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Microbial consortia in mine water bioremediation: principles, design and practical applications

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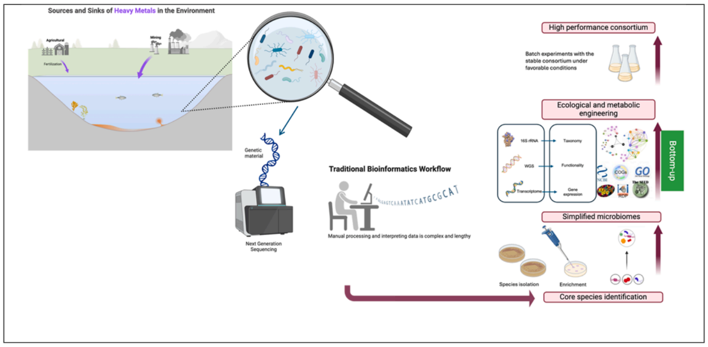
HIGHLIGHTS

- Functional focus may overlook microbial interactions limiting bioprocess efficacy.
- Key design principles (complementarity and stability) are often disregarded.
- Metagenomics–network integration identifies keystones for mine-water consortia.
- A multi-step framework supports predictive, next-generation consortia design.

GRAPHICAL ABSTRACT



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ABSTRACT

The impact of mining activities on water sources is a global concern, especially in water-scarce countries such as Southern Africa, Mediterranean region, western Asia, and South America. Bioremediation emerges as a feasible and attractive alternative to address this environmental issue. However, while biological sulfate reduction and the emerging use of using microbially induced carbonate precipitation (MICP) for remediating polluted mine water have gained attention, strategies for designing effective microbial consortia have seen little advancement. The performance of microbial consortia in these treatments can be quite variable. Most improvement strategies have focused mainly on bioreactor design and selecting suitable carbon sources, addressing technical aspects while neglecting the central players in this process: the microbes themselves. Enhancing consortium effectiveness requires revisiting foundational concepts such as monoculture, co-culture, division of labor, and bottom-up versus top-down approaches. While these concepts offer significant theoretical potential to improve consortium performance, they have seldom been applied in practice for mine water bioremediation. In this literature review, we revisit these principles and explore the integration of novel tools such as metagenomics and bioinformatics. These approaches can deepen our understanding of indigenous microbial diversity, uncover dynamic

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interactions among microbial species, and identify keystone species as potential candidates for bioremediation. By leveraging their genomic potential, it becomes possible to design consortia that are more efficient and better suited to support the recovery of contaminated water sources.

1. Introduction

According to the World Health Organization, many mineral-rich countries are experiencing increasing water stress due to mining. These include the United States, Australia, South Africa, India, China, Mongolia, Russia, Mexico, Chile, and Namibia (Miranda, 2010; Meißner, 2021). Under a business-as-usual scenario, the number of critical mineral sites facing high or extremely high water stress is expected to increase to 20 % by 2050 (Kunz, 2020). While mining activities are often associated with waste production that leads to the formation of alkaline or acid mine drainage, a closer examination reveals that these operations also significantly deplete water sources due to extraction processes (Gunson et al., 2012). For instance, the global annual water consumption associated with coal mining is between 7 and 9 billion m^3 of water (Hasii and Gasii, 2024). In China, on average, 1.32 m^3 of water resources are depleted, 0.88 m^3 of water resources are polluted, and 0.17 m^3 of water ecological environment is destroyed for every ton of coal mined (Alun and Li, 2017; Masood et al., 2020). Metal extraction also contributes to resource depletion and environmental degradation. For example, extracting one ton of copper consumes approximately 370 m^3 of water, while lithium extraction can require an estimated 2200 m^3 (Kaunda, 2020).

A major concern is that the wastewater generated during processing, containing toxic metals and salts, is frequently discharged into pit lakes (Bernasconi et al., 2022). Pit lakes are water bodies created when open pits fill with water after opencast mining has stopped. It is worth mentioning that pit lakes can form from the flooding of open mines, where water-rock interactions critically influence water quality (Blanchette and Lund, 2016; McCullough, 2024). Flooding from mineral processing can exacerbate concentrations of salts and toxic metals (Newman et al., 2023). South Africa has more than 200 pit lakes mainly resulting from coal mining in major coalfields such as Mpumalanga/-Witbank/Highveld, KwaZulu-Natal, and Waterberg (Kennedy and Johnstone, 2018; Johnstone et al., 2019; Magagula et al., 2024). These pit lakes can collectively contain millions of litres of water, with some estimates suggesting that individual pit-lakes may hold volumes ranging from hundreds of thousands to several million m^3 (McCullough et al., 2020). Currently, the recovery of water sources contaminated by mining is a top global priority, and a range of efforts and strategies have been implemented to enhance water quality (McCullough et al., 2020).

Since the 1980s, biological sulfate reduction has gained attention as an attractive solution for treating mine water (Oleszkiewicz et al., 1989; Hamilton, 1998). This technology has been described as a sustainable method for managing acid mine drainage laden with high concentrations of metals. However, in alkaline mine water with moderate metal loads, biological sulfate reduction may not be the optimal choice. In such scenarios, the high concentration of hydrogen sulfide gas can accumulate without reacting, resulting in an extremely toxic byproduct. In these cases, the precipitation of sulfide as elemental sulfur has been proposed as a commercially viable byproduct (Jorjani and Ghahreman, 2017). Currently, microbial-induced calcite precipitation (MICP) via ureolytic processes could serve as an alternative solution for more effectively addressing metal and salt concentrations than sulfate-reducing bacteria (SRB) (Torres-Aravena et al., 2018; Castro-Alonso et al., 2019; Castillo et al., 2023). MICP seems particularly effective for treating alkaline mine water, as it facilitates the removal of calcium and magnesium as carbonate minerals, which act as a sink coprecipitating divalent metals (Kim et al., 2021; Song et al., 2022; Dong et al., 2023). Unfortunately, like SRB, MICP induced by ureolytic reactions generates ammonium with the potential to deter water quality (Lee et al., 2019; Gowthaman et al., 2022; Mohsenzadeh et al., 2022). The accumulation of ammonium can also inhibit ureolytic activity and affect the overall effectiveness of the bioremediation process (Lauchnor et al., 2015). The addition of calcium chloride to inhibit ammonium production during ureolysis is a strategy that can enhance the efficiency of MICP processes (Erdmann and Strieth, 2023).

Most mitigation efforts have emphasized chemical approaches. In contrast, biological process optimization has mainly focused on bioreactor design and carbon-source selection to improve microbial activity (Mohsenzadeh et al., 2022). This approach has overlooked the critical role of the microorganisms that catalyse these processes, highlighting a need for a more holistic understanding of the biological mechanisms involved.

Many existing microbial consortia used in bioremediation strategies remain poorly characterized in terms of their composition, dynamic, and genomics (Kuppan et al., 2024). It is unclear whether these consortia are monocultures (consisting of single microbial species), co-cultures (mixtures of *Multiple species*), or display division of labour (specialized microbial populations working together) (Roell et al., 2019; McCarty and Ledesma-Amaro, 2019; Yu et al., 2021; Wu et al., 2023; Yang et al., 2024). Empirically, these consortia have been built using top-down approaches that rely on trial and error, rather than bottom-up strategies that aim to systematically assemble and engineer microbial populations with a specific purpose (Xinxin Li et al., 2022; Liang et al., 2022). While these concepts hold substantial theoretical promise for enhancing consortium performance, they have rarely been applied in practical scenarios for mine water bioremediation.

Nevertheless, the bottom-up perspective seems to be a more integrative system that considers the genetic capabilities of individual microorganisms and their interactions when designing a synthetic microbial consortium (Lin, 2022; San León and Nogales, 2022; Lyu et al., 2024). Currently, next-generation sequencing (NGS) provides valuable insights into microbial composition, allows for the inference of interactions through mathematical correlation, and reveals the genomic potential of both communities and individual species (Song et al., 2014; Goyal et al., 2022; Camacho-Mateu et al., 2024). This genetic information could be the missing link in the bottom-up approach. In this literature review, we will revisit these concepts and explore the integration of innovative methods such as metagenomics and bioinformatics. These tools can enhance our understanding of indigenous microbial diversity, illuminate the dynamic interactions among microbial species through network analysis, and identify keystone species that may serve as promising candidates for bioremediation. By leveraging the genomic potential of these organisms, we can design consortia that, while not necessarily optimal, are more efficient and could significantly contribute to the recovery of contaminated water sources.

