobic–aerobic systems, recalcitrant pollutants are degraded by microbes in an oxygen deficit [28,29].

Two types of bioremediation methods are widely described in the literature, e.g., in situ bioremediation (bioventing, biosparging) and ex situ bioremediation (landfarming and composting, bioreactors, biopiles) [30].

The microorganisms involved in the bioremediation processes may come from a contaminated area (indigenous, autochthonous microflora) or they may be isolated from different places and inoculated to the contaminated site (allochthonous microflora). The supplementation of the microbial population is very effective where the native soil microorganisms are unable to degrade the pollutants. Introducing microorganisms to a contaminated site to enhance the bioremediation process is known as bioaugmentation [31,32]. The application of natural microbial consortia as inocula is a promising bioremediation method to remove anthropogenic compounds from polluted environments.

An option for bioaugmentation is biostimulation. The indigenous microorganisms are stimulated to grow and become active by changing environmental parameters such as temperature, pH or by adding nutrients, oxygen or surfactants [31,32].

Most of the organic hazardous compounds can be destroyed by the biodegradation process which is often a result of the actions of microbial consortium [33]. Microbes forming a consortium can perform multiple functions in bioremediation processes compared to a single microbe. Microbial consortia have great biotechnological potential for applications in long-term biodegradation. The application of microbial consortia is beneficial because of the synergistic interactions between different microbes that contribute to the overall contaminants degradation [34]. Microorganisms have the ability to degrade the contaminants from the enzymatic system into by-products that can be taken up by other microorganisms, which break them down into less toxic forms and harmless compounds that can be introduced into the biogeochemical cycles. In addition, different processes mediated by microbes, i.e., biomineralisation, biosorption and biodegradation, are part of the bioremediation process. Recently, research has focused on the design of microbial consortia, natural consortia or artificial co-culture systems as new emerging tools for bioremediation technologies. Dvorak et al. [35] presented the development of bioremediation technologies from the use of naturally occurring microorganisms called the era of *Bioremediation 1.0* through the application of recombinant DNA technology that provides changes in the metabolic pathways of selected microbial hosts and the development of a patchwork strategy called *Bioremediation 2.0* to the era of new generations of bioengineering called *Bioremediation 3.0*. These novel engineering strategies combine high throughput technologies, computational methods and “omics” techniques to design and develop microbial biodegradation pathways to plan and execute an effective bioremediation process.

Various types of bioremediation technologies have been described in the literature to minimise the environmental pollution caused by xenobiotics of an anthropogenic origin, such as petroleum hydrocarbons, solvents, pesticides, dyes, antibiotics, heavy metals, explosives such as 2,4,6-trinitrotoluene and other chemicals [36–48]. Most of these compounds belong to the emerging contaminants, i.e., substances that remain in the environment for a long time [8]. Most scientists and researchers note that no single bioremediation method can clean up a contaminated environment because most contaminants occur in the form of mixtures containing organic and inorganic pollutants. In such cases, an effective bioremediation strategy should include the implementation of two or more methods [48,49].

Nowadays, the integration of bioremediation approaches is very popular and researches pay attention to the development of hybrid (synergistic) processes. Wetlands are now considered as a holistic and integrated approach using natural physical, chemical and biological processes such as sorption, precipitation, transpiration, sedimentation, plant activities, such as phytostabilisation, phytoextraction, evaporation, and biodegradation activities performed by associated microbial consortia [50]. Wetland systems are nature-based solutions (also popularly known as green solutions) and are a relatively new area of research. As nature-based solutions, they can provide many different services with high social, economic and environmental values, such as recreation, CO₂ sequestration, coastal protection, groundwater and soil moisture regulation, human well-being, flood regulation, biodiversity support and other benefits, such as the creation of new jobs, etc.

Next to the wetlands, algae-based technology named phycoremediation also belongs to nature-based solutions of wastewater treatment. It has been developed as a technique using algae for treating chemically contaminated water [51,52]. Algae have an ability to assimilate various toxic pollutants such as aromatic hydrocarbons, heavy metals and organochlorine [53,54]. Algae from various non-pathogenic genus such as *Chlorella*, *Spirulina* and *Scenedesmus* have been applied in the phycoremediation of phenolic compounds [55].

Municipal wastewater and industrial wastewater from paper, textile, tannery industries, petroleum refineries, agriculture wastewater, mining wastewater, stormwater and landfill leachate are only a few examples of polluted wastewater that has been treated by different kind of wetlands [56–59]. The application of wetlands for the purification of contaminated wastewater is rapidly increasing in the industrial sector.

5. Wetlands as Natural and Engineering Systems to Clean up Industrial Wastewater

Constructed wetlands (CWs) are defined as engineered systems whose operating principle is mainly based on natural ecosystem processes related to the decomposition and circulation of matter. These facilities use heterotrophic microorganisms and aquatic and hydrophytic plants to treat wastewater. The plants grow on appropriately designed objects, such as ground-root filters or ponds [60,61], which are designed to intensify and direct water purification. An important feature of CWs is that they are a low-cost, non-invasive, multifunctional and, above all, environmentally friendly solution [62]. The multifunctionality of CWs stems from their “natural character”, whereby they blend into the landscape and, similar to their natural counterparts, can serve as urban wildlife refuge, recreational facilities and landscape engineering facilities [63].

In CWs, by creating conditions that allow the growth of hydrophytes, it is possible to intensify the redox processes which, supported by other processes such as sorption, sedimentation and assimilation, allow the removal of significant amounts of pollutants from wastewater. Plants used in these systems such as *Phragmites australis* often form an extensive system of rhizomes and roots around which, due to internal gas transport pathways [64], local oxygen microspheres are formed, surrounded successively by hypoxic and further reductive microspheres [65]. The occurrence of aerobic and anaerobic zones around the roots stimulates the decomposition of organic matter and the nitrification process, occurring thanks to the metabolic activity of microorganisms—bacteria, algae, fungi and protozoa. Another desirable feature of plants used in CWs is the rapid growth of biomass, which, among other advantages, has a beneficial effect on the removal of nutrients from treated waters. To create CW systems, naturally occurring wetland plants are used, which can include: emergent plants (*Phragmites australis*, *Typha latifolia*, *Typha angustifolia* and *Salix viminalis*), submerged vegetation (*Hydrilla verticillata*, *Ceratophyllum demersum*, *Vallisneria spiralis*, *Myriophyllum verticillatum* and *Potamogeton crispus*), free-floating plants (*Nymphaea tetragona*, *Nymphaea peltata*, *Trapa bispinosa* and *Marsilea quadrifolia*) and floating leaves plants (*Eichhornia crassipes*, *Salvinia natans*, *Hydrocharis dubia* and *Lemna spp.*).

Constructed wetlands have become very popular in recent years as an alternative to high-tech wastewater treatment solutions [59,62,66]. These systems are suitable for treating wastewater from both point and area sources [67,68]. Due to their ability to assimilate

nutrients, CWs have great potential in treating municipal wastewater. However, it is increasingly observed that these solutions are used to treat industrial wastewater and leachate [62,69,70]. CWs treat a wide range of pollutants, such as heavy metals, pesticides, petroleum hydrocarbons, explosives, radionuclides and pollutants specific to effluents from a textile dye factory, etc. Table 5 lists experiments using CWs, including small-laboratory-scale systems that have been used to remove various compounds.

CWs can be divided into two types, those operating on a surface flow system (FWS, free water surface) and those operating on a subsurface flow system. FWS treatment plants function as ponds or canals that usually provide a serpentine water flow. In FWS treatment plants, the long retention time (usually between a few days and 2 weeks) and large surface area favour the removal of solids and organic matter [71]. Different types of vegetations are used in this type of treatment plant, but it is worth noting that in Europe, the most commonly used macrophyte is *Phragmites australis* [72,73].

Subsurface flow plants are constructed so the wastewater flows under a layer of material filling the bed. In subsurface flow CWs, the retention times are usually shorter (1–2 days) and active microorganisms are associated with plant root systems and the substrate surface [74]. There are two types of subsurface flow CWs: with horizontal flow (HSF, horizontal subsurface flow) and with vertical flow (VSF, vertical subsurface flow) [75]. It also happens that mixed systems, with both a vertical and horizontal wastewater flow, are used in a single treatment process. The division of constructed wetlands is presented in Figure 2.

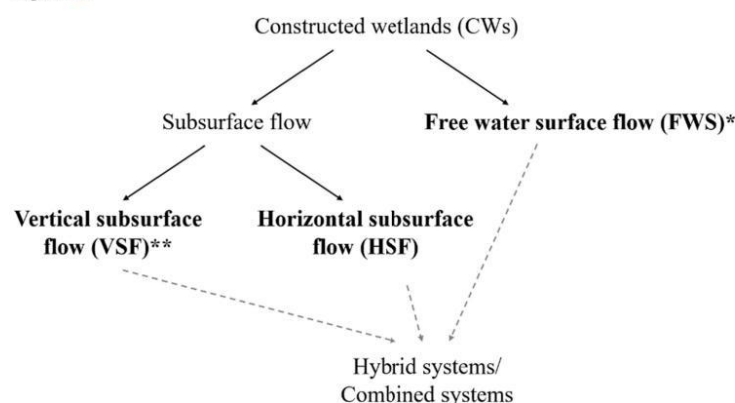


Figure 2. Simplified classification of constructed wetlands (CWs) for wastewater treatment (from [68] with some modifications); * FWS CWs may contain different types of vegetation, such as emergent, submerged, free floating and floating-leaved plants; ** VSF CWs can be characterised by different wastewater flows: up, down and tidal.

In summary, CWs in various configurations can deal with a whole group of different types of pollutants found in industrial wastewater (Table 5). Considering that many of them, such as hydrocarbons, cyanides, phenols and some heavy metals, are present in UCG wastewater, it is reasonable to assume that a properly designed CW should be able to treat UCG wastewater. However, the literature suggests that in the case of high PAH levels, which often occur after the UCG process (Table 4), it would be advisable to aerate the system and extend the hydraulic retention time (HRT) accordingly.

Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych

Table 5. Examples of the experiments described in the literature using wetlands as a bioremediation approach.

