

Abstract

Acid Mine Drainage (AMD) is extremely harmful to the environment because of its polluting potential and its detrimental impact caused by very low pH levels and high amounts of heavy metals. This study focuses on analyzing bacteria found in AMD and investigates their possible use in the bioremediation of acidic gypsum. Using traditional culturing and selective media, acid-tolerant microorganisms were isolated, and their identity was confirmed via biochemical and molecular techniques. To obtain insight into the bacterial diversity present in AMD, the composition of microbial communities from the AMD sites and river water were compared. These acid neutralizing microorganisms were evaluated in artificial gypsums to find out their potential application for the treatment of mitigation of industrial acidic waste. With this knowledge, one approaches the existence of extreme environments through the role of acidophilic microorganisms in environmental remediation. This research advances the implementation of biological approaches to manage waste with high acidity and the effects of AMD pollution.

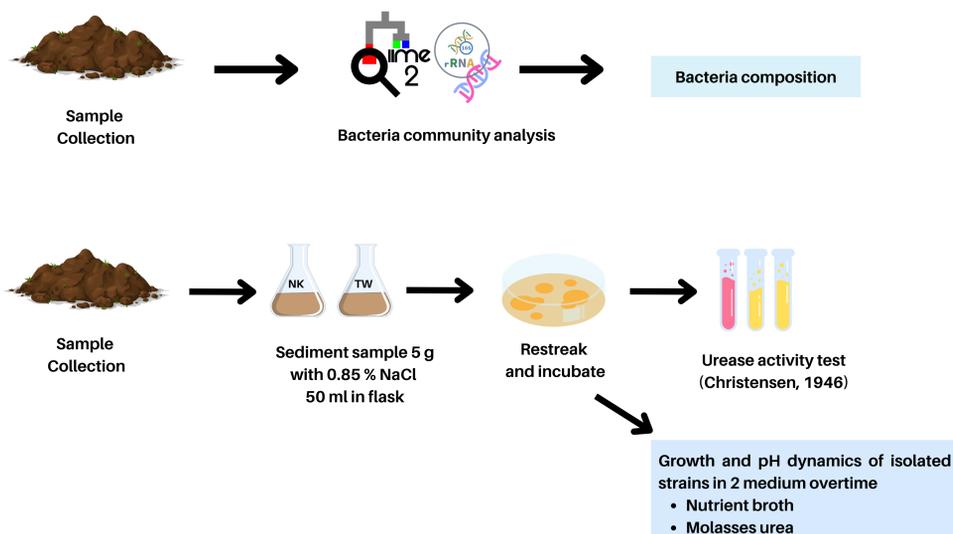
Introduction

AMD is an environmental concern caused by the oxidation of sulfide minerals, resulting in highly acidic water rich in heavy metals. AMD can contaminate rivers and other water bodies, disrupting ecosystems. Acid-tolerant microorganisms that thrive in such extreme conditions offer potential applications in bioremediation (Anekwe & Isa, 2023). This project investigates microbial diversity in AMD and their potential use in treating acidic gypsum, a by-product of certain industrial processes.

Objectives

- To compare the microbial communities of acid mine drainage (AMD) from different sources with those of normal river water.
- To isolate and identify acid-tolerant microorganisms from AMD samples using selective culturing techniques.
- To evaluate the potential of isolated microbes to neutralize acidity in synthetic gypsum.
- To assess the suitability of acid-tolerant microbes for bioremediation applications in acidic environments.

Methodology

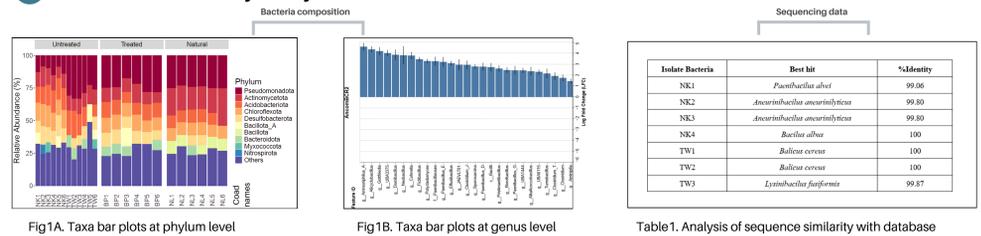


References

- Anekwe, I. M. S., & Isa, Y. M. (2023). Bioremediation of acid mine drainage - Review. Alexandria Engineering Journal, 65, 1047-1075. doi:<https://doi.org/10.1016/j.aej.2022.09.053>
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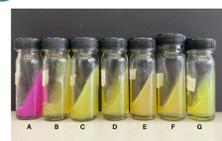
Results