2. What is the importance of understanding indigenous microbial diversity in polluted environments?

Understanding indigenous microbial diversity in polluted environments is crucial, especially in the context of ecosystem health and natural attenuation. In the context of natural attenuation, indigenous microbiota serves as a foundation for developing bioremediation strategies. Autochthonous microorganisms play a pivotal role in the biogeochemical cycle of toxic inorganic compounds and the restoration of ecological balance. These microbes have evolved unique metabolic pathways specifically adapted to degrade pollutants in their surroundings. Research has shown that they can effectively immobilize heavy metals both extracellularly and intracellularly, making them crucial for the natural attenuation of inorganic contaminants (Diels et al., 2010; Kumari et al., 2016; Jing and Kjellerup, 2018). Due to their adaptation to local environmental conditions and the development of resistance mechanisms, indigenous microbes often outperform non-indigenous species in pollutant biotransformation (Mishra et al., 2021; Racić et al., 2023). The diversity of indigenous microbial communities and functionality contributes significantly to the resilience of ecosystems impacted by pollution. These microbes uphold essential ecological functions such as nutrient (including metals) cycling and organic matter decomposition, which are critical for maintaining ecosystem stability (Huang et al., 2019; L. Ma et al., 2020). In particular, the microbiome strongly influences the biogeochemical cycles of metals, which in-/directly induce the immobilization of toxic elements improving the ecosystem health. This natural attenuation, in some cases, has been further enhanced through biostimulation techniques (i.e., addition of external carbon sources) (Dangi et al., 2019; Ayilara and Babalola, 2023). In addition, understanding the dynamics of these indigenous microbial communities can help to optimize natural attenuation strategies. The interactions among indigenous microbial species can significantly affect their collective ability to immobilize inorganic pollutants. Indigenous microbial interactions strongly influence the community's capacity to immobilize inorganic pollutants. Compounds released by some species can promote or inhibit neighbouring microbes, shaping community function and affecting pollutant-microbe interactions (Hibbing et al., 2010; Bittleston et al., 2020). Thus, through interactions, the indigenous consortia act as "functional guides" in which both natural and synthetic microbial communities are assembled. With these guides set in place, synthetic microbial consortia can have their metabolic pathways and social interactions programmed to achieve specific functions, unlike wild microbial consortia.

3. What is the current state of bioremediation strategies for mine drainages?

In bioremediation, microbial communities play a crucial role in the degradation or immobilization of organic and/or inorganic contaminants. Inorganic compounds, such as toxic metals, sulfates, and salts (e.g., calcium and magnesium), can be immobilized through biomineralization processes, with sulfate reduction and ureolysis being among the most commonly employed methods (Lai et al., 2021; Wang et al., 2023; Yu et al., 2023). These biological processes facilitate the precipitation of free metal ions as newly formed mineral phases. The resulting precipitates are often stable, effectively preventing secondary contamination and offering a promising alternative for the decontamination of water sources impacted by mining activity (Torres-Aravena et al., 2018; Dong et al., 2023).

Heavy metal remediation through biomineralization is a highly intricate process involving the interplay of biological, physical, and chemical mechanisms. This process is influenced by multiple factors, including temperature, pH, redox potential, carbon sources, and microbial community dynamics. Two primary strategies can enhance biomineralization efficiency. Bioaugmentation involves introducing native or engineered microbial consortia to degrade or immobilize pollutants, while biostimulation promotes the activity of indigenous microbes by optimizing their growth conditions. Combined, these approaches improve contaminant removal and facilitate mineral formation (Romantschuk et al., 2023). Based on these approaches, numerous bioremediation strategies have been developed to treat mine drainage, focusing on the immobilization of toxic metals. The following is a summary of the early and current state of bioremediation strategies for acid and alkaline mine drainage:

3.1. Early bioremediation strategy: biological sulfate reduction

Prior to the application of biological remediation, acid mine effluents were primarily treated using physical and chemical methods (Anekwe and Isa, 2023). These methods include the soil vapour extraction (SVE), encapsulation method, in-situ isolation, and the use active alkaline chemical neutralization methods such as limestone, dolomite, lime, periclase (Skousen, 2014; Ejechi and Ozochi, 2015). Although effective for short-term pH correction and metal removal, these approaches often fail to achieve long-term remediation (Vidonish et al., 2016). Their limitations include high operational costs from chemical and energy inputs, sludge generation requiring disposal, instability under variable water chemistry, and risks of secondary pollution such as brine or ammonium release (Lee et al., 2019; Anekwe and Isa, 2023; Erdmann and Strieth, 2023; Fu et al., 2023; Lai et al., 2023). These limitations have led to increased interest in biological strategies, such as sulfate reduction (SR), for bioremediation due to their sustainability and cost-effectiveness.

Acid mine drainage (AMD) is not the only source of water pollution associated with mining activities; alkaline mine drainage has also become a significant public concern. This is largely due to the presence of hardness and divalent metals in solution, which can leach into the soil and contaminate groundwater. However, sulfate-reducing bacteria (SRB) are not an effective option for treating alkaline mine drainage. Although sulfate concentrations may be high, the moderate to low levels of divalent metals are insufficient to fully react with the hydrogen sulfide (H_2S) produced. Secondary H_2S formation is a critical operational and environmental risk in biological sulfate-reduction treatments (Kushkevych et al., 2021). H_2S is toxic to aquatic life and humans (acute exposure causes respiratory irritation, neurological effects and at high concentrations can be fatal), and it induces metal corrosion and odour complaints in downstream environments (Batterman et al., 2023; Malone Rubright et al., 2017). In addition to direct toxicity, free H_2S can escape as a gas or react with dissolved metals producing metal sulfides that may either immobilize metals or re-mobilize under changing redox/pH conditions (secondary contamination risk) (Lewis, 2010).

A critical comparison of existing technologies reveals distinct trade-offs in performance, sustainability, and operational demand (Table 1). Physico-chemical treatments are rapid but costly and generate secondary waste; biological processes such as sulfate reduction and MICP offer lower sludge generation and metal selectivity but face stability and rate limitations. This underscores the potential for hybrid or optimized systems that combine biological and chemical processes.

3.1.1. Strategies to enhance SRB activity and treatment efficiency

- *Co-culturing and microbial synergy:*

More recently, hybrid systems that combine active and passive technologies, particularly integrated systems employing sequential or stepwise treatments, have been explored for AMD remediation and valorisation within the framework of a circular economy (Masindi, 2022). Since these systems can efficiently prevent pollution and be utilized for closed-loop and sustainable AMD control, they are cleaner production systems, despite the substantial costs associated with installation and operation.

Research demonstrates that SRB can effectively treat acid mine drainage, thriving in acidic conditions and immobilizing metals as sulfide minerals under anaerobic conditions (Kolmert and Johnson, 2001; Neculita et al., 2007). Sánchez-Andrea et al. (2013) emphasized the potential of acidophilic SRB to effectively reduce sulfate levels and raise pH through the process of dissimilative sulfate reduction. The bioaugmentation of these bacteria could promote the precipitation of insoluble metal sulfides, thereby reducing sulfate concentrations and significantly improving water quality. Co-culturing SRB with other microorganisms can significantly enhance sulfate reduction activity. For instance, Di et al. (2022) explored the dynamic treatment of AMD using *Rhodopseudomonas spheroides* activated lignite immobilized sulfate-reducing bacteria (SRB) particles. The results demonstrated that the combination of *R. spheroides* and lignite continuously supplied a

Table 1
Comparative evaluation of mine water treatment technologies.

Technology	Key mechanism	Operating conditions	Advantages	Limitations / Challenges	Typical removal efficiency	Representative references
Physico-chemical	Chemical neutralization and metal precipitation	pH 7–10; high reagent and energy input	Fast removal; simple operation	High cost; sludge disposal; inefficiency at low metal concentrations; secondary pollution	60–90 %	(Ejechi and Ozochi, 2015, 2015; Erdmann and Strieth, 2023)
Active Biological Sulfate Reduction (BSR)	Anaerobic sulfate reduction forming metal sulfides	pH 5–8; requires electron donor	Selective metal recovery; low sludge; adaptability	Slow start-up; requires carbon source; pH-sensitive	80–99 %	(Yang et al., 2021; Masindi, 2022; Liu et al., 2024)
Passive BSR (constructed wetlands, permeable reactive barriers)	Natural sulfate reduction in organic substrate zones	Ambient; minimal energy input	Low cost; sustainable	Clogging; variable efficiency; limited control	50–85 %	(Neculita et al., 2007; Vidonish et al., 2016; Rakotonimaro et al., 2017; Neff et al., 2021)
MICP (ureolysis)	Biogenic $CaCO_3$ precipitation via microbial metabolism	Aerobic or anoxic; pH >7	Produces stable minerals; CO_2 capture potential	Ammonium accumulation; substrate cost; uneven precipitation	70–95 %	(DeJong et al., 2010; Gat et al., 2017; Lee et al., 2019; Wang and Nackenhorst, 2020)
Hybrid Systems (e.g., lime pre-treatment + BSR or MICP)	Sequential chemical–biological process	Variable; multi-stage	High adaptability; improved pH buffering and stability	Higher CAPEX; design complexity	Up to 99 %	(Hu et al., 2013; Masindi, 2022; Peng et al., 2024)

carbon source for the SRB, enhancing the removal rates of SO_4^{2-}, Cu^{2+}, and Zn^{2+} from AMD, achieving 93.97 %, 98.52 %, and 94.42 %, respectively.