Pollutants	Type of Wastewater	Type of Wetlands and the Plant Species Used	Removal Rate/Comment	References
Heavy metals (Mn, Cd, Zn and Pb), arsenic (As)	Mining (the pilot-scale experiment)	The combination of adsorption (modified iron-ore drainage sludge) and HSF CWs with <i>Phragmites australis</i>	The average removals during four months of operation was as follows: Mn—96.9%, Cd—79.6%, Zn—52.9%, Pb—38.7% and As—96.9%	[76]
Heavy metals (Fe, Mn, Al, Co, Ni and Cr), sulphate (900–1500 mg L ⁻¹)	Synthetic acid mine drainage (laboratory-scale experiment)	HSF-CWs planted with <i>Typha latifolia</i> and organic-rich substrate (cow manure and bamboo chips)	After the 6-month metal removal efficiency: Cr—99.7%, Ni—97.8%, Co—93.7%, Fe—91.6% and Al—59.7%. Microbial sulphate reduction 44–75%.	[77]
Heavy metals (Mn, Cu, Co, Cr and Cd)	Coke plant effluents	Natural wetland. Dominant emergent plants: <i>Colocasia esculenta</i> , <i>Scirpus grossus</i> and <i>Typha latifolia</i>	Natural wetlands seems to be efficient in removal of selected heavy metals from coke-oven effluent	[78]
Heavy metals, phenol	Post-methanated distillery effluent (PMDE)	FWS CWs in India planted with <i>Typha angustata</i> L.	Removal: Cd (34–62), Cr (36–58), Cu (33–54), Fe (33–52), Mn (36–83), Ni (36–59), Pb (33–60), Zn (32–54), phenol—93.75% after 7 days of free water surface flow treatment	[79]
Ammonium, iron and traces of organic compounds	Coke plant effluents (pilot-scale)	HSF-CWs with two-stage gravel bed planted with <i>Phragmites australis</i>	Nitrogen removal efficiency (54–94%). Removal of COD from 35 to 52% of inlet concentrations	[80]
Heavy metals (Cu, Ni, Pb and Zn), cyanides (CN ⁻) and sulphates (SO ₄ ²⁻)	Synthetic electroplating wastewater (laboratory-scale experiment in columns)	Up-flow VSF-CWs (lactates as carbon source, peat or gravel as medium). Some columns planted with <i>Phragmites australis</i>	Maximum removal: Cu, Ni, Zn, CN ⁻ > 90%; Pb > 70%; SO ₄ ²⁻ > 10%. Insignificant effect of vegetation	[81]
Phenol, m-cresol, methyl tertiary-butyl ether (MTBE), benzene	Contaminated groundwater (the pilot-scale experiment)	Three separate HSF CWs. Two of them with <i>Phragmites australis</i> and one without vegetation	Surface load removal rates (SRR; mg m ⁻² d ⁻¹) were as follows: MTBE—20, m-cresol—80, benzene—335, phenol—620. The presence of <i>Phragmites australis</i> significant improved the contaminant removal performance.	[82]
Phenol, ammonium and nitrogen	Domestic wastewater spiked with phenol (laboratory-scale experiment)	HSF CWs planted with <i>Typha latifolia</i> with different substrate	CWs with <i>Typha latifolia</i> were able to remove phenol completely (C ₀ = 500 mg L ⁻¹) after 36 days with rice husk in substrate and by 60% in gravel in substrate. Planted wetland units performed better than the unplanted ones.	[83]
Phenol, organics (COD), thiocyanate and ammonium nitrogen	Synthetic wastewater (laboratory-scale experiment)	HSF-CWs planted with <i>Typha angustifolia</i>	Efficiency removals (operation time period—158 days): Phenol- 99%, COD—93%, ammonia nitrogen—17–30%. Alkalinity improved thiocyanate removal to 91%.	[84]
BTEX	Groundwater from the former refinery site (pilot-scale system consisted of four subsurface flow treatment cells equipped with aeration).	HSF-CWs. Types of plants: <i>Salix</i> , <i>Phragmites</i> , <i>Scirpus</i> , <i>Juncus</i> and <i>Cornus</i>	Removal after one year operating: benzene—80%, total BTEX- 88%	[85]

Table 5. Cont.

Pollutants	Type of Wastewater	Type of Wetlands and the Plant Species Used	Removal Rate/ Comment	References
PAHs (naphthalene; mixture of phenanthrene and pyrene)	A laboratory-scale experiment investigating the effects of PAHs on plant growth and development.	Hydroponics/pot experiment. Species: <i>Baumea juncea</i> , <i>Baumea articulata</i> , <i>Schoenoplectus validus</i> and <i>Juncus subsecundus</i>	The effect of PAHs on plant growth in CWs may be species-specific and can depend on the type of PAHs and the substrate	[86]
PAHs	A laboratory-scale experiment	VSF-CW planted with <i>Iris pseudacorus</i>	The reduction of phenanthrene using biochar-loading copper ions (Cu-BC) was about 94% (HRT lasted 3 days)	[87]
Hydrocarbons and cyanides		FWS-CWs. Shallow basins, initially planted with: <i>Typha latifolia</i> and <i>Schoenoplectus tabernaemontani</i> , subsequently converted to <i>Ceratophyllum demersum</i> and <i>Potamogeton</i> spp.	Removal after 7 days detention: total cyanide—56%, free cyanide—88%, Gasoline, diesel—~67%	[88]

6. Role of Microorganisms in Wetlands

Wetland ecosystems are examples of reservoirs of microbial diversity, mainly bacteria, fungi and actinomycetes [89]. In wetland, most biogeochemical activities are carried out by microbes. In particular, bacteria are the most active in processes occurring in wetland nutrient cycling and biodegradation processes because of their specific properties such as small genome size, short replication time, rapid evolution, relative simplicity of structure, metabolic properties and adaptation to new and extreme environmental conditions. It is known that microbial communities occurring in wetlands intensively enhance various processes. Investigating the interactions of the microbial structure and functions with wetland plants is an important part of specific transformations, biodegradation, biogeochemical cycles (including nitrogen, carbon, phosphorus and sulphur cycles), wetland survival and restoration. Many processes of nitrification, denitrification, mineralisation, humification and absorption occur through physical, chemical and microbial activity. Processes mediated by microorganisms are of great importance for the bioremediation function of wetlands. In wetland, aerobic and anaerobic microbial activity and microbial communities are mainly established by plant roots. Literature reviews by Liu et al. [90] and Wang et al. [91] show that some parameters such as temperature, redox potential and dissolved oxygen (DO) are influential factors for pollutants' degradation in wetland systems. DO is a vital factor that could influence microbial activities and the efficiency of pollutants' removal. Special attention is given to the mechanism of radial oxygen loss (ROL), that is responsible for contaminant removal in wetlands and the activity of the root-associated microbiome (rhizobiome).

The first research papers characterising various types of wetlands (such as rice paddies, acidic wetlands—peatlands, freshwater wetlands, black mangroves, salt marshes, boreal wetlands) and their microbes were published in the 21st century [92–100]. These papers have mainly focused on the characterisation of a specific group of bacteria involved in the nutrient cycles. Current knowledge on the diversity and functioning of wetland microbial communities is insufficient and there are many gaps that need to be assessed in future wetland studies. The characterisation of wetland microbial communities is fundamental for understanding the working rules of such a complex system as a wetland ecosystem. Up to now, phenomena and principles of microbes' activities and plant-microbial interactions in wetlands are priorities in the development of bioremediation research strategies [101].

The synergistic effects of microorganisms and plants have been applied to develop bioremediation processes [102]. With respect to remediation purposes, root-associated

bacteria (rhizobacteria) are considered most relevant. They form the area surrounding plant roots, called the rhizosphere, that is influenced by the growth, respiration and secretions of the plant roots. Most of the bacteria adhere to the root surface and form the microbial biofilm which is named the rhizoplane. The root-associated microbial community, called the rhizomicrobiome, contains around 10^{11} microbial cells per gram of root tissue and more than 30,000 prokaryotic species [103]. Most of the root-associated bacteria belong to plant growth-promoted bacteria (PGPB) which enhance plant growth by the secretion of specific phytohormones such as siderophores, aminocyclopropane-1-carboxylase (ACC) and indole acetic acid (IAA). The enzyme ACC deaminase is known as an ageing hormone and for the improvement of plant nutrient uptake through nitrogen fixation and phosphate solubilisation [104]. Recently, the beneficial role of rhizobacteria in the promotion of plant growth and pollutant removal has been reviewed in detail by Orozco-Mosqueda et al. [105]. Rhizobacteria also produce various metabolites such as antibiotics, biosurfactants, enzymes, cellulases and HCN. The role of bacterial biosurfactants in environmental bioremediation processes has gained importance due to their low toxicity, high biodegradability and ecological acceptability. They are excellent emulsifiers and foaming and dispersing agents due to their surface activity. Biosurfactants such as rhamnolipids, sophorolipids and surfactins can effectively solubilise, emulsify and mobilise both heavy metals and organic pollutants. Biosurfactants are also known as antimicrobial agents inhibiting the growth of bacterial and fungal pathogens. Nowadays, interest in the plant root microbiota, the relative metabolites, and its activity is rapidly growing. However, there are still a huge number of cultivable and uncultivable microbes whose ecology and biochemistry are unknown. Recently, the research on changes in the structure and function of microbial communities has been developed by the advancement in science and technology, especially in the field of molecular techniques [106–108]. There are two main types of methods used to assess the structural and functional diversity of wetland microbes, i.e., culture-dependent and culture-independent methods. Culture-dependent methods provide the detection, enumeration and determination of physiological profiles of microbial species using traditional plate count methods, while culture-independent molecular approaches are used to characterise the unculturable fractions of wetland microorganisms [109]. Figure 3 shows a flowchart of the methods used to characterise the functional and structural diversity of wetland microbial species and communities.

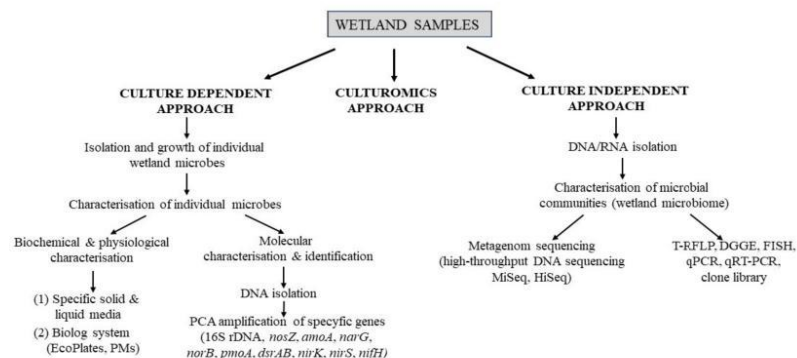


Figure 3. Flowchart of the methods used to characterise the functional and structural diversity of wetland microbial species and communities.

Wetland communities are characterised by changes to the community composition (structure) and by assessing changes in community function. The 16S rDNA gene as a target is currently used for the taxonomic classification of microbes. In contrast, the community function can be assessed by microbial enzyme activity, the degradation of various substrates or evaluation of functional genes. The most popular method to evaluate

the microbial or community function is community-level physiological profiling (CLPP), conducted using BiologTM EcoPlates. This method was used by Salomo et al. [110] to characterise the metabolic potential of a microbial community in a constructed wetland with a vertical flow. Jamwal and Shirin [111] conducted a laboratory-scale experiment to identify microflora using the traditional plate method in planted and unplanted constructed wetland systems. Authors focused on the characterisation of microbes from the rhizosphere region of *Typha domingensis* using traditional culture methods, e.g., serial dilution and plate methods. Bacterial strains involved in the nutrient cycles and playing a significant role in the degradation of organic compounds such as *Pseudomonas* spp., *Bacillus* spp., *Staphylococcus* spp., *Corynebacterium* spp., *Streptococcus* spp., *Lactobacillus* spp. and *Proteus* spp. were isolated and characterised. In contrast, Xu et al. [112] combined metagenomic analysis and enzyme activity measurements to evaluate the distribution of the bacterial community in a vertical flow constructed wetland (VFCW) system. *Proteobacteria* were the most abundant phyla, followed by *Bacteroides*, *Firmicutes*, *Acidobacteria* and *Chloroflexi*. However, about 10% of unknown bacteria were also detected. The results showed that there was no relationship between enzyme activity and microbial populations. Some relations were observed between the functional bacterial community and the activity of particular enzymes, e.g., between *Nitrospira* and urease. Dynamic changes in enzyme activity were observed in different layers of CWs. In the paper of Xiang et al. [113], a constructed wetland system with *Acorus calamus* was established to investigate the bioremediation process of petroleum-contaminated wastewater. In this study, a high-throughput sequencing system was used to characterise bacterial diversity and the community structure. The results showed that the *A. calamus* root system forms a rhizosphere effect, which provides good conditions for the growth of specific microbes in the wetland. The dominant bacteria in the constructed wetland were as follows: *Acinetobacter*, *Pseudomonas*, *Rhizobium* and *Rhodobacter*. Pearson correlation analysis revealed a strong correlation between the values of richness and diversity indices and the removal efficiency of petroleum pollutants. The identified bacteria are involved in organic matter decomposition, nitrogen removal and the decontamination of petroleum pollutants. They have specific organic compound degradation properties such as biosurfactant production.

