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Bioprecipitation as a Remediation Strategy for Soil and Groundwater Contamination

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ABSTRACT

Bioprecipitation is a soil and groundwater remediation technique. It uses microorganisms as a catalyst to enhance the chemical precipitation process. The method aims to immobilize soluble contaminants as less soluble hydroxide, carbonate, phosphate and/or sulfide precipitates. The process can function with numerous microorganism species. However, it is often applied with sulfate-reducing bacteria, nitrate-reducing bacteria or ureolytic bacteria. These species typically require an electron donor to facilitate the process. Application of bioprecipitation can occur as an *ex-situ* or *in-situ* operation, whereby *in-situ* operations offer a more sustainable approach. In every scenario, a comprehensive site assessment and laboratory testing is required to establish feasible designs.

Keywords: Bioprecipitation, AMD, Metal(loid)s, Alkaline Waste, Remediation, Microorganisms

1 INTRODUCTION

Anthropogenic activities, specifically mine related operations, cause harm to the environment. Contamination is derived from the extraction process, mineral processing, and/or mineral storage (i.e., tailings). Changes to the environment via mining activities can cause metals and/or metalloids (metal(loid)s) to leach into soil, groundwater and surface water sources (Figure 1). This can cause direct contamination to water, soil, and agriculture, as well as air and vapour contamination via volatilization. It can also cause

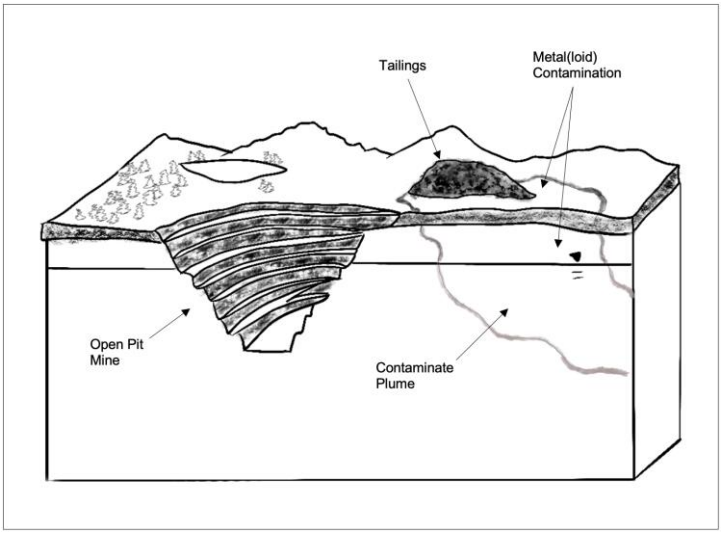


Figure 1. *Soil and groundwater contamination from stockpiled waste from an open pit mining operation (adapted from Hamrin, 1997).*

indirect contamination to the food chain. While this is detrimental to the environment and the biodiversity of surrounding ecosystems, it also has implications to human health through ingestion, inhalation and dermal contact.

In addition to metal(loid) contamination, mining operations can create acidic or alkaline waste equally harmful to the environment. More prevalent in literature is acid rock drainage (ARD), otherwise referred to as acid mine drainage (AMD), which is generated by the oxidation of sulfide minerals (Skousen et al., 2000). AMD is developed during mine operations via mineral exposure to air, water, and microbial activity (Johnson, 2003; Johnson & Hallberg, 2005; Naidu et al., 2019). It is characterized by low pH (and high acidity) and high concentrations of metal(loid)s and sulfates (Johnson, 2003; Naidu et al., 2019). Pyrite (FeS_2) and pyrrhotite (FeS) represent the most abundant sulfide minerals contributing significantly to AMD (see Equation 1) (Egiebor & Oni, 2007). However, other sources of AMD include marcasite (FeS_2), chalcopyrite ($CuFeS_2$), covellite (CuS), galena (PbS) and sphalerite (ZnS) (Skousen et al., 2000).

$$FeS_2 + 3.75O_2 + 3.5H_2O \leftrightarrow Fe(OH)_3 + 2SO_4^{2-} + 4H^+ \quad (1)$$

where FeS_2 oxidizes to form ferric hydroxide ($Fe(OH)_3$ and sulfuric acid (SO_4^{2-} and H^+) (Taylor et al., 2005).