- *Substrate optimization and organic carbon sources:*

Research has also explored various substrates and organic materials to enhance the efficiency of bioremediation processes. For example, Jamil and Clarke (2013) reported that the use of organic solid waste as carbon sources for SRB can improve the performance of bioremediation systems. Such approaches, use tobacco waste, horse manure, or legume compost as a substrate for SRB (Castillo et al., 2012; Dovorogwa and Harding, 2022). Horse manure was the most successful organic substrate to promote SRB activity (sulfate removal of 61 %), followed by vegetal cover (49 %) and legume compost (31 %). Leveraging organic solid waste as a carbon source offers a promising approach to promote sulfate reduction, especially when combined with lignolytic microbes to boost the biological activity (Di et al., 2022).

- *Engineered and hybrid reactor configurations:*

The integration of engineered systems, including bioreactors, has further improved sulphate reduction. In particular, passive bioreactors have shown potential to achieve high metal removal rates while minimizing operational costs (Neculita et al., 2007; Grettenberger et al., 2017). One notable hybrid design is the Barium Carbonate Disperse Alkaline Substrate (BDAS) system, which couples biological sulfate reduction with chemical alkalinity buffering (Gómez-Arias et al., 2021; Castillo et al., 2023). In this system, dispersed barium carbonate provides both an alkaline medium and a sustained carbonate supply, supporting microbial sulfate reduction while promoting metal sulfide and carbonate precipitation. This integration enhances pH stability, minimizes sulfide toxicity, and improves overall treatment resilience under variable mine-water conditions. Thus, BDAS serves as an example of a sustainable hybrid system that efficiently remediates AMD with lower sludge generation and reduced operational demands (Gómez-Arias et al., 2021).

3.2. Current bioremediation approach: microbially induce carbonate precipitation (MICP)

Microbially induced calcite precipitation (MICP) has emerged as a promising alternative for treating alkaline mine drainage when SRB are ineffective. Thus, MICP promotes carbonate mineral formation, providing a stable sink for toxic metals and aiding in their immobilization. This process can be driven by several metabolic pathways (Table 2), including denitrification (Van Paassen et al., 2010; Erşan et al., 2015), sulfate reduction (Van Lith et al., 2003; Bontognali et al., 2014), and urea hydrolysis (Taharia et al., 2024; Torres-Aravena et al., 2018).

In denitrification-driven MICP, nitrate-reducing bacteria oxidize organic substrates while reducing nitrate (NO_3^-) to nitrogen gas (N_2), generating alkalinity that promotes carbonate precipitation. This process is suitable for oxygen-limited environments and avoids ammonium generation, making it environmentally preferable to ureolysis. Denitrifiers such as *Pseudomonas stutzeri* and *Paracoccus denitrificans* have demonstrated high $CaCO_3$ yields and efficient heavy-metal co-precipitation (Anbu et al., 2016; Fang et al., 2022). However, controlling nitrate dosing and avoiding nitrite accumulation remain practical challenges for large-scale deployment. Similarly, sulfate reduction-driven MICP, mediated by SRB, induce carbonate precipitation by generating bicarbonate (HCO_3^-) and hydrogen sulfide (H_2S) during sulfate reduction. The resulting increase in alkalinity supports $CaCO_3$ formation while simultaneously precipitating metals as sulfides. This dual mechanism offers long-term stabilization of contaminants in mine-water treatment systems. However, sulfide toxicity and system sensitivity to oxygen intrusion can limit sustained operation, prompting interest in integrating SRB-driven MICP with buffering agents or hybrid reactor designs.

While denitrification and sulfate-reduction pathways hold promise for passive and hybrid mine-water treatment systems, ureolysis (Fig. 1) provides benefits such as inexpensive substrate, high efficiency, and straightforward reaction control when compared to other approaches (Liu et al., 2021; Lai et al., 2023). Furthermore, regardless of metal valence, toxicity, or redox potential, ureolytic microbes may efficiently immobilize metals in carbonate minerals by precipitation or coprecipitation when used for heavy metal remediation (Kumari et al., 2016).

During ureolysis, one mole of urea is catalytically hydrolyzed intracellularly by urease (EC 3.5.1.5) to one mole of ammonia and one mole of carbamic acid (Anbu et al., 2016).

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \tag{1}$$

The carbamic acid is then spontaneously hydrolyzed to ammonia and carbonic acid.

Table 2
Comparison of the three main microbially induced calcium carbonate precipitation (MICP) pathways (ureolysis, denitrification, and sulfate reduction) highlighting their key mechanisms, advantages, limitations, and suitability for mine-water bioremediation applications.

Pathway	Key mechanism	Advantages	Limitations	Best use context
Ureolysis	Urease-producing microbes hydrolyze urea → ↑ pH, ↑ CO_3^{2-} → $CaCO_3$ precipitation	• Rapid $CaCO_3$ formation • Well-characterized and controllable • Works efficiently in alkaline environments	• Produces NH_4^+ → potential groundwater contamination and eutrophication • Requires urea supply	Controlled, engineered systems where ammonium removal (e.g., via anammox or HN–AD) is integrated
Denitrification	Nitrate-reducing microbes generate alkalinity during NO_3^- → N_2 reduction	• No ammonium by-products • Operates in low-O_2 environments• Efficient heavy-metal co-precipitation	• Requires nitrate source and carbon donor • Risk of nitrite accumulation	Subsurface or semi-anaerobic mine-water systems; environmentally sensitive sites
Sulfate reduction	SRB produce HCO_3^- and H_2S → induces $CaCO_3$ and metal sulfide precipitation	• Dual metal removal via sulfide and carbonate precipitation • Works in sulfate-rich mine water • Produces stable minerals	• Sensitive to oxygen • H_2S toxicity and odor • Slow startup	Acidic, sulfate-rich mine drainage; systems integrated with hybrid reactors or biofilters

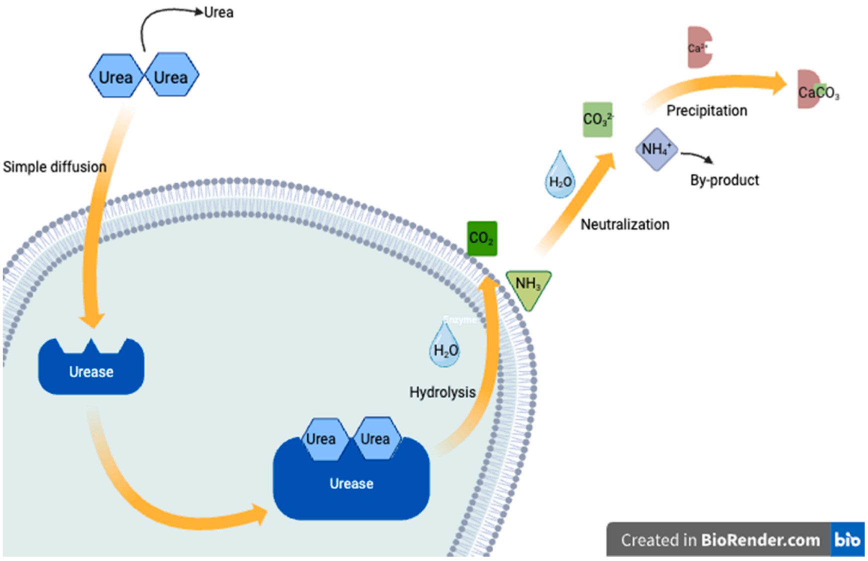

Fig. 1. A schematic diagram of microbially induced carbonate precipitation (MICP) based on urea hydrolysis. Urease-producing bacteria hydrolyze urea, generating carbonate ions and increasing pH. The resulting alkaline conditions promote calcium carbonate ($CaCO_3$) precipitation, which binds soil particles or fills pores, while also enabling the co-precipitation or immobilization of metals within or on the carbonate minerals, reducing their mobility and bioavailability.

$$NH_2COOH + H_2O \rightarrow H_2CO_3 + NH_3 \tag{2}$$

The ammonia diffuses out of the cell and dissociates, forming hydroxide ions.

$$2NH_3 + 2H_2O \leftrightarrow 2NH^{4+} + 2OH^- \tag{3}$$

This raises the pH in the area surrounding the cell, which shifts the carbonic acid balance, resulting in the formation of carbonate ions.

$$2OH^- + H_2CO_3 \rightarrow CO_3^{2-} + 2H_2O \tag{4}$$

When sufficient ionic activity is present, carbonate can precipitate out of solutions in the presence of divalent cations (DeJong et al., 2010; Dhami et al., 2013; Zhang et al., 2023). Heavy metal ions with ion radio similar to Ca^{2+} (1.0 Å), such as Cu^{2+}, Cd^{2+}, Pb^{2+}, Zn^{2+}, and Sr^{2+}, can be incorporated into the $CaCO_3$ (Achal et al., 2011; Jain and Arnepalli, 2019). However, not all divalent metals seem to have a strong affinity to precipitate or coprecipitate as carbonate minerals. For example, Kim and colleagues (2021) found that at a low concentration of pollutants (<0.05 mM) the effectiveness to remove Cu, Zn, and Cd dropped to 30–60 %, while Pb and Sr achieved removal efficiencies of over 99 % (Kim et al., 2021).