The study by Ma et al. [114] used Illumina high-throughput sequencing to identify the profile of microbial communities in wetlands constructed at a pilot-scale during saline wastewater treatment. Variations in the diversity and composition of microbial communities between the roots of *Salicornia bigelovii* and wetland layers were evaluated. Among twelve phyla detected in all samples, eleven were bacterial phyla and only one represented the archaeal phylum—*Thaumarchaeota*. Microbiological differences were observed between the root zones and the wetland layers. *Proteobacteria*, *Firmicutes*, *Cyanobacteria* and *Bacteroidetes* were dominated in the root zones, while *Cyanobacteria*, *Proteobacteria*, *Firmicutes*, *Verrucomicrobia* and *Bacteroidetes* were widespread in the wetland layers. These differences were probably related to the oxygen concentration and root secretions. Furthermore, the analysis indicated that specific functional genera such as *Nitrosopumilus*, *Vibrio*, *Pseudoalteromonas*, *Nitrospina* and *Planctomyces* were present in the wetland system. These play a key role in the promotion of the growth of wetland plants and in the removal of nitrogen and phosphorus pollutants.

The review article of Lv et al. [115] summarises the results of a study related to the search for 16S rRNA gene sequences from wetland ecosystems deposited in two public databases: GenBank and RDP. This meta-analysis showed that a total of 12677 bacterial and 1747 archaeal sequences from various wetland ecosystems were collected in the GenBank database in 2012. All bacterial sequences were assigned to 6383 operational taxonomic units (OTUs), representing 31 known bacterial phyla. The predominant bacterial phyla were: *Proteobacteria*, *Bacteroidetes*, *Acidobacteria*, *Firmicutes* and *Actinobacteria*. The genus *Flavobacterium* (11.6% of bacterial sequences) was the dominant bacterium in the wetland, followed by *Nitrospira* and *Nitrosomonas*. Archaeal sequences were mostly assigned to the

Euryarchaeota and *Crenarchaeota* phyla. The dominate archaeal genera were *Fervidicoccus* and *Methanosaeta*.

The review by Mellado and Vera [107] focuses on the description of microbes detected in both natural and constructed wetlands that belong to the Bacteria and Archaea domains. Microorganisms were identified and characterised using a high-throughput, culture-independent sequencing technique. The 16S rRNA gene was used as a target to classify Bacteria and Archaea in wetlands. Functional genes have also been used as genetic markers to identify the specific metabolic groups of microbes. Among the bacteria, *Proteobacteria* was the most abundant phylum, both in natural and constructed wetlands. *Proteobacteria* members participate in most metabolic processes, such as the degradation of organic matter, organic compounds, biochemical cycles such as the methane nitrogen cycle, the sulphur and phosphorus cycle and methanogenic oxidation. The specific group of microorganisms perform non-conventional pathways such as heterotrophic nitrification, anammox and autotrophic denitrification to remove organics and nutrients [116–118]. Based on some recent reviews, there is an increasing research interest in studying microbial communities to track the success of constructed saltmarsh and wastewater treatment processes [119,120].

7. Conclusions

Wetland systems are an example of eco-industry which focuses on environmentally friendly clean-up technologies using the natural processes of plant-associated microorganisms. The valorisation of contaminated industrial wastewater, also in the context of clean coal technologies to which the UGC process belongs, appears to be one of the pillars of a modern and responsible industry. Wetlands have recently received a lot of interest from researchers in the treatment and removal of anthropogenic pollutants from wastewater as they are considered as nature-based solutions whose application would have a positive impact on the environment.

Despite this, the most important information about the operation and processes of wetlands is still unknown. Knowledge about the type of microorganisms and their role in designing efficient wetland systems for bioremediation processes is still needed.

This review article systematises the latest knowledge and recent developments in the treatment of industrial wastewater in wetland ecosystems, particularly in terms of the removal of organic and inorganic pollutants present in difficult matrix wastewaters, such as those from UGC processes. The review suggests that constructed wetlands could be a multi-green remediation approach for treating industrial effluents, including wastewater from coal gasification processes. It can be expected that properly designed CWs can purify wastewater from underground coal gasification, however, it should be noted that high concentrations of certain pollutants that often accompany UGC, e.g., PAHs, may require the additional aeration of the wetland system and the hydraulic retention time extension.

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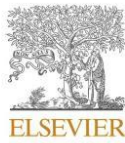
Opracowanie procesu oczyszczania ścieków z podziemnego zgazowania węgla z zastosowaniem metod biologicznych

A hybrid system based on the combination of adsorption, electrocoagulation, and wetland treatment for the effective remediation of industrial wastewater from underground coal gasification (UCG).

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Research article

A hybrid system based on the combination of adsorption, electrocoagulation, and wetland treatment for the effective remediation of industrial wastewater from underground coal gasification (UCG)

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ABSTRACT

The study verified the effectiveness of treating post-process underground coal gasification (UCG) wastewater, containing high loads of inorganic and organic pollutants, using constructed wetlands (CW) enhanced by hybrid adsorption and electrocoagulation (EC) techniques. Four different system configurations were tested: wetland, EC/wetland, adsorbent/wetland, and EC/adsorbent/wetland. Each experiment lasted 60 days. The feed and effluents from each treatment step were analysed for their basic physicochemical parameters such as metals and trace elements, phenols, sulphides, cyanides, total organic carbon (TOC), chemical oxygen demand (COD), biological oxygen demand (BOD), benzene, toluene, ethylene, xylene (BTEX), and polycyclic aromatic hydrocarbons (PAHs).

Systems with electrocoagulation proved to be effective in the case of metal removal. The best results were obtained for Fe, Ni, Sb and As (up to 96%, 98%, 94% and 82% respectively). The systems were ineffective in removing Mn. All tested systems showed the greatest effectiveness in the treatment of wastewater from phenols, BTEX and CN (almost 100% removal). CWs without preliminary electrocoagulation showed practically 100% effectiveness in removing BTEX after 14 days of treatment. Electrocoagulation was particularly effective in reduction of large quantity of PAH compounds (from 1228 µg/l to below 0.050 µg/l). Effective toxicity reduction from V class to II class after 60 days in comparison with meeting the requirements contained in the Best Available Techniques (BAT) document, showed that all tested systems were favorable in terms of UCG wastewater treatment. A significant decrease in toxicity was observed in just 14 days (around 90% reduction of toxicity measured in TU values for systems without adsorbent and around 75% for systems with adsorbent). The wastewater were still toxic due to the formation of degradation intermediates of organic compounds (BTEX, PAHs, phenol compounds), despite a significant decrease in the concentration of contaminants, therefore toxicity assessment should be one of the evaluation criteria for industrial wastewater treatment.

1. Introduction

The technology of UCG involves the in-situ conversion of coal into a valuable gas product, which is suitable for chemical synthesis or can be used in electricity and heat production. Although UCG is considered one of the economical and technically feasible clean coal technologies, there are still some issues to be concerned about. Critical issues related to environmental safety include the production of wastewater that

contains a large load of organic and inorganic pollutants (Camp and White, 2015; Xu et al., 2021). The characteristics of UCG post-processing water are similar to coke wastewater and, as a potential source of environmental pollution, require proper treatment. Process water generated during UCG may originate from moisture contained in the coal bed, groundwater infiltrating from the surface, water chemically bonded in minerals present in the coal seam, or water steam fed to the gasifier (Grabowski et al., 2021). The formation and composition of wastewater depend on the type of gasified coal and process conditions,

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Abbreviations

BAT	Best Available Techniques
BOD	biochemical oxygen demand
BTEX	benzene, toluene, ethylene, xylene
COD	chemical oxygen demand
CW	constructed wetland
EC	electrocoagulation
EC50	concentration causing 50% reduction in the bioluminescence of the bacterium <i>Aliivibrio fischeri</i>
HRT	hydraulic retention time
PAHs	polyaromatic hydrocarbons
RE	removal efficiency
TOC	total organic carbon
TU	toxicity unit
UCG	underground coal gasification
VFCW	vertical flow constructed wetland
WW	wastewater
WWTPs	wastewater treatment plants

including pressure, temperature, gasification agent, reaction time, use of a scrubber, etc. (Perkins, 2018). Because wastewater from UCG contains, among other substances, metals, cyanides, sulphides, phenols, polyaromatic hydrocarbons (PAHs), and benzene, toluene, ethylene, xylene (as BTEX) compounds (Kapusta and Stańczyk, 2011; Wang et al., 2024), cleaning such a complex matrix is a challenge, both technological and financial.

Generally, the primary goal of wastewater treatment is to fulfil the regulatory framework for water discharge with the least possible impact on the environment and human health. The purification methods proposed in the literature often include adsorption and electrocoagulation (Pratiwi et al., 2021; Chandra Nainwal, 2024). These treatments have been discussed separately across a wide range of applications; however, it is now known that their limitations can be surpassed through combination with constructed wetlands (Ji et al., 2022).

The phenomenon of adsorption on solids is well-known and commonly used on an industrial scale as one of the most effective, universal, environmentally friendly, and low-cost methods for blocking contaminants' mobility (Babel and Kumiawan, 2003; De Gisi et al., 2016; Kamari et al., 2014; Park et al., 2016). Adsorption is suitable for different pollutants and can be effectively applied over wide ranges of concentrations (Qin et al., 2020; Xiang et al., 2020; Ambaye et al., 2021). A wide range of solid sorption materials have been developed and used for removing solutes from solution (Gupta et al., 2009), both of natural origin (clay, natural zeolites, chitosan, pumices, plant-based adsorbents) (Qin et al., 2020; Yadav et al., 2021) and laboratory-synthesised (like activated charcoals or silica gels). Detailed reviews of adsorption techniques involving the removal of emerging contaminants from wastewater was conducted by Rathi & Kumar (2021) and Jagadeesh and Sundaram (2023). Ambaye et al. (2021) reported that biochar has good adsorption capacity for typical industrial wastewater pollutants such as potentially toxic metals, organic pollutants, phosphorus, and nitrogen compounds. Morin-Crimi et al. (2022) reviewed advanced sorption for the removal of emerging contaminants (antibiotics, hormones, pesticides, surfactants, industrial products, microplastics, nanomaterials, and many others), while toxic dye removal by sorption was described by Teo et al. (2022) and Lan et al. (2022). Adsorption depends on the degree of surface development, spatial structure, and the chemical nature of the adsorbent. For example, gasification of the coal seam may increase the number of oxygen groups involved in sorption processes, and consequently, the sorption capacity of the post-process UCG char may be higher than that of raw coal (Strugała-Wilczek et al., 2020). The second of mentioned treatment

options was electrocoagulation (EC). It also is a versatile method for the simultaneous multicomponent treatment of liquid effluents, which utilizes five main mechanisms: coagulation and flocculation, chemical oxidation, pH adjustment, electroflotation, and microbial activity. The balance between each depends on the process conditions and the type of applied electrode. Iron and aluminium are mainly used when seeking the coagulation and flocculation mechanism (Bani-Melhem and Rasool Al-Kilani, 2023; Mahmud et al., 2016); however, iron is often chosen also for its effectiveness in removing heavy metals and phosphate ions. Other electrode materials such as stainless steel (Mahmud et al., 2016), zinc (Gong et al., 2022) titanium (Trompette, 2022), or graphite (Orori et al., 2010) can be used when the process conditions are harsh (e.g., pH, temperature, salinity) or when chemical oxidation or flotation is desired (Ambaye et al., 2021).

The potential for tailoring the EC process widens its applicability, and hence EC can be successfully introduced in any process where the following types of contaminants need to be tackled: chemical oxygen demand (COD) (Bote, 2021), heavy metals (eg. Kumar et al. (2022) for Pb or Solís-Rodríguez et al. (2022) for V), organic compounds/solvents/hydrocarbons (eg. Gong et al., 2017, for PAH or Abdelwahab et al., 2009, for phenol index). The list is numerous and just to give the reader a reference, EC is also known to be able to reduce dyes, suspended solids, emulsified oils, fertilizer compounds like phosphorus and nitrogen, pesticides, and pathogens.