Although studied considerably less, alkaline wastes also pose a major threat to its surrounding environment. Alkaline waste is characterized by the hydration of sodium oxide (Na_2O), calcium oxide (CaO) or magnesium oxide (MgO) (see Equation 2) to form soluble hydroxides (OH^-) (Gomes et al., 2016). It typically has a high pH, high salinity, high sodicity, fine particle size (Santini & Banning, 2016), and high content of alkaline earth metals (Gomes et al., 2016). This type of waste is often associated with nickel, chrysotile, kimberlite and red mud mining (Khudhur et al., 2022), as well as gold, alumina, chromite and uranium processing (Santini & Banning, 2016).

$$MgO + H_2O \leftrightarrow Mg(OH)_2 \leftrightarrow Mg^{2+} + 2OH^- \quad (2)$$

where MgO hydrates to produce magnesium hydroxide ($Mg(OH)_2$) followed by dissolution to ionic species (Gomes et al., 2016).

With a growing global population, there is a growing demand for minerals. Therefore, mining activities will likely continue to increase. However, with over-extraction the cut-off grades (minimum ore grade economically feasible to mine) will likely decrease ensuring significant stripping ratios (ore: waste) and therefore larger tailings piles (Santini & Banning, 2016). Sustainable treatment and management techniques, such as biological treatment methods, are therefore required for the large quantities of mining waste and potential leachate in the future. These methods typically have a smaller environmental footprint (i.e., consume less energy and water, and release fewer greenhouse gas (GHG) emissions) in comparison to other treatment methods.

Biological precipitation, also named bioprecipitation, is an emerging biological soil and groundwater remediation technique. Bioprecipitation is a biological enhancement to chemical precipitation, whereby microorganisms are used to ameliorate the precipitation process acting as a catalyst to induce insoluble metal(loid) precipitation (Wilcox et al., 2023). These precipitates often include hydroxides, carbonates, phosphates and sulfides (Jeyakumar et al., 2023; Kumar et al., 2013). The microorganisms release organic acids, electron donors and enzymes to facilitate precipitation (Jeyakumar et al., 2023). While the process sometimes relies on microbial metabolic activity, the microorganisms also induce precipitation through environmental (i.e., pH and redox potential) changes (Jeyakumar et al., 2023). The process offers a sustainable solution to soil and groundwater contamination as it works to enhance the naturally occurring processes. This paper is meant as an overview for bioprecipitation as a remediation technique focusing on its biochemical mechanisms and operational designs.

2 BIOPRECIPITATION MECHANISMS

The basis of bioprecipitation revolves around the chemical precipitation process. Chemical precipitation is both a thermodynamic and kinetic process (Karpiński & Bałdyga, 2019). To occur, a solution must be in a supersaturated state, such that the solute exceeds the liquid-solid equilibrium of solution (Lewis, 2017). Therefore, the solubility must be exceeded for precipitates to form (Lewis, 2017). There are three kinetic mechanisms involved: nucleation, growth, and agglomeration, referring to the birth and

enlargement of crystal particles (Lewis, 2017). Another governing factor influencing chemical precipitation is pH, where precipitation favors high pH/ alkaline environments.

There are several variations of bioprecipitation based on the type of microorganism present and the oxidative-reduction reactions occurring. Figure 2 depicts some microbial activities related to bioprecipitation. Precipitation can be a biologically controlled mechanism (BCM; based on microbial activity in the intracellular environment; red) or a biologically induced mechanism (BIM; based on microbial activity in extracellular environment; blue) (Dhami et al., 2013; Joshi et al., 2020; Mujah et al., 2016). The microorganisms can induce passive diffusion and secretion of cations, anions etc. out of the cell membrane. For BCMs, this can influence precipitation in the extracellular, intracellular and intercellular environment (Joshi et al., 2020). Since the cell membrane typically has a net negative surface charge due to the presence of carboxyl groups, amino groups, phosphoryl groups and sulfo groups (Kapahi & Sachdeva, 2019), positive metal(loid) ions can precipitate out of soil and groundwater to form insoluble compounds. This is considered a BIM, since precipitation is dependent on the extracellular environmental conditions. Furthermore, the negative bacterial cell wall also provides sites for nucleation and growth (Beveridge & Fyfe, 1985). In the context of engineered bioprecipitation for soil and groundwater remediation, BIMs are of focus.