Oyetibo et al. (2021) utilized ureolysis in highly polluted coal mine drainage at Onyeama, Nigeria, to explore its potential for sequestering toxic heavy metals. The urease-producing consortium, which included *Sporosarcina koreensis, Bacillus cereus, Exigobacterium aurantiacum, Pseudomonas citronellolis, Acinetobacter pitti*, and Enterobacteriaceae, achieved removal efficiencies of 100 % for Cd and Pb, 70.1 % for Co, 95.2 % for Ni, and 91.2 % for As when biostimulated with urea (Oyetibo et al., 2021). It is worth noting that pH is one of the key factors affecting remedial efficiency of ureolytic bacteria such as *Sporosarcina pasteurii* strains and *Bacillus* sp., which have been reported to sequestrate metals optimally at pH 6–9.3 (Jiang et al., 2019; Chung et al., 2020; Kim et al., 2021). Similarly urease-producing consortium used in copper mine waste, worked optimally at neutral pH (Yin et al., 2021; Song et al., 2022). Certain heavy metals can be also considered as factors that influence the effectiveness of the ureolytic MICP process (Song et al., 2022). For instance, Mugwar and Harbottle (2016) reported the inhibition of *Sporosarcina koreensis'* MICP by concentration ranging between 0.03 - 0.06 mM, 0.2 - 0.5 mM, and 1 mM of Cd^{2+}, Zn^{2+} and Pb^{2+}, respectively.

Although data on ureolytic MICP remain limited the application of MICP in alkaline mine waste appears promising. Firstly, the inherent alkalinity of some mine waste creates optimal conditions for carbonate mineral precipitation (Wang et al., 2020). As noted by Han et al. (2022) an alkaline environment is a prerequisite for the precipitation of carbonate minerals. Thus, microorganisms can modify the physical and chemical conditions of the solution, such as pH and dissolved inorganic carbon (DIC) content, to promote mineral saturation (Han et al., 2022). This characteristic is crucial for MICP, as the precipitation of $CaCO_3$ is favored in alkaline conditions, allowing for the effective removal of heavy metals and other contaminants present in mine waste (Fu et al., 2023; Stocks-Fischer et al., 1999). Secondly, alkaline mine waste often contains high concentrations of calcium ions, which are essential for the MICP process (Fang et al., 2021; Zhang et al., 2024). The presence of calcium facilitates the formation of calcium carbonate when carbonate ions are produced through the ureolytic activity of bacteria such as *Sporosarcina pasteurii* (Erdmann and Strieth, 2023). This bacterium's capacity to precipitate calcite makes it particularly well suited for MICP applications in alkaline environments (Tobler et al., 2011; Jain and Arnepalli, 2019; L. Ma et al., 2020; Xinxin Li et al., 2022). However, the bioaugmentation of a single microbial species may pose several risks, including increased susceptibility to environmental changes and competition with other microorganisms, ultimately leading to poor performance of monocultures when exposed to real conditions (Tigue et al., 2019). Naturally adapted to their environments and tolerant to contaminants, indigenous microorganisms are expected to perform efficiently (Bruins et al., 2000; Diels et al., 2010). Tobler et al. (2011) highlighted that alkaline wastewater microbial communities can be harnessed to enhance MICP, particularly when coupled with biostimulation to promote microbial growth and activity.

Like many other bioremediation techniques, ureolysis has its limitations, yet these are frequently overlooked in numerous studies. For instance, the production of ammonium as a by-product during ureolysis process has generally been neglected (DeJong et al., 2010; Gat et al., 2017; Lee et al., 2019). It has been shown that the accumulation of ammonium can potentially contaminate groundwater, posing risks to water quality and aquatic ecosystems (Wang and Nackenhorst 2020). Subsequently, elevated levels of ammonium can lead to eutrophication in water bodies, which can result in harmful algal blooms and subsequent oxygen depletion, adversely affecting aquatic life. Another critical concern is the cost associated with managing ammonia volatilization (Khodadadi et al., 2017; Sigurdarson et al., 2018).

Alternative approaches to ammonium mitigation include physicochemical methods such as ion exchange, chemical precipitation, and biofiltration. While these approaches can reduce ammonium levels, they often suffer from high operational costs, chemical usage, secondary waste production, and energy-intensive processes. For example, chemical precipitation (Farghali et al., 2024) can generate large volumes of sludge requiring disposal, while ion exchange systems (Maggetti et al., 2024) demand frequent regeneration and chemical input. Biofiltration (Zulkifli et al., 2022), though more sustainable, may not fully integrate with ureolysis-based carbonate precipitation and could limit process efficiency.

In contrast, a multi-species microbial consortium offers a sustainable and cost-effective solution. By employing division of labor and cross-feeding strategies, distinct microbial species can be selected to simultaneously carry out ureolysis (carbonate production) and ammonium removal (Yin et al., 2021). This synergistic metabolism allows for the in situ elimination of ammonium without the need for external chemical inputs, minimizing secondary pollution and operational costs (Chen et al., 2025). Additionally, microbial consortia can enhance process robustness, resilience to environmental fluctuations, and overall bioremediation efficiency compared to monocultures or single-strategy approaches. Thus, the integration of a multi-species consortium represents a strategic advancement in ureolysis-based bioremediation, addressing the dual challenge of effective carbonate precipitation and ammonium by-product mitigation in an environmentally sustainable manner (Lee et al., 2019; Qu et al., 2023).

Anammox bacteria, such as *Candidatus* Brocadia and *Candidatus* Jettenia, are capable of converting ammonium (NH_4^+) and nitrite (NO_2^-) directly into nitrogen gas (N_2) under anaerobic conditions (Van Niftrik and Jetten, 2012; Liu et al., 2022). Zhu et al. (2011) highlighted that anammox is an environmentally friendly and cost-effective method for nitrogen removal, especially in high-ammonium-loaded wastewaters, such as those from anaerobic digesters (Zhu et al., 2011; Hu et al., 2013). Combining anammox with MICP can help regulate ammonium levels while also encouraging carbonate precipitation (Fig. 2). However, ureolytic reactions occur under aerobic conditions. Utilizing a *Pseudomonas aeruginosa* that performs simultaneous heterotrophic nitrification and aerobic denitrification may offer a more viable solution for ammonium removal (Huang et al., 2022).

Pseudomonas putida has also been shown to possess the capability for heterotrophic nitrification and aerobic denitrification, allowing for the conversion of ammonium to nitrogen gas while utilizing organic substrates (Xu et al., 2017; Yang et al., 2021). This versatility can be advantageous in systems where organic matter is present alongside ammonium, providing a dual pathway for nitrogen removal. Ammonium removal in MICP can be enhanced by designing microbial consortia that incorporate nitrogen-removal pathways, thereby reducing the environmental impacts of ammonium accumulation and improving the overall efficiency of MICP-based bioremediation.

4. Designing microbial consortia

4.1. Early approaches for designing a synthetic consortium

Most bioremediation research has focused on pure cultures involving a single microbial species (monocultures) (Fig. 3a). However, monocultures exhibit several limitations, including narrow substrate specificity, sensitivity to pH and temperature changes, and low operational flexibility (Özbelge et al., 2007; Regueira et al., 2021; Xue et al., 2022). Their genetic uniformity further constrains metabolic efficiency and ecological adaptability, particularly in complex mine-water environments where low diversity impedes pollutant degradation (Sandrin and Maier, 2003). These drawbacks underscore the need for more robust and cooperative bioremediation strategies (Table 3).

Mixed microbial consortia (Fig. 3b) overcome many of these limitations by establishing metabolically complementary interactions among species. Advances in sequencing over the past four decades have clarified how mixed consortia form stable networks that enhance treatment efficiency (Regueira et al., 2021; Thompson et al., 2015). For example, a pure culture of *Acidithiobacillus ferrooxidans* achieved only 51 % removal of U(VI) from mine water within 24 h (Romero-González et al., 2016), whereas a mixed culture of U(VI)-reducing microorganisms isolated from South African uranium tailings achieved 60 % removal (Chabalala and Chirwa, 2010). Field-scale and pilot studies have similarly demonstrated the value of community-based systems: an ureolytic consortium (*Sporosarcina, Bacillus, Pseudomonas, Acinetobacter*) induced carbonate precipitation and removed Pb, Cd, and Ni from coal-mine drainage (Oyetibo et al., 2021); lignite-activated SRB systems achieved >90 % sulfate, Cu, and Zn removal (Di et al., 2022); and a metagenome-guided sulfur-oxidizing system maintained stable performance in Cu-tailings effluent (Peng et al., 2024).