The most well studied areas of EC application are known to be linked to treatment of drinking water and coagulation of industrial effluents; however, EC is known for its merits also in mining (Bow et al., 2013; Bow and Tagwa, 2014; Mamelkina et al., 2017), oil and gas (Kadiy et al., 2022), petrochemicals (Akkaya, 2022), pharmaceuticals (Alam et al., 2021), semiconductors (Lee et al., 2023), food processing (Barrera-Díaz et al., 2006), milk and dairy (Ankoliya et al., 2023), agriculture and livestock (Rakhmania et al., 2022), landfilling (Sedqi et al., 2021). The major advantages of electrocoagulation can thus be summarised as simple, compact, highly reliable, cost-effective, efficient, optimisable for a given contaminant, and generating lesser amounts of waste (sludge). Often, EC is used in conjunction with other treatments, e.g., as a pretreatment before biological treatment or as a final polishing step before releasing the effluent into the environment (Szul et al., 2024).

Constructed wetlands are artificial engineered systems simulate the natural wetlands. The proven advantages of CW as green and sustainable technology have been discussed by many researchers and resulted in numerous applications (Saeed and Khan, 2019; Waly et al., 2022; Lam et al., 2024). The symbiosis between plants and rhizospheric bacteria constitutes the main factor during bioremediation process in CW systems. Plant growth promoting rhizobacteria (PGPR) promote plant growth through secretion of various compounds such as indoleacetic acid (IAA), abscisic acid (AA) and auxins. Importantly, the efficiency of wetlands in removing substantial amounts of organic and inorganic contaminants has been demonstrated by many authors (David et al., 2023; Lott et al., 2023; Saeed et al., 2021; Spiniello et al., 2023; Zhao et al., 2021). Bioremediation by constructed wetlands is increasingly applied worldwide for the removal of metals from many types of industrial effluents (Guittonny-Philippe et al., 2014). However, it is important to consider the potential challenges faced by wastewater treatment plants (WWTPs) located near industrial facilities, which may affect vegetation and inhibit plant metabolic functions. Although particulate matter emissions, including coal dust or soot emitted from coking plants, have decreased significantly in recent years, their potential impact on plants in vegetated WWTPs may still be significant, especially in regions with high air pollution (Roy et al., 2024).

The integration of physicochemical and biological technologies for the treatment of effluents from various industries has been extensively discussed. Pinedo-Hernández et al. (2024) proposed an electrocoagulation-wetland system for the treatment of landfill leachate, and Mella et al. (2017) researched a combination of

coagulation-flocculation, adsorption, and ozonation for the treatment of leather dyeing wastewater. However, there is still a limited number of research publications presenting the results of using similar approaches for the treatment of wastewaters generated from coal processing. In this context, Pratiwi et al. (2021) studied the use of electrocoagulation, adsorbents, and wetland technologies for the treatment of coke oven wastewater, while An et al. (2023) described the efficiency of coupling pulse electrocoagulation with chemical precipitation methods for the treatment of greywater from coal gasification. Zhai et al. (2024) pointed out that physisorption is more suitable for in situ UCG wastewater treatment than chemical coagulation. Until now, only Jałowicki et al. (2024) have tested the use of constructed wetlands for the treatment of UCG wastewater.

To address the aforementioned research gap, the authors sought a relatively inexpensive and convenient purification technology capable of efficiently treating UCG wastewater. Therefore, this study proposed and tested four different system configurations: wetland, electrocoagulation/wetland, adsorbent/wetland, and electrocoagulation/adsorbent/wetland. The adsorbent used was char, a by-product from the same coal gasification process as the contaminated water. A key strength of this study lies in the comprehensive analysis of effluents from each step for their physicochemical properties, including metals and trace elements, phenol index, sulphides, cyanides, total organic carbon (TOC), COD, biological oxygen demand (BOD), BTEX, PAHs, and biotoxicity.

2. Materials and methods

2.1. Wastewater and char generated from underground coal gasification process

The large-scale experimental simulation of underground coal gasification was conducted under ambient installation pressure in a surface reactor located in „Barbara” Experimental Mine, a part of GIG - National Research Institute (NRI) (Katowice, Poland). The bulk samples of bituminous coal were obtained from the „510” seam located at a depth of 850 m in „Wesola” coal mine. The UCG process was carried out using pure oxygen as the gasification reagent. During the 72 h of the process, 292.1 kg of coal was gasified, and the initial mass of the coal block was 1,365 kg. After the completion of UCG process and emptying the reactor of solid residues, the representative char samples were taken for further experiments according to the ISO 18283 standard (2006), crushed on a laboratory crusher to fractions below 10 mm and stored at 4 °C until analysis. More detailed information about the conducted experiment and char can be found in Wiatowski et al. (2023).

The total amount of wastewater produced during the gasification experiment was 1269 kg, however the real quantity of post-process wastewater obtained from coal gasification after subtraction of water volume that was added to the scrubber to cool the process gases was 261 kg (Wiatowski et al., 2023). UCG wastewater was collected in a plastic container and transported to the laboratory, where sample was filtered through a 0.45 µm pore diameter membrane filter under vacuum and stored at 4 °C until analysis.

2.2. Physico-chemical analysis of liquid and solid samples

Optimally techniques and analytical methods were selected appropriately according to the expected high concentrations of pollutants in the post-process UCG wastewater and effluents from the wetland columns. All analysis were carried out in GIG-NRI in accordance with the currently applicable standards. Key laboratory equipment used in analytical studies included but was not limited to: ICP-OES analyser Optima 5300 DV (PerkinElmer) for the determination of metals and metalloids; Ion Chromatography System Dionex ICS-5000 (Thermo Fisher Scientific) for the determination of inorganic anions; HS-GC-MS analyser (Agilent Technologies) for BTEX analysis; HPLC analyser 1200 series with FLD detector (Agilent Technologies) for the

determination of PAHs compounds.

The initial “Wesola” coal sample was tested in the GIG-NRI (Katowice, Poland) and the char sample was analysed at the Instituto de Ciencia y Tecnología del Carbono (Oviedo, Spain).

2.3. Electrocoagulation system setup

Continuous electrocoagulation experiments were conducted using a test rig developed at the Institute of Energy and Fuel Processing Technology (ITPE). The reactor consists of two chambers arranged in series. While the treated liquid flows upward in each chamber, the setup allows for independent control of the intensity of aeration and mixing. In the case of flotation, forming froth can be continuously removed using a scraper system. A stable inflow of treated liquid is maintained using a peristaltic pump, while its recovery after electrocoagulation can be accomplished either by gravity or with the assistance of peristaltic pumps. The treated wastewater is directed into the receiving tank from which effluent sampling is performed.

Two laboratory power supplies can be utilized in this system: the Twintex TP-2305 (30V DC, 2 × 5A) or the Elektro-Automatik GmbH & Co. KG PS8080-60 T (80V DC, 60A). The choice depends on the expected current demand and the operating mode of the reactor (single-stage or two-stage). Two illustrative photos of the setup are presented in Fig. 1 while Fig. 2 depicts a schematic diagram of the electrocoagulation reactor used here to pretreat UCG wastewater before phytoremediation. In Fig. 1(b) the flow is from the bottom left side of the left chamber upwards to the overflow, and similarly for the subsequent second (right) EC chamber.

The UCG wastewater was treated using a single-stage, constant-current mode. The wastewater was introduced into the reactor from an inlet located at the bottom of the reactor's side wall (left side in the figures), and the flow was kept constant at 20 dm³/h. EC was carried out using a single pair of horizontally aligned electrodes, both having equal total surface areas of 788 cm². Both electrodes consisted of four flat Fe (Ammco) plates connected by rods. The iron steel used in this research was >99.5% Fe grade 04 J according to PN 89/H-84023/02 standard, having C_{max} = 0.035%, Mn_{max} = 0.25%, Si_{max} = 0.02%, P_{max} = 0.025%, S_{max} = 0.030%, Cr_{max} = 0.10%, Ni_{max} = 0.10%, Cu_{max} = 0.10%, Al_{max} = 0.02%–0.07%. The anode and cathode were separated by 25 mm, while the power was supplied via electrical connectors located in the sidewalls of the reactor.

In this arrangement, it was found that the location of the cathode plays a role in removing the sludge that forms on the surface of the anode and causes resistance to the flow of current, affecting the structure of the flocs produced (floating flocs or sedimenting precipitate). Thus, during this series of experiments, the cathode was located below the anode. Moreover, a radial mixer was used to ensure good mixing of the feedstock entering the reactor and to guarantee turbulent flow through the electrode zone.

During the experiments, the temperature of the effluent ranged from 15 to 18 °C. The constant flow of current was regulated and maintained by the power supply. The 4.6 A current allowed here to feed into the wastewater the dose of Fe equal to 204 mg/dm³.

The basic physicochemical characteristics of raw UCG wastewater and UCG wastewater after the EC treatment were evaluated by the Laboratory of Analytical Chemistry at the Institute of Energy and Fuel Processing Technology (Zabrze, Poland).

2.3.1. Introducing sulphides and cyanides into the wastewater before the electrocoagulation treatment

Cyanides and sulphides are known to be notoriously difficult contaminants to measure due to their reactive characteristic. Hence their concentrations in solutions and speciation tend to change over time.

Based on experience, wastewaters produced during gasification of hard coals can contain well over 15 mg/dm³ of cyanides and 40 mg/dm³ of sulphides. However, due to the time gap between the UCG gasification

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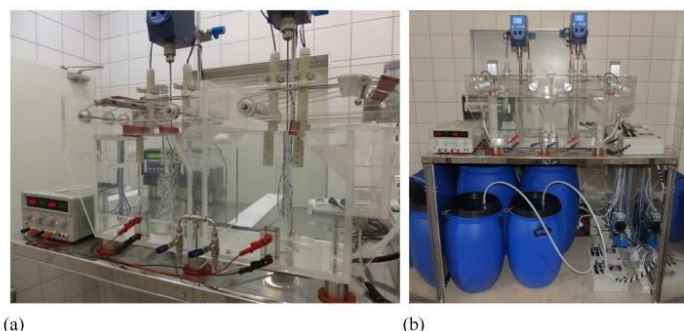


Fig. 1. Test stand for testing continuous electrocoagulation processes: (a) a close-up of both reactor chambers, agitators, scrapers, electrodes, power supply and agitators; (b) a general view of the reactor, supply and receiving tanks and pumps.

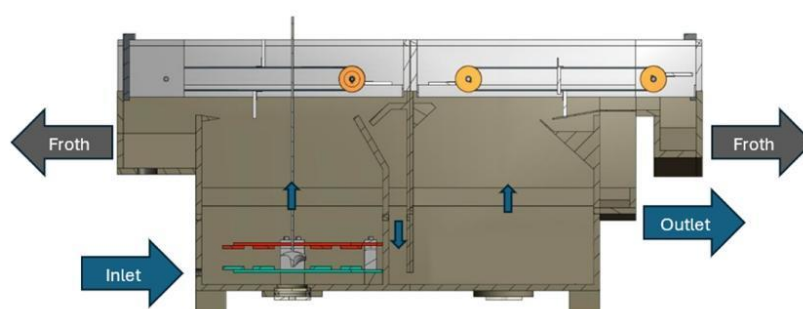


Fig. 2. Diagram of the EC reactor system used to pre-treat the UCG wastewater before its phytoremediation.

experiment and the phytoremediation studies, the samples of raw UCG wastewater intended to be treated using EC contained only traces of these contaminants. Specifically, the sulphides concentration did not exceed the lower detection limit herein used UV-VIS method ($<0.1 \text{ mg/dm}^3$), while the cyanide concentration was measured to be equal to 1 mg/dm^3 . Therefore, it was decided to artificially introduce the above-mentioned concentrations of cyanides and sulphides into the UCG wastewater before treating the effluent in EC reactor.