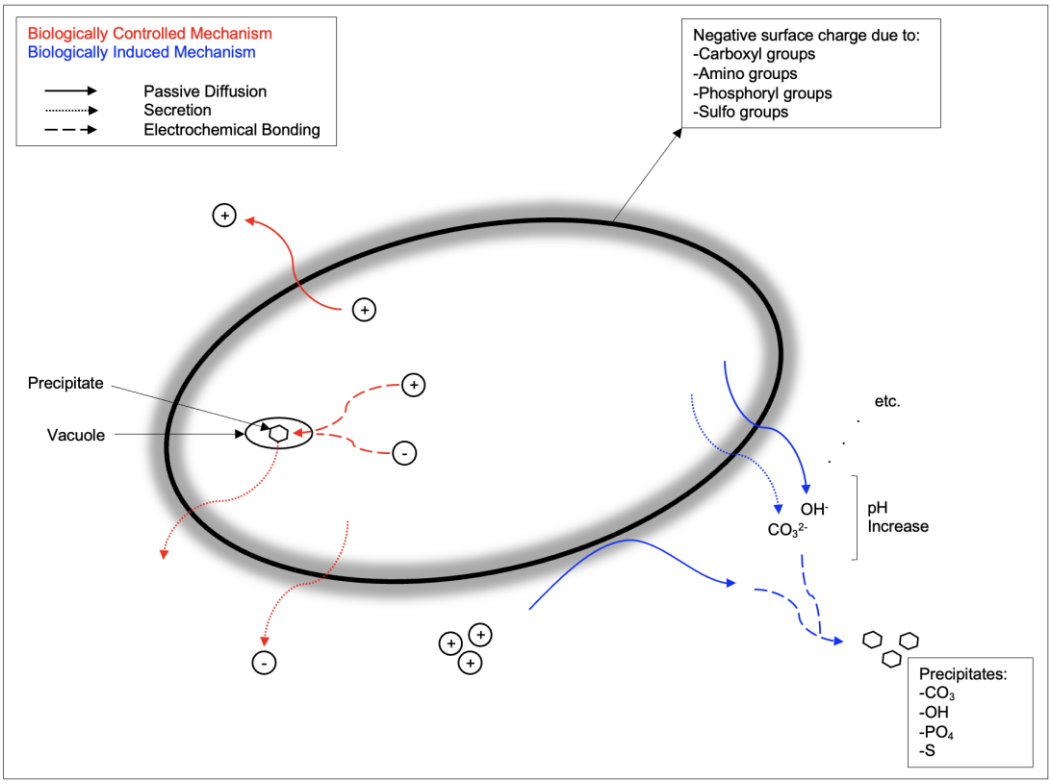


Figure 2. *Microbial mechanisms involved in biological precipitation of metal(loid)s (adapted from Joshi et al., 2020).*

While many microorganisms suffer in contaminated soil and groundwater conditions, some can adapt to the presence of toxic metal(loid)s. The following microbial mechanisms are used to resist toxic effects of metal(loid)s: exclusions by permeability barrier, intracellular sequestration, extracellular sequestration, active transport efflux pumps, enzymatic detoxification and reduction in metal sensitivity of cellular targets (Bruins et al., 2000). Metal(loid) tolerance is essential to the bioprecipitation process ensuring microorganisms aren't inhibited but thrive in their surrounding environment. There are numerous microbial groups and metabolic pathways capable of bioprecipitation. These microorganisms and their respective metabolism include: cyanobacteria algae (photosynthesis), ureolytic bacteria (ureolysis), nitrate-reducing bacteria (denitrification), myxobacteria (ammonification), sulfate-reducing bacteria (sulfate reduction), methanogens (methane oxidation) (Zhu & Dittrich, 2016). The more common microorganisms used for bioprecipitation are detailed below.