Synthetic microbial consortia deliberately harness division of labour (Fig. 3c), allowing distinct strains to share metabolic tasks and reduce individual burdens (Hays et al., 2015; Zhou et al., 2015; Zhang and Wang, 2016; Roell et al., 2019). Wu et al. (2023) co-cultured *Paenarthrobacter nitroguajacolicus* C1 and *Pseudomonas putida* C2, achieving 95 % Cr(VI) reduction within five days significantly outperforming either strain alone. While cooperative systems can experience competitive interference or resource competition (Freilich et al., 2011; Zhang et al., 2018), predictive social-interaction and computational modelling (Duncker et al., 2021) are improving the design of stable, functional consortia. Collectively, these developments reflect a transition from single-strain optimization to community-aware engineering, highlighting that ecological stability, metabolic complementarity, and adaptive potential are key to next-generation bioremediation design.

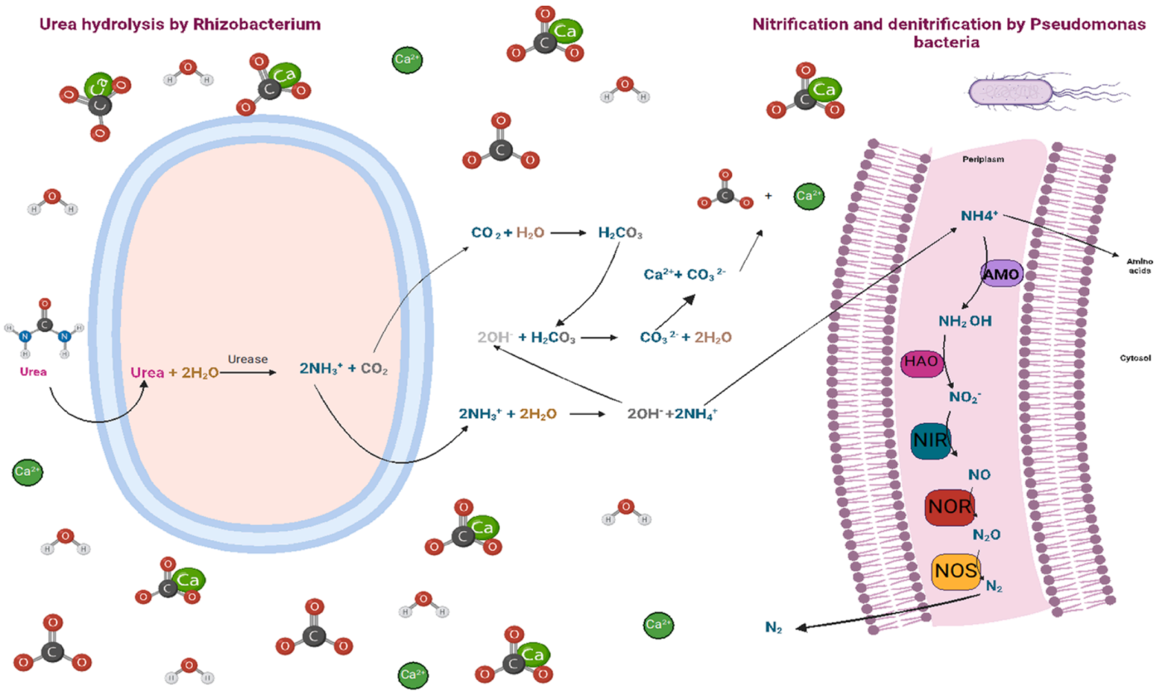

Fig. 2. Microbially induced carbonate precipitation (MICP) through ureolysis can be effectively coupled with the removal of the by-product ammonium via heterotrophic nitrification and aerobic denitrification, thereby achieving simultaneous carbonate biomineralization and nitrogen removal under aerobic conditions.

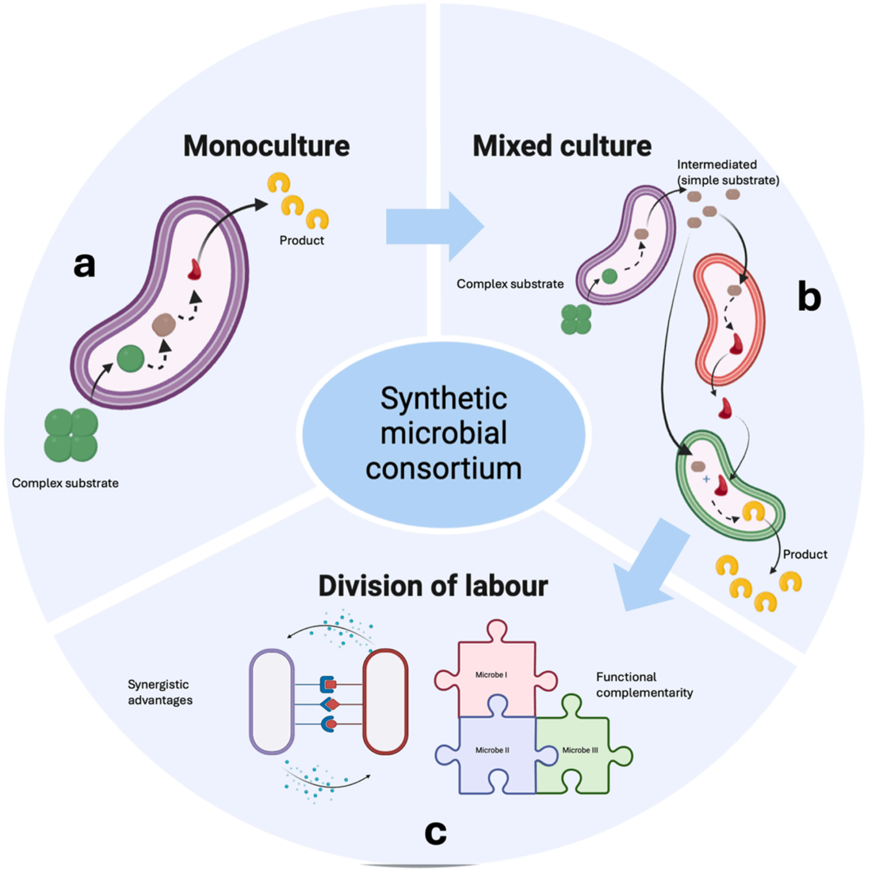

Fig. 3. Designing an efficient synthetic microbial consortium based on interspecific relationships involves using monoculture systems (a) as simple prototypes, on which cooperative mixed culture consortia can be developed, focusing on cross-feeding (b) and division of labour (c). Mixed cultures rely on collective function with emergent interactions, while division of labour explicitly assigns tasks to individual species to maximize efficiency. In bioremediation, division of labour can enhance targeted pollutant removal, whereas mixed cultures offer robustness and adaptability in variable environments.

Table 3
Comparative overview of monoculture, mixed culture, and division of labor strategies for mine water bioremediation.

Feature	Monoculture	Mixed culture	Division of labor (Synthetic consortium)
Definition	Single microbial strain	Multiple microbial species interacting naturally	Multiple strains engineered to share metabolic tasks
Advantages	Easy to study metabolic pathways; controlled genetics	Metabolic complementarity; enhanced treatment efficiency; resilience to environmental fluctuations	Optimized performance; reduced individual metabolic burden; targeted pollutant removal; predictive design possible
Limitations	Narrow substrate specificity; sensitive to pH/ temperature; low operational flexibility; limited ecological adaptability	Possible competition among species; less predictable behavior than engineered systems	Complexity in design; potential for competitive interference; requires predictive modeling
Applicability to mine water	Limited; struggles with complex, multi-pollutant environments	Effective; can remove multiple contaminants (e.g., U(VI), Pb, Cd, Ni, sulfate, Cu, Zn) (Chabalala and Chirwa, 2010; Oyetibo et al., 2021; Di et al., 2022; Peng et al., 2024)	Highly suitable; can achieve superior removal rates (e.g., 95 % Cr(VI) reduction with co-cultured strains) (Wu et al., 2023)

4.2. Current approaches for designing a synthetic consortium

Synthetic microbial consortia are defined as artificially created co-cultures of two or more species in well-defined medium (Großkopf and Soyer, 2014; Li et al., 2021; Liang et al., 2022). Microbial consortia-based biotechnologies employ either top-down or bottom-up approaches (Fig. 4) for microbiome engineering, aiming to leverage the metabolic capabilities of microorganisms and their synergistic interactions (Li et al., 2021; Hu et al., 2022; San León and Nogales, 2022; Lyu et al., 2024).