The pH of the UCG wastewater was in the range of 8–9, hence sulphides mainly exist in the solution in the form of undissociated, soluble H_2S (gas) and HS^- . During electrocoagulation, the pH increases, leading to an increase in the amount of available free sulphide ions. To limit the emission of gaseous hydrogen sulphide and avoid the need to artificially increase the pH of the UCG wastewater prior to its EC, sulphides were added to the solution just before the EC experiments. Cyanides are known to form complexes over time and react with heavy metals, but they can also undergo oxidation with hydrocarbons available in the solution. Compared to sulphides though, their nature is much less volatile.

The aforementioned concentrations of 15 and 40 mg/dm^3 of cyanide and sulphide ions, respectively, were achieved by adding potassium cyanide (KCN, manufactured by Thermo Scientific) and hydrated sodium sulphide (Na_2S , manufactured by Warchem Sp. z o.o.) to the solution.

2.4. Wetland columns setup

Four experimental vertical flow constructed wetlands (VFCWs) were

established, each containing 12 seedlings of common reed (*Phragmites australis*). The 0.3 m diameter columns were filled with layers of compost (0.01 m), sand (0.02 m) and gravel (0.03 m). Two columns contained an additional 0.05 m thick layer of UCG coal sandwiched between the sand layers, while the volume of the remaining layers was reduced accordingly. The wastewater was diverted through each column into 100-L tanks. After a month of stabilization with tap water, the wastewater was discharged. The hydraulic retention time (HRT) in each column was maintained at 2 days. Four experimental systems combined physicochemical and biological treatment: (1) VFCW with recirculation of raw wastewater, (2) VFCW with recirculation of wastewater after the electrocoagulation process, (3) VFCW with UCG char and recirculation of raw wastewater, and (4) VFCW with UCG char and recirculation of wastewater after electrocoagulation (Fig. 3). Physicochemical analyses of the recirculated water have started two weeks after wastewater discharge.

2.5. Toxicity assessment

Toxicity analysis of UCG wastewater, UCG wastewater after electrocoagulation and effluents from the wetland columns were carried out in accordance with the Microtox assay system (MAS) according to the standard ASTM Standard Method D5660-96, 2009 entitled "Standard Test Method for Assessing the Microbial Detoxification of Chemically Contaminated Water and Soil Using a Toxicity Test with a Luminescent Marine Bacterium". The assay measures the luminescence of the bioluminescent marine bacterium *Aliivibrio fischeri* (ATCC 49387). The measures were done using the Microtox M500 toxicity analyser (ISO

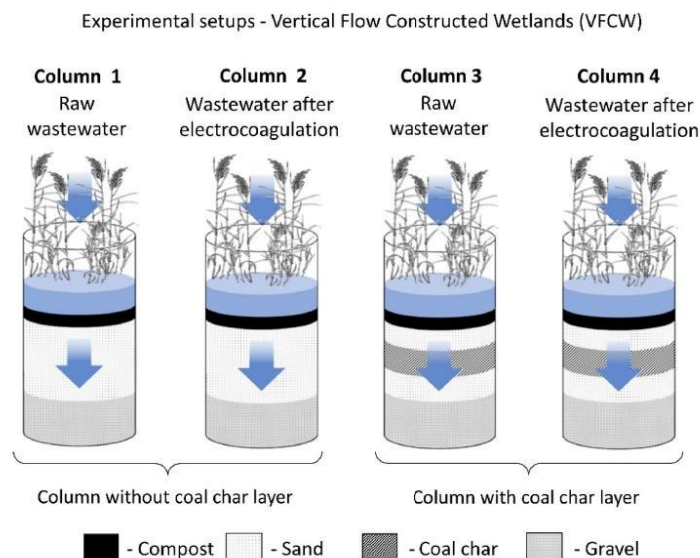


Fig. 3. Diagram of the experimental systems setup – Vertical Flow Constructed Wetlands (VFCW).

11348-3, 2007) serving both as an incubator and a photometer. The analyses were done in two stages. Initially, the basic test on non-diluted samples was carried out following the standard procedures of the manufacturer. Next, toxicity was assessed on sample solutions diluted with 2% NaCl to achieve more than a 50% effect over the initial screening. The luminescence inhibition after 15 min was taken as the endpoint. The 15 min Microtox™ test is slightly more sensitive than the 5 min test (Sönmez and Sivri, 2020). The EC50 values (concentration causing 50% reduction in the bioluminescence of the bacteria) and the corresponding 95% confidence intervals were computed using the MicrotoxOmni® Software. The lower the EC50 value, the greater the toxicity of the sample. The toxicity unit (TU) was calculated in accordance with the following equation:

$$TU = (1/EC50) \times 100 \quad \text{Eq. (1)}$$

Finally, obtained results were ranked based on TU values into one of five classes described by Persoone et al. (2003). Table 1 presents the toxicity classification based on TU values.

To evaluate the results the toxicity criteria based on the EC50 value proposed by Vasseur et al. (1986) was also used. The following toxicity criteria are as follows.

- EC50 > 100% – non-toxic samples
- 10% < EC50 < 100% – slightly toxic samples
- 1% ≤ EC50 ≤ 10% – toxic samples
- EC50 < 1% – highly toxic samples

Table 1
Toxicity classification based on TU values.

Toxicity classes	Toxicity Unit (TU)	Toxicity assessment
Class I	0	Non-toxic
Class II	<1	Slightly toxic
Class III	1–10	Toxic
Class IV	10–100	Highly toxic
Class V	>100	Extremely toxic

Each sample was run in triplicates assay. Toxicity test was evaluated in the microbiological laboratory of the Institute for Ecology of Industrial Areas (IETU) (Katowice, Poland).

3. Results and discussion

3.1. Raw coal and post-process UCG char characteristics

The results of proximate and ultimate analysis of gasified coal and post-process UCG char are presented in Table 2. The elemental analysis of the UCG char is presented in Table 3.

Due to the high carbon content and relatively small number of heteroatoms (Table 2), the gasified “Wesola” coal is poorly reactive. It is worth paying attention to the relatively high content of Cr, Zn, Cu and Pb in obtained UCG char (Table 3). Despite the UCG process being carried out in a reactive atmosphere of pure oxygen, the resulting char is characterized by small specific surface area S_{BET} ($9 \text{ m}^2/\text{g}$), whereas the total pore volume V_T was $0.005 \text{ cm}^3/\text{g}$, and the N_2 micropore volume was $0.004 \text{ cm}^3/\text{g}$. The textural parameters of the adsorbent were determined based on the nitrogen adsorption isotherm at a temperature of 77.3 K (Wiatowski et al., 2023).

Table 2
Analysis of coal and UCG post-process char (Wiatowski et al., 2023).

Parameter	Sample	
	Coal	Post-process UCG char
<i>Analytical state</i>		
Moisture W (%)	3.49	0.80
Ash A (%)	2.15	6.24
Volatile matter content V_f (%)	30.12	–
Total sulphur S_t (%)	0.21	0.21
Calorific value Q^f (kJ/kg)	31,458	–
Carbon C (%)	82.01	90.81
Hydrogen H (%)	5.18	0.77
Nitrogen N (%)	2.24	1.42

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Table 3

Elements content in the mineral matter of char derived from UCG of "Wesola" coal (Wiatowski et al., 2023).

Parameter	Post-process UCG char
Fe (%)	11.2
Mn (%)	0.2
As (ppm)	11.9
Cr (ppm)	450
Zn (ppm)	494
Cu (ppm)	562
Pb (ppm)	102
Ti (%)	0.3

3.2. Electrocoagulation pretreatment

After treating the raw UCG wastewater with EC, the percentage removal efficiency η_i was calculated using the following formula:

$$\eta_i = \left(1 - \frac{C_{i,\text{outlet}}}{C_{i,\text{inlet}}}\right) \times 100 \quad (\%) \quad \text{Eq. (2)}$$

where:

$C_{i,\text{inlet}}$ – concentration of component i at the inlet to the reactor (mg/dm³),

$C_{i,\text{outlet}}$ – concentration of component i at the outlet from the reactor (mg/dm³).

Some of the parameters and components tested in this work were noticed to have higher value at the outlet (treated effluent) than measured for the inlet (effluent fed). In such case the value of η_i has negative value, representing gain instead of removal.

As indicated before, laboratory analyses were performed on the day of the experiment and immediately after electrocoagulation pretreatment at ITPE. The concentration of BTEX and PAHs was determined in the GIG-NRI laboratories. Table 4 presents all process and efficiency data measured during EC of the UCG wastewater.

The electrocoagulation pretreatment resulted in CN⁻, S²⁻, and COD removal efficiencies of 95%, 84%, and 32%, respectively. A minor decline was also observed in the phenol index; however, the change was minimal (16%). Additionally, a beneficial effect was noted on the removal of most trace metals. The primary contaminating metal remaining in the solution was attributed to the excess of iron, while a slight increase in the concentration of Ni was also observed. Interestingly, EC also facilitated the removal of BTEX from the effluent of the UCG process. Consequently, it was determined that the continuous EC system applied here allowed for the reduction of BTEX to a level of 0.32 mg/dm³, resulting in a 91% reduction. The highest effectiveness of removal was recorded in the case of removing PAHs compounds (>99.996%), which is consistent with the research conducted by Gong et al. (2017) who found that EC treatment effectively removed a total of 86% of PAHs by mass in the effluent after 30 min treatment of industrial wastewater.

3.3. Treatment of the UCG effluents in CW setup

The raw UCG wastewater was biologically treated with a set consisting of four constructed wetland columns, and in the case of two columns, water was introduced into the system after the initial electrocoagulation procedure. The effluents from the constructed wetlands were collected and analysed after 14 and 45 days of treatment. The toxicity analysis of effluents was performed after 14, 30, 45 and 60 days. UCG wastewater treatment experiments were carried out in four different systems: CW1 (wetland), CW2 (electrocoagulation/wetland), CW3 (UCG char/wetland), CW4 (electrocoagulation/char adsorbent/wetland). Raw post UCG process wastewater was fed to the CW1 and CW3 systems, while wastewater after initial pretreatment by EC was fed to the CW2 and CW4 systems. The composition of raw water and water

Table 4

Collation of the EC process and efficiency parameters for pretreatment of UCG wastewater.