2.1 Sulfate-reducing bacteria (SRB)

Biological sulfate reduction (BSR) is the most documented form of bioprecipitation, whereby redox reactions lead to precipitation of metal(loid)s. It is frequently used as a remediation strategy for AMD from mine tailings. The sulfuric acid in AMD will undergo dissimilatory sulfate reduction or assimilatory sulfate reduction, in which the electron acceptor (sulfate, SO_4^{2-}) is transformed to soluble sulfides (H_2S, HS^-, S^{2-}) using SRB (Sánchez-Andrea et al., 2014). This leads to an increase in pH followed by precipitation of metal divalent cations (e.g., Fe^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}) into insoluble metal sulfide precipitates (Sánchez-Andrea et al., 2016). However, SRB can also reduce other metal(loid)s (i.e., Cr, As, Al, Te, and Sb (Willis & Donati, 2017)) into a less toxic, less soluble form, mitigating the hazard produced by contamination (Sánchez-Andrea et al., 2016; Willis & Donati, 2017). Further, these reduced chemical species are available for precipitation of various sulfides or hydroxides.

To facilitate BSR, an energy or carbon source is required. There are numerous electron donors suitable for BSR, ranging from synthetic to organic in nature, both simple and complex (Wilcox et al., 2023). Common electron donors are: hydrogen, formate, methanol, ethanol, molasses, lactate, acetate, propionate, butyrate, sugar, hydrocarbons and organic waste (Liamleam & Annachhatre, 2007). Often multiple carbon sources are used simultaneously to enhance sulfate reduction and maintain it active over a longer period (Liamleam & Annachhatre, 2007).

2.2 Nitrate-reducing bacteria (NRB)

Biological nitrate reduction (BNR) uses denitrifying bacteria to oxidize ferrous iron (Fe^{2+}) or to precipitate carbonates. In both scenarios, under anaerobic conditions, nitrate (NO_3^-) acts as an electron acceptor and undergoes dissimilatory nitrate reduction to produce nitrogen gas (N_2) (Rahman et al., 2020). For iron oxidation, Fe^{2+} acts as the electron donor transforming into ferric iron (Fe^{3+}) which enables the precipitation of numerous ferric hydroxides (Kiskira et al., 2017). This remediates iron rich mine wastewaters leaching to soil and groundwater.

Again, the precipitation of carbonates is similar via NRB. A carbon source will act as an electron donor for dissimilatory nitrate reduction producing carbon dioxide (CO_2) and hydroxide (OH^-) (Rahman et al., 2020). The increase in alkalinity will allow metal(loid) divalent cations in soil and groundwater to precipitate metal carbonates (MCO_3).

2.3 Ureolytic bacteria

Ureolytic bacteria is the most common form of microbially induced calcium carbonate precipitation (MICP). The process uses urease producing microorganisms to facilitate urease hydrolysis, whereby urea ($CO(NH_2)_2$) reacts with water (H_2O) to produces carbonate (CO_3^{2-}) and ammonium (NH_4^+) ions (Rahman et al., 2020). The NH_4^+ increases pH, which enables calcium carbonate ($CaCO_3$) precipitation. The process typically incorporates a cementation solution (typically composed of calcium ions (Ca^{2+})) to immobilize contamination. This is a solidification/stabilization technique that aims to entrap the contaminants in a cement matrix or to co-precipitate metal carbonates.

In addition to soil and groundwater remediation, carbonate precipitation has the potential to act as a carbon sequestration method. The bacterial enzyme, carbonic anhydrase, can act as a catalyst to hydrate atmospheric CO_2 (Kaur et al., 2016). This means inorganic atmospheric CO_2 can precipitate calcium carbonate acting as a carbon sink for GHG contamination (Kaur et al., 2016). While this can be incorporated into the remediation of various mine wastes, it has specific application with alkaline waste, which mimics the natural weathering process (Gomes et al., 2016). The dissolution of alkaline silicate minerals reacts with carbonate from hydrated atmospheric CO_2 to precipitate MCO_3 (Khudhur et al., 2022). This process has great potential to sequester large quantities of mine generated GHG contamination via bioprecipitation of alkaline waste.