The first step in the **top-down** (complex to simple) method is selecting a potential microbial community, followed by adjusting parameters such as temperature and pH to shape the community structure and functions toward the intended result (Lawson et al., 2019). The top-down approach has been used for the bioremediation of heavy metals from acid mine drainage by heterotrophic acidophiles that mitigate the attachment of iron oxidising bacteria that accelerates pyrite dissolution. Typically, the microbial consortia are treated as a single functional unit, excluding the metabolic pathways and species specialisation. In theory, optimal functionality can be attained without considering the molecular mechanisms underlying the intended phenotypes and doesn't necessitate a thorough computational analysis (León and Nogales, 2022).

However, it should be emphasized that this is not achieved by chance. This is because mathematical models are used to perform mass balance analysis of chemicals and relevant microorganisms in the system and simulate chemical and biochemical transformation rates by the microbial consortia (Li et al., 2021). The metabolic roles of microbial guilds involved in carbon, nitrogen, and sulfur cycling can be simulated using stoichiometric and kinetic parameters. These include growth yields, substrate affinity, and maximum specific growth rate (Henze et al., 1999; Batstone et al., 2015; Muñoz-Tamayo et al., 2016). Besides, modelling helps improve the efficiencies of removing pollutants and controls the costs of bioremediation. Usually, the models use reactor

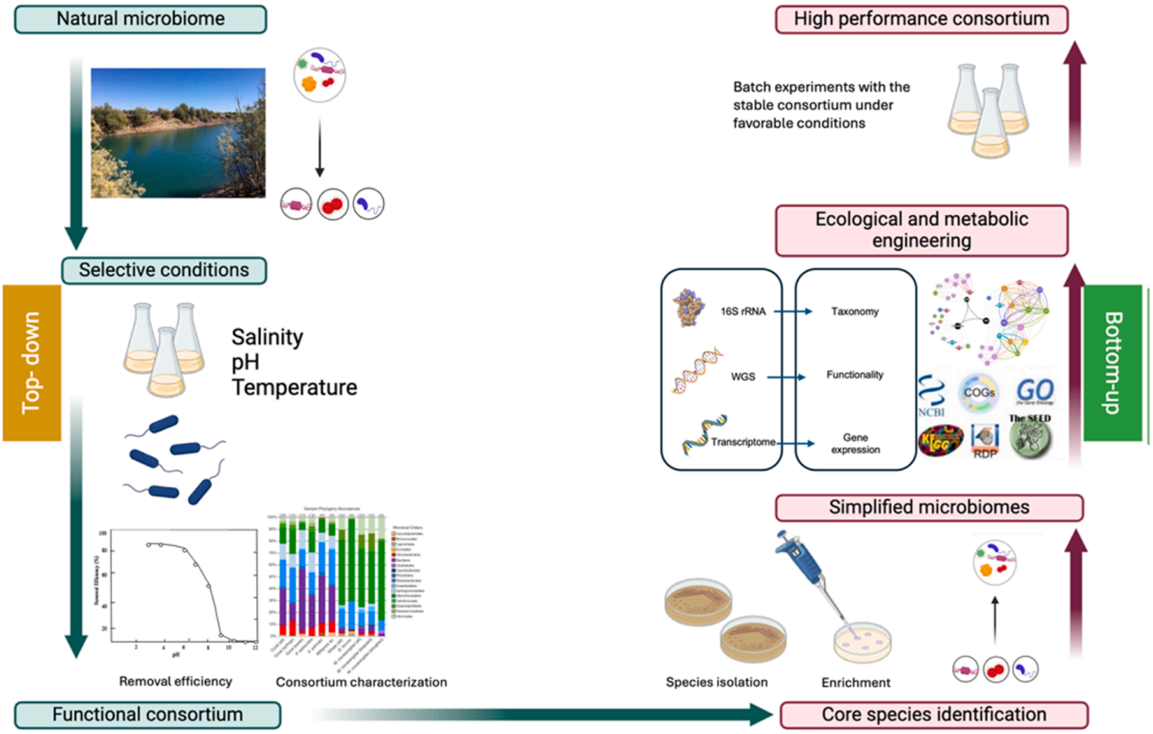

Fig. 4. Schematic representation of the two main approaches used to design synthetic microbial consortia for bioremediation: the top-down approach, which involves modifying or optimizing existing natural communities to enhance desired functions, and the bottom-up approach, which assembles defined microbial strains with complementary metabolic capabilities to achieve targeted pollutant degradation.

engineering or biostimulation to induce the growth of essential species while microbial consortia self-assemble (Liang et al., 2022; Duncker et al., 2021).

Despite being effective for bioremediation, the traditional top-down design approach overlooks the intricate interspecies electron transfer and complex in situ metabolic networks that drive microbial and link chemical transformations (Löffler and Edwards, 2006; Batstone et al., 2015). Consequently, design decisions frequently overlook molecular-scale microbiome activities, which restricts the ability to optimise systems through molecular-scale mechanistic understanding. It also ignores processes that rely on complex interactions between community members, such as syntrophic interactions (Rotaru et al., 2014; Yang et al., 2024). However, with advances in multi-omics and silico technologies, researchers have found ways to capitalise on the metabolic frameworks and microbial interactions by using bottom-up approaches (Embree et al., 2015; Gude and Taga, 2020).

The removal of contaminants is determined by the metabolisms of the individual species that formulate a consortium, and so are the products produced from their breakdown (Cao et al., 2022). An output prediction can be made based on interacting networks and metabolic fluxes (Nikoloski et al., 2015). In microbial consortia, strain genome sequences are typically required to reconstruct metabolic pathways, and consortia dynamics are examined using quantitative models (Li et al., 2021). An essential component of bottom-up engineering is the design of microbial consortia with well-defined social interactions (Kong et al., 2018; Lin, 2022). Competition and cooperation are examples of microbial social interactions prevalent in microbial communities and play a crucial role in defining the dynamics of ecosystems (Faust and Raes, 2012). A social-interaction programming-based model can accurately forecast the dynamics and behaviour of a community with up to four species (Kong et al., 2018).

The **bottom-up** (simple to complex) method aims to create a *de novo* consortium with distinct interconnections and a metabolic process (Kong et al., 2018). It is possible to build and optimise a synthetic with particular functions using bottom-up engineering. A variety of bottom-up techniques have been employed, such as computational model-based design (Haruta and Yamamoto, 2018; Ibrahim et al., 2021), combinatorial evaluation (Kapoore et al., 2022), and enrichment or community reduction (Lee et al., 2013). Although omics-based methods have many advantages, they face challenges when species have incomplete or uncertain metabolic data. Knowledge gaps in gene function and protein annotation remain major barriers to designing reliable microbial consortia (Sanchez et al., 2023; Diaz-Colunga et al., 2024).

The current state of designing microbial consortia for bioremediation reflects a sophisticated understanding of microbial interactions and metabolic capabilities, enabling the development of effective strategies to address environmental contamination. Recent advancements in this field emphasize the importance of mimicking natural microbial metabolisms to enhance the removal of pollutants. However, the interactions among different microorganisms within a consortium can significantly influence their functional stability and effectiveness in bioremediation (Zhou et al., 2023). Wang et al. (2018) demonstrated that the cooperative interactions between *Bacillus cereus* and *Brevundimonas naejangsanensis* enhanced hydrogen production and starch utilization, indicating that carefully designed consortia can optimize metabolic processes for pollutant removal. This highlights the importance of selecting compatible microbial partners to improve bioremediation outcomes.

Combining both strategies (**top-down and bottom-up**) can lead to a more robust bioremediation framework. For instance, the integration of artificial microbial consortia, as discussed by Efremenko et al. (2024), allows for the design of specific microbial groups tailored to target a range of pollutants, including heavy metals (Emenike et al., 2023; Efremenko et al., 2024). This approach can be enhanced by employing biostimulation techniques to create optimal conditions for these consortia to thrive. Moreover, the synergistic effects observed in mixed microbial populations can lead to improved metal sequestration capabilities, as evidenced by the positive interactions among species within consortia (Kvasnová et al., 2017; Henagamage et al., 2022). Studies have shown that synthetic microbial consortia can effectively utilize the metabolic pathways of various microorganisms to facilitate heavy metal removal (Brune and Bayer 2012).

Building on these principles, a pragmatic framework for consortium design combines top-down selection with bottom-up reconstruction: (1) Top-down screening use site surveys (amplicon/metagenomic profiles) and reactor enrichments to identify communities that already perform the desired transformation under real conditions; (2) Metagenomic / MAG analysis recover functional genes and MAGs from enriched communities to identify candidate strains and metabolic modules (e.g., urease operons, sulphate reduction pathways, nitrogen removal genes); (3) Selective enrichment & culturomics use targeted media and high-throughput culturing (guided by predicted metabolic needs and KOMODO/known-media data) to isolate keystone strains or stable co-cultures; (4) Bottom-up assembly construct defined consortia from isolates or enriched mixed cultures, testing division-of-labour configurations (e.g., ureolytic producer and ammonium-removing heterotrophic denitrifier or anammox partner) and iterating under controlled reactor conditions; (5) Scale and monitoring pilot under realistic hydraulic/chemical regimes with time-series omics and functional monitoring to validate stability. Combining both approaches accelerates translation from field-observations to tractable consortia while keeping resilience to site variability.