Stream	Feed ^a	Treated ^a	Removal efficiency (%)
pH (–)	8.65	8.98	–
Conductivity (µS/cm)	2000	1860	–
Redox (mV)	–135	–153	–
Free cyanide (mg/dm ³)	14.60	0.72	95.06
Sulphide (mg/dm ³)	39.20	6.18	84.24
COD (mg/dm ³)	355.70	243.2	31.63
Phenol index (mg/dm ³)	23.10	19.4	16.02
Al (mg/dm ³)	2.52	<0.125	>95.04
Fe (mg/dm ³)	0.179	2.67	–1391
Mn (mg/dm ³)	0.333	<0.02	>93.99
Ni (mg/dm ³)	0.492	0.586	–19.11
Sb (mg/dm ³)	0.080	0.07	12.5
Zn (mg/dm ³)	0.213	<0.02	>90.61
Sum of metals (mg/dm ³)	3.817	3.491	8.54
Naphthalene (mg/dm ³)	0.294	<0.000050	>99.98
Acenaphthene (mg/dm ³)	0.0105	<0.000050	>99.52
Fluorene (mg/dm ³)	0.036	<0.000050	>99.86
Phenanthrene (mg/dm ³)	0.142	<0.000050	>99.96
Anthracene (mg/dm ³)	0.042	<0.000050	>99.88
Fluoranthene (mg/dm ³)	0.135	<0.000050	>99.96
Pyrene (mg/dm ³)	0.155	<0.000050	>99.97
Benzo(a)anthracene (mg/dm ³)	0.054	<0.000050	>99.91
Chrysene (mg/dm ³)	0.154	<0.000050	>99.97
Benzo(b)fluoranthene (mg/dm ³)	0.039	<0.000050	>99.87
Benzo(k)fluoranthene (mg/dm ³)	0.024	<0.000050	>99.80
Benzo(a)pyrene (mg/dm ³)	0.036	<0.000050	>99.86
Dibenzo(a,h)anthracene (mg/dm ³)	0.018	<0.000050	>99.72
Benzo(g,h,i)perylene (mg/dm ³)	0.052	<0.000050	>99.90
Indeno(1,2,3-cd)pyrene (mg/dm ³)	0.035	<0.000050	>99.86
Sum of PAHs (mg/dm ³)	1.228	<0.000050	>99.99(6)
Benzene (mg/dm ³)	2.464	0.263	89.33
Toluene (mg/dm ³)	0.802	0.048	94.01
Ethylbenzene (mg/dm ³)	0.042	0.00012	99.71
m,p-xylene (mg/dm ³)	0.153	0.0033	97.84
o-xylene (mg/dm ³)	0.139	0.0042	96.98
Sum of BTEX (mg/dm ³)	3.599	0.320	91.11

^a Mean value.

after electrocoagulation, as well as leachates collected after 14 and 45 days from wetland columns CW1/CW3 and CW2/CW4 are presented in Table 5 and 6.

As in the case of electrocoagulation, also here the removal efficiency η_i was calculated according to Equation 2. The percent removal efficiency after 14 and 45 days of treatment for metals and metalloids, inorganic contaminants and organic contaminants is presented in Fig. 4a, b and 4c, respectively. The exact calculated values are available in the supplement in Table A.1 and Table A.2.

Due to the lack of legal regulations regarding the composition of post-process UCG wastewater and the similarity of its matrix to wastewater from the coke industry, the obtained values of individual pollutants were compared to the Best Available Techniques (BAT) conclusions document (BAT, 2012). The maximum concentrations of pollutants in treated coking wastewater recommended in BAT are: COD <220 mg/dm³; BOD <20 mg/dm³, free sulphides <0.1 mg/dm³, thiocyanides <4 mg/dm³, free cyanides <0.1 mg/dm³, PAHs <0.05 mg/dm³, phenol index <0.5 mg/dm³, and total nitrogen <15–50 mg/dm³. Concentrations obtained in the experiments that exceed the BAT levels are marked in bold in Tables 5 and 6. In relation to these regulations, all tested systems successfully treated the wastewater (WW). The only instances where limits were exceeded after 45 days of treatment involved the COD and BOD values, specifically observed in the constructed wetland (CW1). Small discrepancies in the characteristics of wastewater after the EC process can be noticed between Tables 4 and 6. These discrepancies arise from the fact that the analyses were conducted at separate times, and during storage, the samples were only refrigerated and not

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Table 5
Physicochemical composition of raw UCG post-process wastewater and changes in composition after CW1 and CW3 treatment. (CW1 – wetland, CW3 - UCG char/wetland).

Parameter	Unit	Raw WW	CW1		CW3	
		day 0	day 14	day 45	day 14	day 45
pH	–	8.4	7.8	7.2	7.8	7.7
Conductivity	µS/cm	1910	1260	919	1540	1430
Redox	mV	176	225	212	232	237
Ammonia	mg/dm ³	170	55	15	24	1.2
Total nitrogen	mg/dm ³	150 ^a	82 ^a	23	80 ^a	15
Nitrate	mg/dm ³	0.60	1.9	10	170	40
Nitrite	mg/dm ³	<0.006	2.6	0.31	23	0.90
Chloride	mg/dm ³	279	228	240	275	284
Sulphate	mg/dm ³	54.7	45	29	123	116
Total cyanide	mg CN/dm ³	2.3	0.020	0.013	0.037	0.0040
Sulphide	mg/dm ³	0.022	0.023	<0.02	<0.02	<0.02
Total phosphorus	mg P/dm ³	<0.065	3.36	0.50	0.18	0.18
BOD	mg O ₂ /dm ³	120 ^a	120 ^a	22 ^a	47 ^a	3.8
COD	mg O ₂ /dm ³	303 ^a	217	240 ^a	98	105
TOC	mg C/dm ³	80	86	90	37	45
Phenol index	mg/dm ³	15 ^a	<0.001	0.002	0.010	<0.001
Fe	mg/dm ³	0.015	4.11	2.30	0.17	0.053
Mn	mg/dm ³	0.36	0.48	0.43	0.18	0.21
Sb	mg/dm ³	0.090	0.014	0.005	0.033	0.020
As	mg/dm ³	<0.05	0.027	0.0097	0.011	0.012
B	mg/dm ³	0.30	0.32	0.29	0.45	0.41
Cr	mg/dm ³	<0.005	0.0027	0.0020	0.0011	<0.0005
Zn	mg/dm ³	0.24	0.11	0.020	0.12	0.014
Al	mg/dm ³	0.18	0.20	0.14	0.012	0.032
Cd	mg/dm ³	<0.001	<0.0005	<0.0005	0.0005	<0.0005
Co	mg/dm ³	<0.005	0.016	0.0080	0.0062	0.0059
Cu	mg/dm ³	<0.01	0.0072	0.0043	0.010	0.0073
Mo	mg/dm ³	0.0051	<0.005	<0.005	0.0078	0.012
Ni	mg/dm ³	0.54	0.073	0.020	0.071	0.013
Pb	mg/dm ³	<0.02	0.017	<0.005	0.0072	<0.005
Hg	mg/dm ³	<0.001	<0.001	<0.001	<0.001	<0.001
Se	mg/dm ³	<0.05	<0.005	<0.005	0.011	<0.005
Ti	mg/dm ³	<0.005	0.013	0.0072	0.0017	0.0014
Naphthalene	ug/dm ³	294	0.12	<0.050	<0.050	<0.050
Acenaphthene	ug/dm ³	10.5	<0.050	<0.050	0.093	<0.050
Fluorene	ug/dm ³	36	<0.050	<0.050	<0.050	<0.050
Phenanthrene	ug/dm ³	142	<0.050	0.087	<0.050	<0.050
Anthracene	ug/dm ³	42	0.08	<0.050	0.10	<0.050
Fluoranthene	ug/dm ³	135	<0.050	<0.050	<0.050	<0.050
Pyrene	ug/dm ³	155	0.10	0.091	0.12	<0.050
Benzo(a)anthracene	ug/dm ³	54	<0.050	<0.050	<0.050	<0.050
Chrysene	ug/dm ³	154	<0.050	<0.050	<0.050	<0.050
Benzo(b)fluoranthene	ug/dm ³	39	<0.050	<0.050	<0.050	<0.050
Benzo(k)fluoranthene	ug/dm ³	24.5	<0.050	<0.050	<0.050	<0.050
Benzo(a)pyrene	ug/dm ³	36	<0.050	<0.050	<0.050	<0.050
Dibenzo(a,h)anthracene	ug/dm ³	18	<0.050	<0.050	<0.050	<0.050
Benzo(g,h,i)perylene	ug/dm ³	52	<0.050	0.059	<0.050	<0.050
Indeno(1,2,3-cd)pyrene	ug/dm ³	35	<0.050	<0.050	<0.050	<0.050
PAHs (15 compounds)	ug/dm ³	1228 ^a	0.30	0.24	0.31	<0.050
Benzene	ug/dm ³	2464	<0.020	0.21	0.49	0.21
Toluene	ug/dm ³	802	0.51	<0.020	2.01	<0.020
Ethylbenzene	ug/dm ³	42	0.15	0.20	0.24	<0.020
m,p-xylene	ug/dm ³	153	0.42	2.82	0.65	<0.040
o-xylene	ug/dm ³	139	0.13	1.01	0.44	<0.020
BTEX (incl. styrene)	ug/dm ³	3599	1.21	4.2	3.8	0.21

^a concentrations exceeding the recommended values from the BAT document (2012).

otherwise stabilised. Nevertheless, these differences do not affect the interpretation of the observations discussed later.

WW after electrocoagulation was characterized by higher concentrations of NH₄, total nitrogen, chlorides and sulphates, Fe, B, Co, Mo, Ni (Table 6). Phenol index was rather constant compared to raw wastewater (Table 5).

Regarding metals and metalloids, better removal efficiency was observed in the electrocoagulated systems CW2 and CW4 (Fig. 4a). The exception was Mn, which appeared in the CW2 and CW4 effluents, and its concentration increased with the time of the experiment (Table 6). It is worth noting that Mn is released in both the CW system and the CW-adsorbent hybrid system, and only the introduction of EC achieved net

removal of this contaminant. Cd, Pb and Hg were not detected in raw water and purified leachates, which is advantageous from the environmental point of view, because these elements belong to heavy metals with highly toxic properties. An increase in concentration was observed in the case of Cu (CW4) and Zn (CW2) after 14 days of the experiment, but it occurred at a trace level (Table 6). The best effects of wastewater treatment after electrocoagulation were observed for Fe, Ni, Sb, As, and the weakest for B, Mo, and Co (Fig. 4a). B remains at a very low level and none of the tested methods demonstrated its effective and reproducible removal. It is worth noting that the longer experiment time did not have a positive effect for all elements - a decrease in removal efficiency after 45 days occurred in the case of Fe, As, Cr (CW4), Zn, Al, Co (CW2) and Ti

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Table 6

Physicochemical composition of UCG post-process wastewater (WW) after electrocoagulation (EC) and changes in composition after CW2 and CW4 treatment (CW2 - electrocoagulation/wetland, CW4 - electrocoagulation/UCG char/wetland).

Parameter	Unit	WW after EC	CW2		CW4	
		day 0	day 14	day 45	day 14	day 45
pH	–	9.1	7.8	7.4	8.2	7.6
Conductivity	µS/cm	1670	1490	1610	1720	1760
Redox	mV	100	221	204	197	202
Ammonia	mg/dm ³	190	43	0.40	56	1.0
Total nitrogen	mg/dm ³	170 ^a	75 ^a	34	80 ^a	5.5
Nitrate	mg/dm ³	<0.5	21	120	6.0	1.2
Nitrite	mg/dm ³	<0.006	32	0.53	2.6	0.12
Chloride	mg/dm ³	298	273	310	277	372
Sulphate	mg/dm ³	85.0	187	222	184	240
Total cyanide	mg CN/dm ³	8.1 ^a	0.072	0.41	0.30	0.44
Sulphide	mg/dm ³	0.14 ^a	<0.02	<0.02	<0.02	<0.02
Total phosphorus	mg P/dm ³	<0.07	0.33	0.19	0.082	0.38
BOD	mg O ₂ /dm ³	110 ^a	35 ^a	2.3	82 ^a	8
COD	mg O ₂ /dm ³	237 ^a	72	49	140	165
TOC	mg C/dm ³	65	24	25	50	67
Phenol index	mg/dm ³	16 ^a	0.018	0.0013	0.0010	0.0021
Fe	mg/dm ³	3.79	0.16	0.22	0.34	0.70
Mn	mg/dm ³	<0.005	0.024	0.11	0.12	0.44
Sb	mg/dm ³	<0.05	0.0068	<0.005	0.014	<0.005
As	mg/dm ³	<0.05	0.0065	0.0091	0.015	0.017
B	mg/dm ³	0.46	0.29	0.29	0.43	0.3
Cr	mg/dm ³	<0.005	0.0005	<0.0005	0.0009	0.0013
Zn	mg/dm ³	<0.05	0.088	0.018	0.023	0.032
Al	mg/dm ³	<0.1	<0.01	0.031	0.027	0.047
Cd	mg/dm ³	<0.001	<0.0005	<0.0005	<0.0005	<0.0005
Co	mg/dm ³	0.015	0.011	0.014	0.0099	0.0086
Cu	mg/dm ³	<0.01	0.0090	0.0065	0.016	0.0057
Mo	mg/dm ³	0.010	0.0083	0.0081	0.0096	0.0087
Ni	mg/dm ³	0.67	0.083	0.051	0.060	0.016
Pb	mg/dm ³	<0.02	<0.005	<0.005	<0.005	<0.005
Hg	mg/dm ³	<0.001	<0.001	<0.001	<0.001	<0.001
Se	mg/dm ³	<0.05	<0.005	<0.005	0.0076	<0.005
Ti	mg/dm ³	<0.005	0.0009	0.0013	0.0018	0.0033
Naphthalene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Acenaphthene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Fluorene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Phenanthrene	ug/dm ³	<0.050	0.057	<0.050	<0.050	<0.050
Anthracene	ug/dm ³	<0.050	0.12	0.98	0.080	<0.050
Fluoranthene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Pyrene	ug/dm ³	<0.050	0.10	<0.050	0.053	0.11
Benzo(a)anthracene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Chrysene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Benzo(b)fluoranthene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Benzo(k)fluoranthene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Benzo(a)pyrene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Dibenzo(a,h)anthracene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Benzo(g,h,i)perylene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	<0.050
Indeno(1,2,3-cd)pyrene	ug/dm ³	<0.050	<0.050	<0.050	<0.050	0.16
PAHs (15 compounds)	ug/dm ³	<0.050	0.27	0.98	0.13	0.27
Benzene	ug/dm ³	263	0.16	<0.020	0.76	<0.020
Toluene	ug/dm ³	48	0.73	<0.020	0.66	<0.020
Ethylbenzene	ug/dm ³	1.2	0.19	<0.020	0.19	<0.020
m,p-xylene	ug/dm ³	3.3	0.50	<0.040	0.61	1.80
o-xylene	ug/dm ³	4.2	0.17	<0.020	0.31	0.50
BTEX (incl. styrene)	ug/dm ³	320	1.75	<0.040	2.53	2.30