3 DESIGN

The selection of an appropriate design is an iterative process. Figure 3 demonstrates a typical engineering

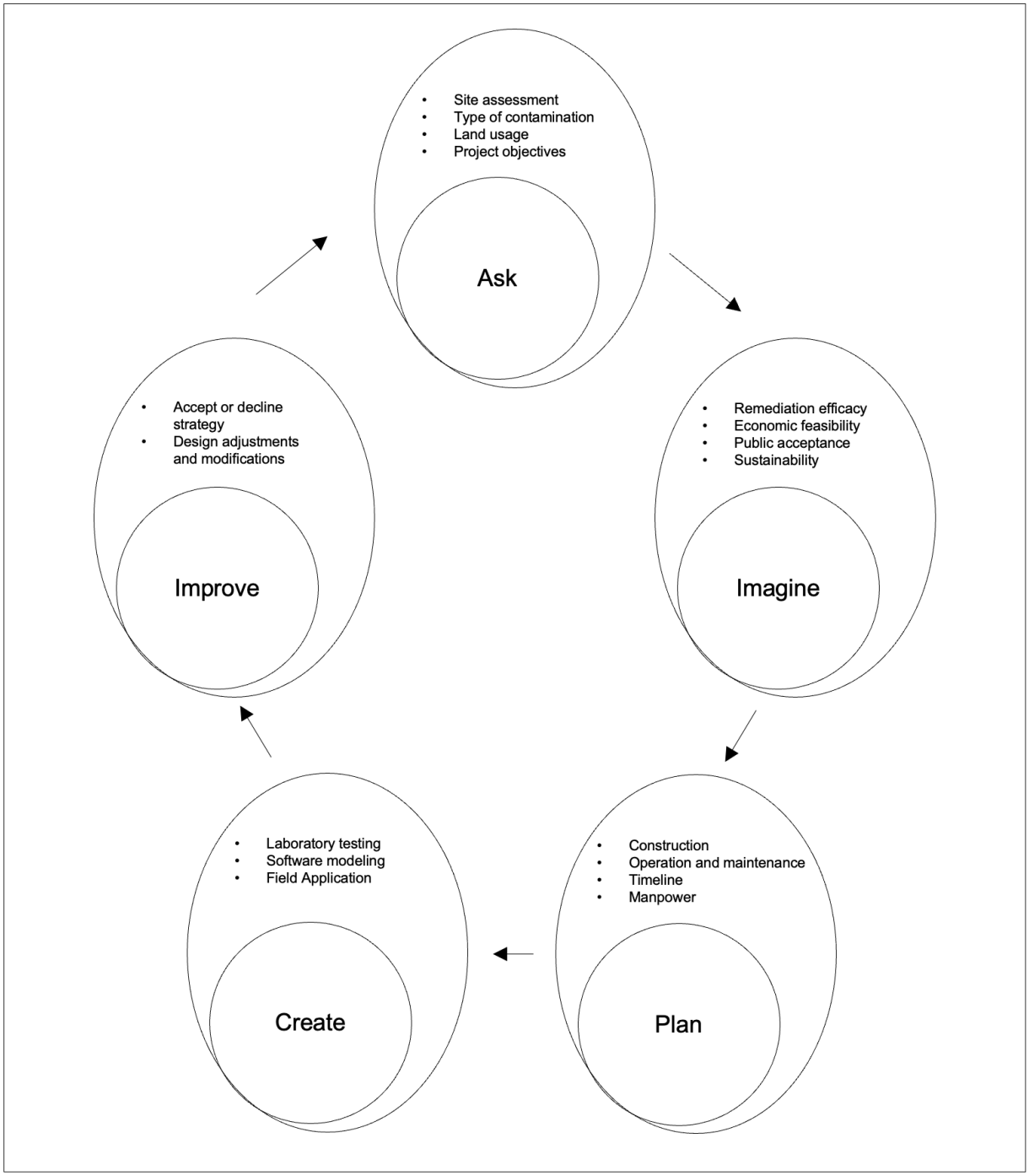


Figure 3. *Engineering design cycle incorporating selection criteria for remediation techniques (adapted from Wilcox et al., 2023).*