5. How could metagenomics and bioinformatics contribute to designing an optimal consortium?

Limited knowledge of metabolic pathways and interaction networks involved in the transformation of metals through bioprecipitation significantly hinders the design of microbial consortia for bioremediation (Malla et al., 2018). The metabolic black whole may limit our knowledge of genes involved in improving or declining resistance to heavy metals, reduction of their uptake, inhibition of other metabolic functions, oxidative stress induction, and synergistic effects (Giner-Lamia et al., 2014; Igiri et al., 2018; Mathivanan et al., 2021).

To address this challenge, research relies on meta-omics to build a foundational understanding of pathways and microbial interactions, which could effectively design consortia suitable for biomineralization (Haruta and Yamamoto, 2018; Michealsamy et al., 2021; Sharma et al., 2022). Synthetic microbial consortia can be precisely designed and optimized by harnessing the power of omics-based techniques to achieve improved stability and efficiency (Delgado-Baquerizo et al., 2018; Kumar and Thakur, 2022; Sharma et al., 2022). Omics approaches (e.g., targeted genomic (meta)genomics, (meta)transcriptomics, (meta)proteomics, and metabolomics) (Fig. 5) have been fundamental in analyzing monocultures, defined consortia, enrichment cultures, and environmental microbial communities (Che and Men, 2019). The omics data provides insights into microbial interactions by elucidating microbial networks, metabolite profiles, functional diversity, and gene expression levels (Malla et al., 2018). Integrating multiple omics analyses offers a comprehensive view of microbial interactions, providing a deeper interpretation of complex microbial dynamics.

Bridging metagenomic data and practical consortium development requires a structured "data-to-strains" workflow. Metagenomic and metatranscriptomic analyses are first used to identify key functional genes and metabolic pathways (e.g., *urease, dsrAB*, or denitrification clusters) and to prioritize metagenome-assembled genomes (MAGs) with complete target pathways (Liang et al., 2022; Liu et al., 2023). These MAGs inform the design of selective enrichment and culturomics experiments, where predicted nutritional and physiological traits guide media formulation (L. Huang et al., 2023). Active taxa can then be validated through stable isotope probing (SIP) or bio-orthogonal

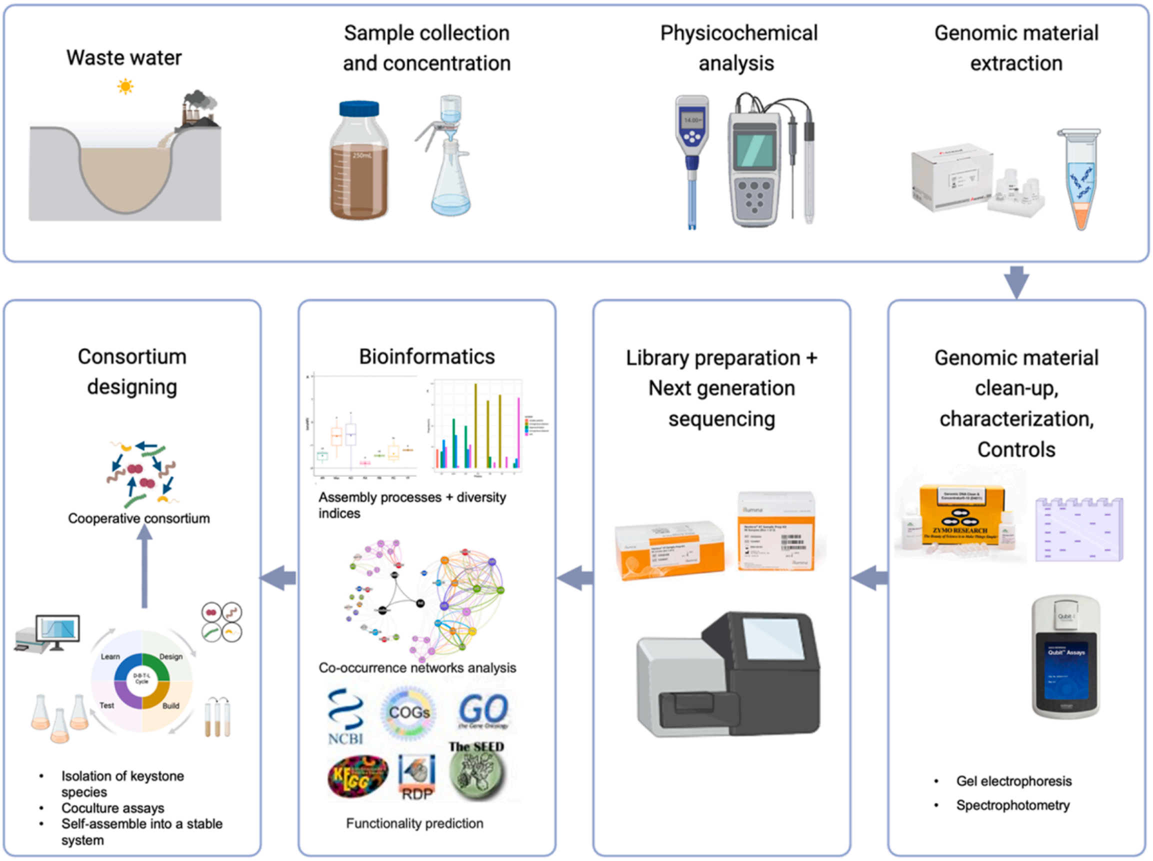

Fig. 5. Proposed workflow for the construction of a synthetic microbial consortium for mine-water bioremediation. Microbial communities from AMD-impacted sites are characterized using multi-omics analyses to identify key taxa and functional pathways linked to contaminant removal. Network and metabolic modeling guide the selection of keystone microorganisms for isolation via selective enrichment and high-throughput culturomics. Isolates are genomically and physiologically characterized, then assembled into synthetic microbial consortium based on complementary functions such as sulfate reduction, ureolysis, or denitrification. Predictive modeling and iterative omics feedback refine community composition to enhance stability and efficiency. The optimized synthetic microbial consortium are validated in laboratory or pilot reactors and ultimately deployed in field systems, where chemical sensors and time-series omics enable performance monitoring and adaptive management.

non-canonical amino acid tagging (BONCAT) to directly link genes to functions (Amekan et al., 2025; Mulay et al., 2025). Isolates corresponding to key MAGs are cultured and assembled into synthetic consortia based on complementary metabolic roles and division of labour (Ibrahim et al., 2021; Wu et al., 2023). Iterative validation using multi-omics and reactor-scale testing ensures ecological stability and target pollutant removal efficiency (Peng et al., 2024). This integrative approach accelerates translation from sequencing data to deployable bioremediation consortia.

Stable isotope probing (SIP) is increasingly applied to link microbial identity with active metabolic functions in bioremediation systems (Alcolombri et al., 2022). By tracing isotopically labeled substrates (e.g., ^{13}C-urea, ^{15}N-nitrate, or ^{34}S-sulfate), SIP identifies taxa directly involved in contaminant transformation, bridging metagenomic predictions with real biochemical activity (Vyshenska et al., 2023). In mining environments, SIP has revealed microorganisms mediating the oxidative dissolution of orpiment and realgar (Sun et al., 2024) and the transformation of arsenic and antimony in tailings (Zheng et al., 2025). Integrating SIP with metagenomics thus enables targeted enrichment of functionally active strains for constructing efficient synthetic consortia in mine water bioremediation.

5.1. *Characterizing microbial composition and inferring microbial interactions using network analysis*

Prior to genomic-era tools, microbial communities were mainly investigated through culture-dependent approaches and treatability experiments, which assessed the rate of pollutant immobilization under controlled laboratory conditions (Malla et al., 2018). In many cases, while researchers can identify the pollutants being broken down or immobilized (e.g., oil, heavy metals, or pesticides), the exact microbial species or strains performing the work may remain unclear, especially if they are part of a diverse community working together (Briffa et al., 2020). This lack of specificity is partly due to the complexity of microbial ecosystems and the difficulty in isolating and characterizing individual species, particularly when many microbes are not easily cultivated in a lab setting (Cross et al., 2019; Kodera et al., 2022).