1. Bacteria community analysis



QIIME and ANCOM-BC were used for bacterial identification and classification. Taxa bar plots were generated to display the bacterial composition of each sample type at the phylum and genus levels. Codenames represent the designated names for sampling points at each site (Fig. 1A). ANCOM-BC analysis identified the enrichment of *Alicyclobacillus* and *Paenibacillus* within the *Bacillota* phylum in the non-acid-affected (natural waterway) sites. Therefore, we speculate that these bacteria may have potential pH-increasing capabilities (Fig. 1B). To further investigate this, we targeted the isolation of these bacteria from acid-affected sites. As a result, we obtained six isolates belonging to the *Bacillota* phylum (Table 1).

2. Urease activity test



Urea agar test results. Urea agar slants were inoculated with TW3, which showed a rapid urease-positive reaction (Fig. 2A), while TW1, TW2, NK1, NK2, NK3, and NK4 were urease-negative (Fig. 2B-G). All samples were incubated at 37 °C for 24 hours.

3. Growth and pH dynamics of isolated strains in nutrient broth overtime

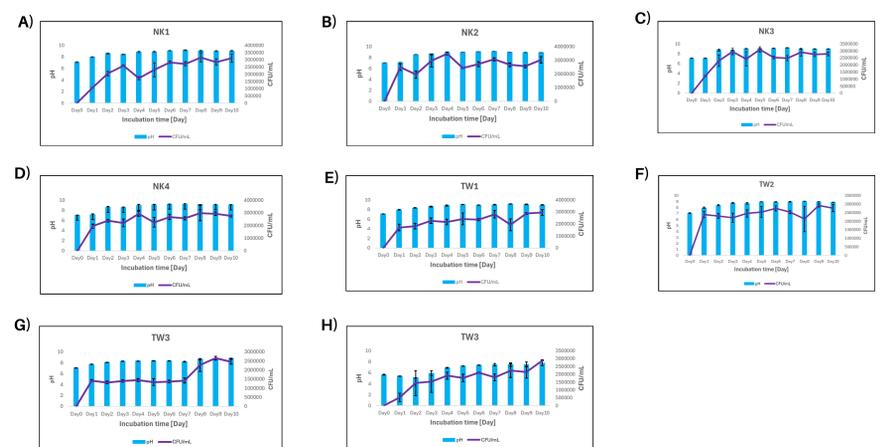


Figure 3: Graph of pH-increasing potential and CFU/mL of bacteria in 7 isolates, separated from sediment samples (NK1, NK2, NK3, NK4, TW1, TW2, and TW3), after 10 days of incubation in nutrient broth (Fig3A-G). Graph of pH-increasing potential and CFU/mL of bacteria in 1 isolate (TW3) after the addition of gypsum to the media and incubation for 10 days (Fig3H).

4. Growth and pH dynamics of isolated strains in molasses urea medium overtime

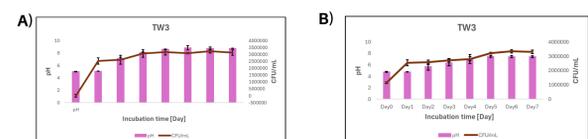


Figure 4: Graph of pH-increasing potential and CFU/mL, separated from a sediment sample (TW3), after 10 days of incubation in molasses urea (Fig4A). Graph of pH-increasing potential and CFU/mL, separated from a sediment sample (TW3), after the addition of gypsum to the media and incubation for 7 days (Fig4B).

Conclusion

- From the analysis, the target microorganisms for acid neutralization belong to the *Bacillota* phylum.
- All bacteria in the *Bacillota* phylum can increase the pH in the media.
- TW3 is the most promising candidate for treating acidic gypsum.
- Molasses and urea supports TW3 growth, aiding acid neutralization, while being a cost-effective alternative to nutrient broth.