design cycle with remediation technique considerations. A set of criteria is developed based on the specific project in question, whereby pertinent input and output data are clearly defined. The selected design must meet the project objectives and satisfy all governmental and/or industrial requirements. Each design should consider the complexity of the solution, the availability of the strategy, the relevant research and field application pertaining to the design and the reliability of the system.

Bioprecipitation can be implemented as an engineering strategy either as an *ex-situ* or *in-situ* operation (see Figure 4 for design configurations). *Ex-situ* operations rely on bioreactors to facilitate bioprecipitation. Waste is transported from its original site and treated with the required additives. This method can achieve high rates of efficacy, since it is easily controlled. However, it is energy demanding and cost intensive. More sustainable designs consider *in-situ* operations. These operations are passive relying on natural processes.

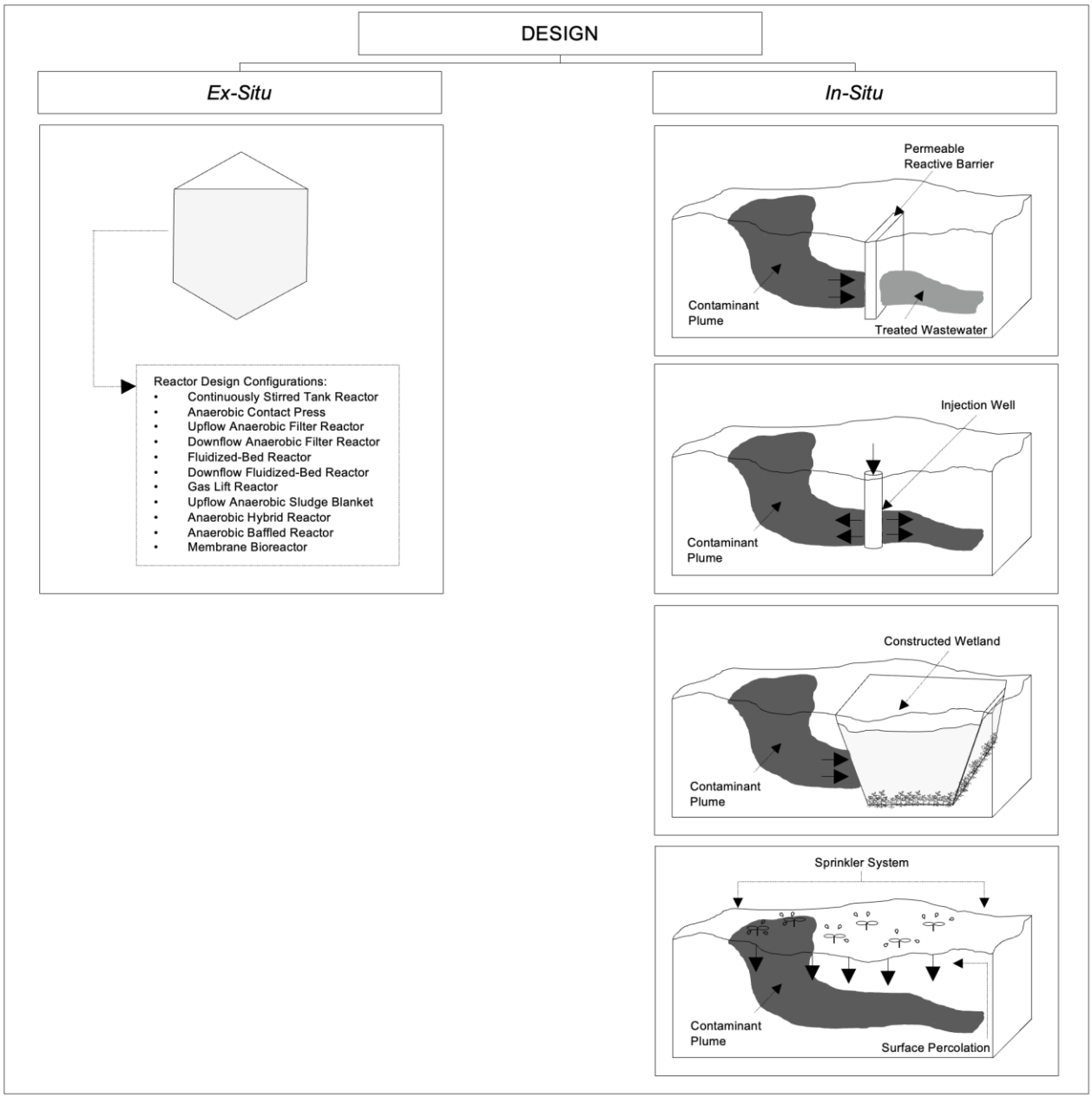


Figure 4. *Design configurations for ex-situ and in-situ application of bioprecipitation (adapted from Wilcox et al., 2023).*

They have low energy demand, low costs, and are inherently more eco-friendly. The following designs are *in-situ* bioprecipitation operations: reactive barriers, injection wells, wetlands, biofilters and surface percolation systems (Wilcox et al., 2023). Each of these designs offer a different application to promote the biochemical reactions involved. Designs are selected based on considerations and criteria noted in Figure 3.

Implementation of bioprecipitation is site specific. Thorough site assessment and laboratory experimentation will determine optimal operating parameters. In all scenarios, an appropriate microorganism (or mixture) and electron donor (or combination) should be selected based on the mining waste in question and the environmental conditions (i.e., pH, temperature, moisture, etc.) at the site. All designs should consider project objectives, economic feasibility, government regulations and environmental sustainability.

In addition to design optimization, experimental data can also be used for computer simulation software prior to field application at large scale. Both experimental design and computer modelling exercises can assess the method and predict the efficacy of bioprecipitation treatment. Experimental techniques are