^a concentrations exceeding the recommended values from the BAT document (2012).

(Fig. 4a).

Better removal efficiency was observed in the system after electrocoagulation, which did not contain post-process char (CW2), what may indicate either the potential leaching of metals from this fine-grained material, or better wetland efficiency, which compared to CW4 due to the lack of an additional adsorbent layer occupied a larger active area.

It is worth noting, however, that raw water without preliminary electrocoagulation was purified of metals more effectively in the presence of adsorbent (CW3). Interestingly, the degree of purification increased with the time of the experiment, contrary to what happened in the system with a sorbent and after electrocoagulation, CW4 (e.g. for Ti, Zn, Cr). Systems without a preliminary electrocoagulation process did

not remove Fe as effectively, while Mn was removed to a relatively small extent only in the system with the CW3 adsorbent (the fractional removal of Mn was at the level of 50% and 41.7% after 14 and 45 days, respectively). The constructed wetland itself (CW1 system) relatively effectively removes most metals except Fe, Mn, B, Co, and Ti.

It seems that columns CW1 and CW3 better remove phenolic compounds from wastewater (Table 5), while the phenol index in leachates from columns CW2 and CW4 remained at a trace level (Table 6). Total nitrogen was best removed by the CW4 column, and the worst by the CW2 column, but these differences were not significant. CW2 and CW4 columns, i.e. systems in which electrocoagulation was used, were more effective in removing sulphides (Fig. 4b). In the case of nitrates, a

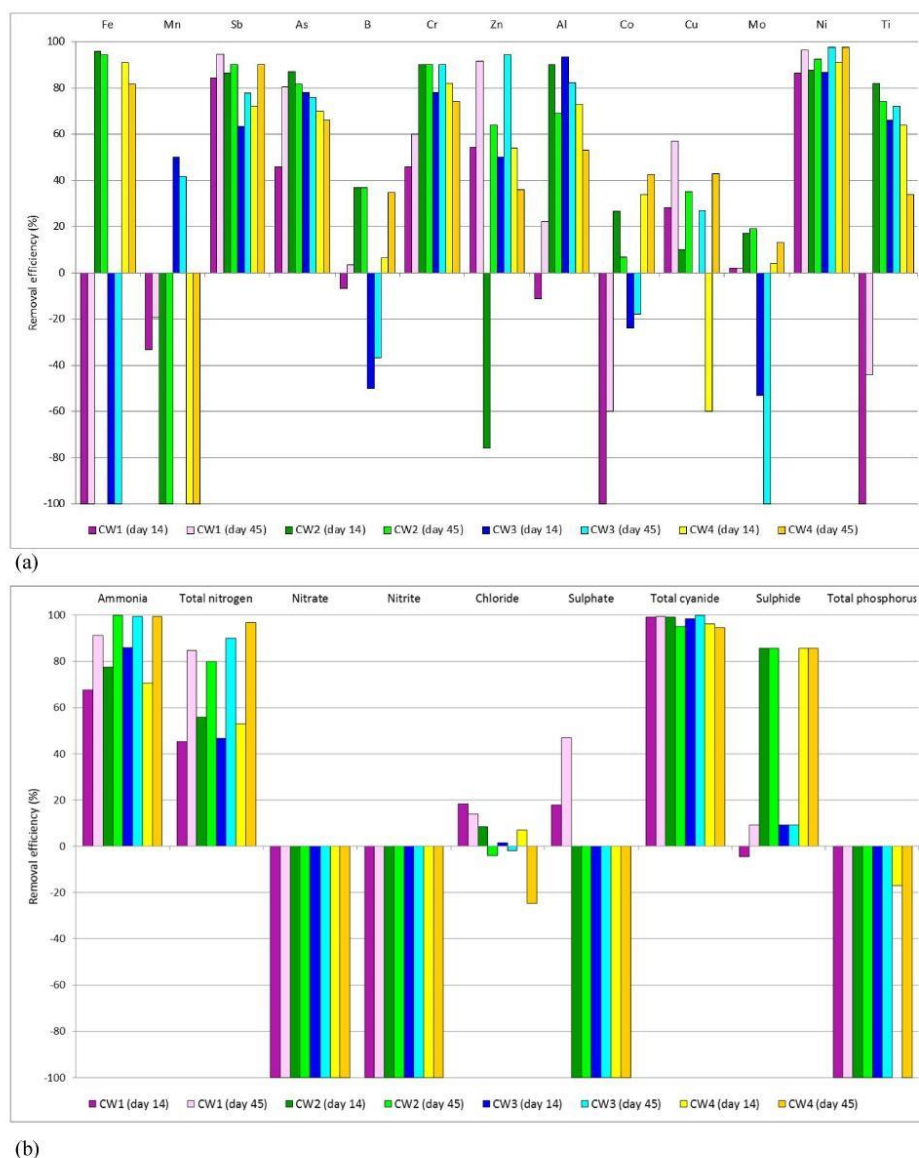
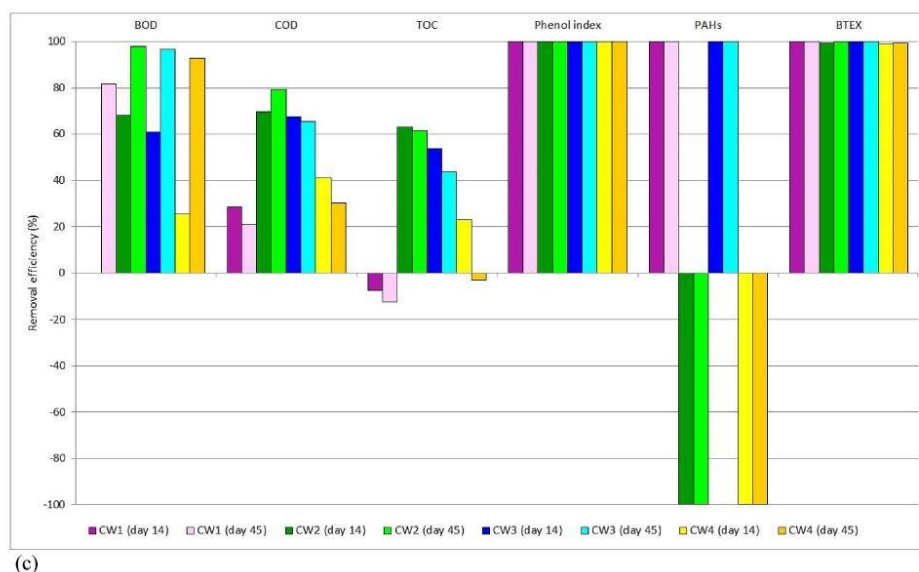


Fig. 4. Removal efficiency in tested systems: (a) metal and metalloids; (b) inorganic contaminants; (c) organic contaminants (CW1 – wetland, CW2 - electrocoagulation/wetland, CW3 - char/wetland, CW4 - electrocoagulation/char/wetland; the lower y-axis scale has been limited to 100% to maintain the readability of the chart).

significant increase in concentration after 14 days and then a decrease on day 45 was observed in the presence of the sorbent (column CW3). An initial increase and then a decrease in concentration was observed for each of the tested systems except the CW2 column (electrocoagulation/wetland). In the CW2 column, a significant increase in the concentration of nitrates in the leachate was recorded over time (Table 6), which

indicates the occurrence of conditions unfavourable to denitrification processes.

Although electrocoagulation turned out to be particularly effective in the removal of PAH compounds (from 1228 $\mu\text{g/l}$ to below 0.050 $\mu\text{g/l}$), trace amounts of these compounds appeared in the leachates obtained after the CW2 and CW4 column (Table 6), and their content increased



(c)

Fig. 4. (continued).

with over the duration of the experiment. Interestingly, raw water without electrocoagulation introduced to the CW1 and CW3 columns was purified by almost 100%, and the PAHs content decreased with the duration of the experiment, even to a level below the detection limit of $<0.050 \mu\text{g/l}$ after 45 days in the case of the CW3 column (Table 5). In the electrocoagulation process, 91.1% of compounds from the BTEX group were removed (Table 4), and then wastewater was further cleaned on CW2 and CW4 columns to the level of 99.3% and 100%, respectively (Table 6). A similar or even better effect was achieved in the CW1 and CW3 systems, which after 14 days showed practically 100% effectiveness in removing BTEX without preliminary coagulation (Table 5). The use of a preliminary coagulation procedure to purify wastewater from PAH and BTEX compounds does not generate additional benefits (Fig. 4c). The use of a fine-grained adsorbent in the form of post-process char does not affect the change in the concentration of these compounds in wastewater, although, according to Zhao (2021), sediments have a strong affinity for hydrophobic organic pollutants and therefore can be responsible for PAHs mechanism removal in CW.

Total organic carbon (TOC) reflects the total organic matter content in wastewater and can be a comprehensive indicator of organic pollution. Changes in the content of organic contaminants are shown in Fig. 5a. As can be seen, the most effective and lasting TOC decrease was recorded in the case of the CW2 system, i.e. electrocoagulation integrated with constructed wetlands. In turn, the addition of an adsorbent causes an initial decrease, but then an increase in the organic matter content in the sample.