High-throughput sequencing of the 16S ribosomal RNA gene has shed light on many previously uncharacterized and uncultured microorganisms, significantly helping to fill gaps in the tree of life (Parks et al., 2017; Castelle and Banfield, 2018; Zamkovaya et al., 2021). Moreover, advancements in next-generation sequencing have significantly enhanced the depth and resolution of genomics and metagenomics, leading to a rapid expansion of bioinformatics analysis tools (Oulas et al., 2015; Navgire et al., 2022; Satam et al., 2023). However, meta-omic analyses may encounter challenges linked to the nature of the DNA (which is dependent on quality) and the methods of analysis used (including the type of sequencer, read length, taxonomic assignment software, and database selection). Amplicon sequencing of marker genes, such as 16S rRNA or ITS regions, is susceptible to primer bias, which can distort estimates of microbial diversity and hinder accurate identification of key taxa in complex communities (Brooks et al., 2015).

For decades, the assessment of a member's essentiality within a community has been largely based on its abundance, with the notion that a more abundant member is deemed more important (Props et al., 2017; Bruijning et al., 2023). However, community member abundance is not necessarily indicative of ecological importance. Sun et al. (2022), demonstrated that low-abundance species can be crucial for maintaining biosystem stability under changing reactor conditions. Similarly, Alalawy et al. (2021) found that taxa representing as little as 0.1 % of the community play key roles in sustaining stability and function in anaerobic digesters. Both rare and dominant members can influence community dynamics, either stabilizing systems through cooperation or destabilizing them through competition (Sarsan et al., 2021).

By applying correlation methods, network metrics, and graph-based models, researchers can uncover patterns of co-occurrence, identify keystone species, and understand the intricate relationships within microbial communities (Proulx et al., 2005; Vidal et al., 2011; Layeghifard et al., 2017). Altogether, this information is instrumental in designing an effective consortium, as complex interactions and keystone species play a pivotal role in determining the stability, resilience, and overall performance of the synthetic microbial consortium. Although microbial co-occurrence networks offer deep insights into community interactions, they carry intrinsic biases.

First, correlations indicate associations rather than causation and can therefore misrepresent the underlying biological relationships (Kurtz et al., 2015). Network topology may also be distorted by sequencing depth, compositional effects, rare taxa, and uneven sampling, leading to false or missing links (Dubart et al., 2021; Ghannam and Techtmann, 2021; L. Huang et al., 2023). Moreover, environmental gradients such as pH, redox potential, and carbon availability can create apparent correlations unrelated to microbial interaction. To reduce these artefacts, newer methods like SparCC, SPIEC-EASI, and Bayesian network models are increasingly adopted often supported by temporal data or experimental validation (Faust and Raes, 2012; Weiss et al., 2017). Recognizing these biases is essential when using network analysis to guide synthetic consortium design, ensuring that inferred “keystone taxa” are treated as hypotheses to be tested rather than confirmed causal agents.

5.2. *Application of metagenomics in bioremediation strategies*

Metagenomic approaches, including shotgun sequencing, allow researchers to explore the functional potential of microbial communities. These methods provide both a broad overview of community function and specific insights through metagenome-assembled genome (MAG) analysis (Sar and Islam, 2012; Thomas et al., 2012; Malla et al., 2018; Peng et al., 2024). Although integrating gene abundance data into correlation-based networks may not yield optimal outcomes (Kumar et al., 2018; Cappellato et al., 2021), identifying functionalities linked to resistance and tolerance to metals, as well as potential metabolic pathways for bioremediation, can shed light on the community's bioremediation potential. Additionally, MAGs enable a more in-depth investigation of the genomic machinery of individual microbial members. This functional insight is critical for selecting suitable strains for bioremediation applications (Breton-Deval et al., 2020). Furthermore, identifying previously uncharacterized pathways in microorganisms with a higher likelihood of isolation could expedite the development of effective consortia (Martiny, 2020).

Metagenomics has revolutionized our understanding and application of microbial communities in bioremediation by uncovering key taxa and metabolic pathways that enhance system performance. For instance, metagenome-guided selection of ureolytic consortia improved metal sequestration in coal mine drainage (Oyetibo et al., 2021); rare but keystone taxa identified in anaerobic digesters boosted reactor stability (Sun et al., 2022); and MAG-based isolation facilitated targeted biomineralization (Peng et al., 2024). Today, integrated omics and enrichment approaches simplify the process of going from field sampling to creating a pilot microbial inoculant, producing more reliable results than traditional top-down methods. However, several pitfalls and limitations should be considered when designing consortia and interpreting metagenomic studies. Key challenges include: 1) the complexity of data analysis (Petrucci et al., 2020), 2) potential biases in sampling and sequencing (Felczykowska et al., 2015), and 3) the limitations of functional predictions based solely on metagenomic information (Felczykowska et al., 2015).

6. Future directions - synthesizing network and metagenomic insights for optimal consortium designing

Based on a comprehensive review of the literature, it becomes evident that designing a consortium for bioremediation poses significant challenges. The variable performance of treatment approaches based on biological sulfate reduction and Microbially Induced Calcium Carbonate Precipitation (MICP) underscores the need for an alternative strategy. This raises a critical question: **what is the optimal approach to designing a synthetic community that can effectively remediate polluted water?**

Traditional methodologies have mainly focused on selecting microorganisms based on specific functional traits, such as sulfate-reducing capacity, often overlooking a more holistic perspective that considers the long-term establishment, interactions, and survival of these microorganisms. To address this challenge, a novel strategy that harnesses the potential of metagenomics and bioinformatics is needed. This integrated approach should involve a multi-step process, taking into account several key factors that should be considered:

1. **Source of the microorganisms:** Indigenous microorganisms are often more effective than non-native strains because they are already adapted to the target environment, a phenomenon known as the ‘home-field advantage’. Consequently, using targeted metagenomic techniques to identify microorganisms in water sources impacted by mining activity is crucial.
2. **Composition of the consortium:** Previous strategies often overlooked the synergistic and antagonistic interactions among consortium members, which can significantly affect bioremediation outcomes. By unravelling these microbial interactions, more optimal and stable consortia can be designed. The construction of co-occurrence networks offers a holistic, visual representation of potential relationships between microorganisms, highlighting key species with bioremediation potential.
3. **Resilience to environmental changes:** Developing a robust consortium requires identifying key species that are stress-tolerant, suitable for dry formulation, and crucial for maintaining overall consortium resilience.
4. **Culturing the keystone species:** As noted earlier, only about 1 % of the microbes in Earth are culturable, making it challenging to isolate all desired species for a consortium in pure culture. Identifying the optimal culture media is therefore crucial for obtaining effective microbial inoculants. Shotgun metagenomics provides a comprehensive view of all genomes present in a sample, and when combined with tools like the KOMODO database (https://komodo.modelseed.org), it allows prediction of nutrient requirements and media compositions for culturing keystone microbes.

Bridging metagenomic data with consortium design requires a clear data-to-strains workflow: (1) identify target genes or pathways from metagenomic profiles (e.g., urease, dsrAB, nitrification genes); (2) select high-quality MAGs containing these functions; (3) predict culture media requirements using MAG-based metabolic data and databases like KOMODO; (4) enrich target functions under controlled redox conditions; (5) isolate and validate strains or co-cultures for key activities; and (6) assemble and test synthetic microbial consortia with complementary roles, refining them through omics-based monitoring. This approach offers a framework for using sequencing data to guide the development of microbial inoculants with potential effectiveness in field conditions.

5. **Bioactivity and stability of the consortium:** Overlooking potential conflicts between synthetic consortia and native microbes can lead to incompatible communities and antagonistic interactions, undermining the intended benefits of the consortia. Ecological networks can be used to track changes in water microbiomes in response to the addition of the consortia.

The behaviour of a biological consortium is inherently difficult to predict because of the numerous factors that influence its performance. However, insights from metagenomics and bioinformatic technics can guide more informed decisions during consortium design, enhancing both its effectiveness and stability. Optimizing a consortium requires sufficient time to monitor bacterial succession, which is critical for understanding its stability and resilience. Temporal monitoring provides valuable data on actual performance, helping to validate the effectiveness of the optimization process.

Unfortunately, limiting access to knowledge on consortium design, as well as omics and bioinformatics tools, has led many in situ research to overlook these essential considerations. In practice, potential of these tools for consortium design remains largely unrealized, despite theoretical recognition of their benefits. This literature review aims to raise awareness and provide a foundation applying these approaches in the bioremediation of water sources impacted by mining activities, a pressing global concern. By bridging the gap between theory and practical application, this review seeks to support the development and implementation of more effective consortium design strategies for water bioremediation.

CRediT authorship contribution statement

A. Matu: Writing – review & editing, Writing – original draft, Validation, Investigation, Conceptualization. **A. Valverde:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition, Conceptualization. **E. Cason:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition. **A. Gomez-Arias:** Writing – review & editing, Writing – original draft, Validation. **M. Maleke:** Writing – review & editing, Writing – original draft. **J. Castillo:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Investigation, Conceptualization.

Declaration of competing interest

The authors declare no competing interests.

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Data availability

No data was used for the research described in the article.

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