used to predict site specific outcomes based on input variables. They typically analyze a specific mechanism to derive conclusions on the factors influencing them. However, modeling software's are capable of computing more complex systems to analyze integration of numerous bio-geochemical factors (i.e., biology, chemistry, mechanics, thermodynamics, fluid transport, etc. (Landa-Marbán et al., 2021)). Unfortunately, there is no single, best-practice modeling software used to predict the efficacy of bioprecipitation. However, there are many different software's which focus on different aspects of the bioprecipitation process (e.g., MODFLOW (Prommer et al., 2007), MATLAB® MRST (Landa-Marbán et al., 2021), ASPEN PLUS® 7.1 (Coto et al., 2012), Medusa (Pagnanelli et al., 2009), NNMODEL (Janyasuthiwong et al., 2016)). A combination of multiple test scenarios should be used to predict potential outcomes prior to field application of projects.

4 CONCLUSIONS AND RECOMMENDATIONS

Bioprecipitation is a promising soil and groundwater treatment method. The technique offers both an effective and sustainable solution for mine waste remediation for metal(loid)s, AMD-generating and alkaline waste. The process can be applied as either an *in-situ* or *ex-situ* operation depending on project specifications. It can be tailored to use organic substrates and/or passive design processes to enhance sustainability. Bioprecipitation offers an economically feasible remediation solution with lower energy and water consumption, and less GHG contamination in comparison to other remediation methods. This method is a viable option to remediate the predicted increasing quantities of mine related contamination expected in the future.

Additional research is required to access the viability of bioprecipitation as a large-scale remediation technique. Numerous studies have conducted pilot scale analysis on the efficacy of the operation. However, it's important to assess its feasibility as a practical, field application for real waste. Environmental influences such as rain/snow, wet/dry and freeze/thaw cycles, erosion etc. should be analyzed to assess their overall impact on long-term efficacy. Further, research relating to secondary contamination from by-products is required. This will ensure bioprecipitation is effective as a stand-alone technique.

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