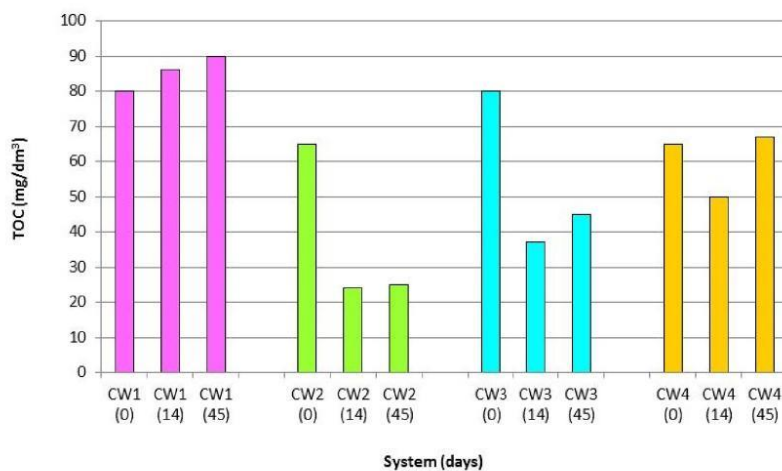
Differing biodegradability may be assessed by the COD/BOD ratio (Fig. 5b), which can be an indicator of organic matter biodegradation (Destro et al., 2020; Lindamulla et al., 2022; Pinedo-Hernández et al., 2024; Mella et al., 2017). A COD/BOD value in the range of 1.5–2.5 indicates the presence of biodegradable organic pollutants, while a higher value indicates the presence of pollutants that are hardly or not biodegradable and is characteristic for industrial wastewater (Lee&Nikraz, 2014). In the case of the tested effluents, all four samples were characterized by a favorable COD/BOD ratio of approximately 2, which, however, increased significantly between the 14th and 45th day

of purification in all tested systems (up to a maximum of 27.6 in the case of CW3) except for CW2, where it remained at 2.

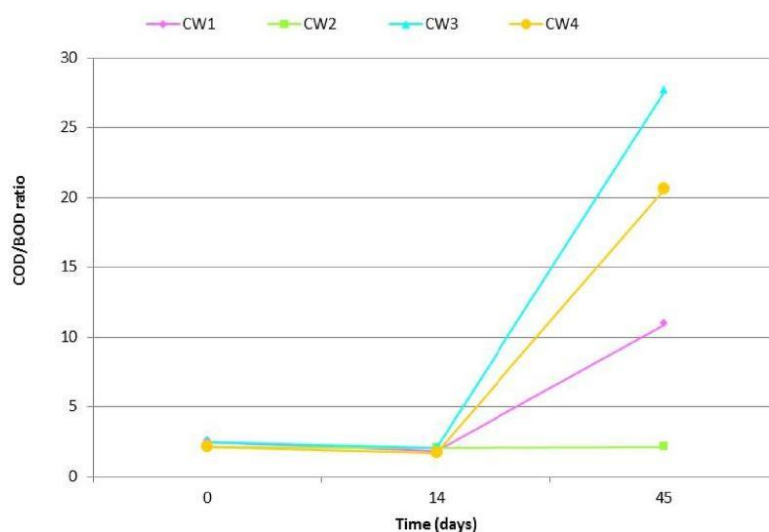
3.4. Effluents toxicity evaluation

The changes in toxicity of effluents from various treatments, as measured with the Microtox™ assay, are summarised in Table 7.

Both, UCG wastewater and UCG wastewater after electrocoagulation had remarkably high toxicity. The toxicity of effluents significantly decreased after 14 days of treatment process in the case of bioremediation treatment (wetland columns CW1 and CW2). The reduction of TU value was around 90% for both treatment systems, and the effluents were classified to IV class of toxicity. In comparison, for the systems combining bioremediation with the use of adsorbent (wetland columns CW3 and CW4) the toxicity of the effluents decreased less remarkably and the effluent maintained the same V toxicity class. In these cases, the observed decrease of leachates toxicity measured by TU values was around 75%. After 30 days of the treatment, the effluents from the columns were toxic, despite a significant decrease in the concentration of the initial compounds in raw post-processing waters (Tables 5 and 6). This is due to the formation of degradation intermediates of BTEX, PAHs, and phenols, most of which are more toxic than the initial substrates. A significant decrease in toxicity was observed after 45 days of treatment in all treatment systems. After this time, the effluents collected from wetland columns CW1 and CW2 belonged to the toxicity class no. II (slightly toxic) according to the classification of Persoone et al. (2003), while the toxicity of the same effluents evaluated based on EC50 parameter were non-toxic. Similar results were obtained after 60 days of the treatment. In just 14 days, the CW1 and CW2 systems achieved a 90 percent toxicity reduction, while over a period of 60 days the lowest toxicity value was measured in the system with the addition of adsorbent (CW4). It is also worth emphasizing that after 45 days, despite exceeding some indicators (COD and BOD), the CW1 system did not show any toxicity. Summarizing, only slightly differences were observed between toxicity assessment among four studied treatment systems and minor differences were observed when using the two toxicity



(a)



(b)

Fig. 5. Changes in organic pollutant indicators: (a) TOC; (b) COD/BOD ratio in wastewater samples treated in four different CW systems (CW1 – wetland, CW2 – electrocoagulation/wetland, CW3 – char/wetland, CW4 – electrocoagulation/char/wetland).

classification systems.

The assessment of the remediation effects of natural and constructed wetland systems has traditionally been based solely on physicochemical parameters. However, several researchers have stressed the need to include bioassays to better monitor the impact of wetland remediation technologies, incorporating various toxicity tests into their studies (Lutterbeck et al., 2018, 2020; Calheiros et al., 2019; Ashraf et al., 2020; Celma et al., 2021; Berego et al., 2022). Among these, the Microtox test

has been identified as particularly sensitive and effective in evaluating the toxicity of both treated and untreated wastewater samples (Calheiros et al., 2019). In our research, the Microtox test has proven to be a valuable complement to the commonly analysed physicochemical parameters, especially for assessing the progress of the wetland remediation process.

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Table 7

Changes of toxicity during the various treatment processes – bioremediation, adsorption, and electrocoagulation (average values, n = 3).

Experimental set up	Toxicity	Time (days)				
		0	14	30	45	60
CW1 (UCG wastewater)	EC50	0.12	1.05	1.17	104	115
	TU	835	98	85	0.96	0.86
	Toxicity classes	V	IV	IV	II	II
	Toxicity assessment based on EC50	Highly toxic	Toxic	Toxic	Non-toxic	Non-toxic
	Toxicity assessment based on TU	Extremely toxic	Highly toxic	Highly toxic	Slightly toxic	Slightly toxic
CW2 (UCG wastewater after electrocoagulation)	EC50	0.08	2.03	0.67	116	122
	TU	1204	74	65	0.8	0.75
	Toxicity class	V	IV	IV	II	II
	Toxicity assessment based on EC50	Highly toxic	Toxic	Toxic	Non-toxic	Non-toxic
	Toxicity assessment based on TU	Extremely toxic	Highly toxic	Highly toxic	Slightly toxic	Slightly toxic
CW3 (UCG wastewater + adsorbent)	EC50	0.12	0.65	1.84	91.0	118
	TU	835	153	54	1.09	0.84
	Toxicity class	V	V	IV	II	II
	Toxicity assessment based on EC50	Highly toxic	Toxic	Toxic	Non-toxic	Non-toxic
	Toxicity assessment based on TU	Extremely toxic	Highly toxic	Highly toxic	Toxic	Slightly toxic
CW4 (UCG wastewater after electrocoagulation + adsorbent)	EC50	0.08	3.01	2.2	98.0	129
	TU	1204	230	150	2.1	0.70
	Toxicity class	V	V	V	III	II
	Toxicity assessment based on EC50	Highly toxic	Toxic	Toxic	Slightly toxic	Non-toxic
	Toxicity assessment based on TU	Extremely toxic	Highly toxic	Highly toxic	Toxic	Slightly toxic

4. Conclusions

Evaluation of the applicability of the integrated process of four systems combining electrocoagulation, constructed wetlands and adsorption for the effective treatment of UCG wastewater showed that all tested systems were effective in removing contaminants. Verifying the effectiveness of hybrid treatment systems on wastewater with such a complex matrix enable their application to contaminated wastewater from other industrial processes. The conducted research allowed for the formulation of several important conclusions.

1. Combination of EC and CW proved effective in removing PAHs. CW was found to be more effective for the removal of phenolic compounds and BTEX. Due to the physicochemical properties of the tested UCG wastewater, EC is not a required pre-treatment for the use of CW.
2. Electrocoagulation is necessary in the case of Fe, B, Co, Mo removal. Regarding the efficiency of both EC and CW for the removal of metals and metalloids, the best effects were observed for Fe, Ni, Sb, As, Cr, Zn, Al, and Ti and the weakest for Mn, B, Co, Cu and Mo.
3. Longer CW treatment time (45 days vs 14 days) does not entail a positive effect on all tested physicochemical parameters and it may have an adverse effect in the case of COD, TOC, and some metals (Fe, Mn, As, Cr, Zn, Al, Co and Ti). Not only the selection of the appropriate method, but also the optimization of treatment duration seems crucial for the wastewater treatment.
4. The use of an adsorbent in the form of post-process UCG char does not change the treatment method by constructed wetlands, however, it may reduce removal of some metals (Sb, As, Cr).
5. For a 14 days treatment, EC-CW system turned out to be the most effective in toxicity reduction. Introducing an adsorbent into the system may improve its efficiency in the long term, as seen in the example of adsorbent-EC-CW system. Effective toxicity reduction from V class to II class after 60 days in comparison with meeting the requirements contained in the BAT document, shows that all tested systems were favorable in terms of UCG wastewater treatment.

6. No significant difference was observed in terms of testing the toxicity of individual systems. Studies have shown that toxicity assessment should be one of the evaluation criteria for industrial wastewater treatment. This approach is essential for effectively evaluating the performance of wastewater treatment based on natural or constructed wetland systems.

Obtained laboratory-scale results can be the basis for designing effective, profitable, and environmentally friendly purification system in a pilot scale.

CRedit authorship contribution statement

Aleksandra Strugała-Wilczek: Writing – original draft, Visualization, Investigation, Conceptualization. **Łukasz Jałowiecki:** Writing – original draft, Methodology, Investigation. **Mateusz Szul:** Writing – original draft, Project administration, Investigation, Conceptualization. **Jacek Borgulat:** Writing – review & editing, Visualization. **Grażyna Plaza:** Writing – original draft, Project administration, Investigation. **Krzysztof Stańczyk:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jenvman.2024.123180>.

Data availability

Data will be made available on request.

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Jałowiecki Ł., Strugała-Wilczek A., Ponikiewska K., Borgulat J., Płaza G., Stańczyk K.,
Constructed wetland as a green remediation technology for the treatment of wastewater from
underground coal gasification proces, Ploze one, 2024, 19(3), 1-19.
DOI: <https://doi.org/10.1371/journal.pone.0300485>.

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Jałowiecki Ł, Borgulat J, Strugała-Wilczek A., Glaser M., Płaza G. Searching of phenol-degrading bacteria in raw wastewater from underground coal gasification process as suitable candidates in bioaugmentation approach, J. Ecol. Eng. 2024; 25(2):62-71.
DOI:<https://doi.org/10.12911/22998993/176143>.

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Jałowiecki Ł, Borgulat J, Strugała-Wilczek A., Stańczyk K., Jastrzębski J., Babis W., Płaza G., A look at biofilm on the coal waste-derived adsorbent enhanced wetland system
Ecohydrology & Hydrobiology 2025, DOI <https://doi.org/10.1016/j.ecohyd.2024.12.005>.

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Niniejszym podaję procentowy wkład autorski w publikacji

Borgulat J., Ponikiewska K., **Jałowiecki Ł.**, Strugała-Wilczek A., Płaza G., Are wetlands as an integrated bioremediation system applicable for the treatment of wastewater from underground coal gasification processes? Energies 2022,15, 4419.

DOI:<https://doi.org/10.3390/en15124419>.

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Strugała-Wilczek A., **Jałowiecki Ł.**, Szul M., Borgulat J., Płaza G., Stańczyk K. A hybrid system based on the combination of adsorption, electrocoagulation, and wetland treatment for the effective remediation of industrial wastewater from underground coal gasification (UCG). Journal of Environmental Management 2024, 371: 123180.
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