Experiences and Future Challenges of Bioleaching Research in South Korea

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Abstract: This article addresses the state of the art of bioleaching research published in South Korean Journals. Our research team reviewed the available articles registered in the Korean Citation Index (KCI, Korean Journal Database) addressing the relevant aspects of bioleaching. We systematically categorized the target metal sources as follows: mine tailings, electronic waste, mineral ores and metal concentrates, spent catalysts, contaminated soil, and other materials. Molecular studies were also addressed in this review. The classification provided in the present manuscript details information about microbial species, parameters of operation (e.g., temperature, particle size, pH, and process length), and target metals to compare recoveries among the bioleaching processes. The findings show an increasing interest in the technology from research institutes and mineral processing-related companies over the last decade. The current research trends demonstrate that investigations are mainly focused on determining the optimum parameters of operations for different techniques and minor applications at the industrial scale, which opens the opportunity for greater technological developments. An overview of bioleaching of each metal substrate and opportunities for future research development are also included.

Keywords: bioleaching; bioremediation; review; waste treatment; metal recovery; South Korea

1. Introduction

Historical registers state that ancient civilizations in Asia (100–200 years BCE) and Europe (around 200 years CE) used oxidizing bacteria to recover metals from mineral ores [1]. Nevertheless, the first formal industrial application of the technology did not appear until the late 1950s when Zimmerley and colleagues patented the first heap-leaching process to leach metals from, among others, sulfide, bearing pyrite and oxide-sulfide copper (Cu) ores [1]. After overcoming multiple operational and technical challenges, bioleaching became an actual alternative for metal recovery; it presents economic, environmental, and operational advantages due to its robustness and minimum use of chemicals during the process. The principle of bioleaching is based on the dissolution of metals by oxidizing agents produced by microorganisms [2].
Statistics show that approximately 7% of the total global annual production of copper is processed through continuous stirred tank reactors, whereas the dump bioleaching input is estimated to be around 20%–25% per year [3]. Cu recoveries higher than 90% have been achieved in bioleaching operations worldwide [4,5]. Similarly, successful industrial experiences for the recovery of nickel (Ni) and zinc (Zn) have been reported. Such is the case of the bio-heap leaching operation at the Talvivaara Sotkamo mine, northeastern Finland, where recoveries of 80% of Zn and 85% of Ni have been reported. The most relevant aspect of this operation is the fact that bioleaching is effective even at environmental temperatures below $-30\,^\circ\text{C}$ [6]. Although design and operating conditions play a fundamental role for heat conservation in this operation, the exothermic characteristics of the mineral oxidation (e.g., oxidation of pyrrhotite at Talvivaara), ultimately allow microorganisms to thrive even at such a low temperature [7]. These extreme conditions, together with the diversity of metal substrates, are examples of the wide range of applications for bioleaching operations. Thus, bioleaching represents a potential technology to recover precious metals from unconventional sources, including mine tailings, electronic scrap, and spent catalysts as well as for waste treatment purposes. From a scientific perspective, researchers focus their efforts on understanding and modeling bio-heap leaching and stirred tank reactors to improve leaching efficiencies [8]; molecular advances are also continuously reported [9].

The aforementioned and other successful operations and studies have promoted the global interest in bioleaching. On one hand, researchers focus their efforts on understanding and modeling bio-heap leaching and stirred tank reactors to improve leaching efficiencies [8]; molecular advances are also continuously reported [9]. From the industrial point of view, on the other hand, process improvement to reduce operative costs is among the main motivations for introducing microorganisms in mining operations. In traditional mining countries, governmental organizations are set to preserve competitiveness in the extractive sector forming alliances between private sector, academia, and research institutions. Some examples of those alliances are: the European programs of BioMine and BIOMOre, the South African MINTEK, and the Chilean-Japanese BIOSIGMA. These institutions and projects hold the sole purpose of developing and enhancing the efficiency of biomining operations. The creation of these organizations, however, is not the norm in most of the countries with metal extractive operations.

In South Korea, the depletion of mineral resources and rapid economic growth have already diverted the main economic interest in local mining operations, leaving behind hundreds of inoperative mines with stockpiled tailings without proper treatment. This not only poses a possible negative impact on the environment but also constrains the opportunity of potential economical revenues that may well be generated from abandoned mine rehabilitation. Therefore, for South Korea, the development of technologies such as bioleaching for proper treatment of abandoned mines is fundamental. Additionally, as stated earlier in this section, bioleaching can also be applied for the treatment of electronic waste and other types of waste that are also considered as part of alternative sources for valuable metal recovery and/or recycling [10–12]. Hence, the South Korean government has included among its operative agenda the development of bioleaching as a feasible and sustainable technology that can be applied for either mine site reclamation or the treatment and recycling of electronic waste. This is done by empowering environmental-related agencies, such as Mine Reclamation Corporation (MIRECO), to invest in the application of the bioleaching technology to meet South Korean specifications. Korean companies have foreign investments such as Korea Resources Corporation (KORES) that executes 32 mining projects in 16 countries [13]. This interest in investing in foreign countries, driven by the ever increasing local demand of metals, also promotes the creation of research funding to implement cutting-edge technologies in their operations.

In consequence, Scientific input to the global discussion about bioleaching from South Korean journals gains importance year to year. Therefore, to assess the literature production on bioleaching, this article intends to compile knowledge of the technology published in Korean journals and to provide future directions to those scholars involved in the area. We also expect that this first literature
review on bioleaching will serve as a catalyst for research related to the technology in South Korea. To reach that objective, we conducted a systematic review and classification of 63 papers registered in a Korean Journal Database using The Thomson Reuters ISI Web of Science.

2. Overview of Organisms and Mechanisms

The environmentally friendliness and cost effectiveness of bioleaching makes it an attractive technology for mining and environmental-related applications. It is based on the solubilization of metals by biological oxidation or complexation reactions from different sources [2]. The biomining microorganisms have been developed to be fully effective in harsh operational conditions, including low solution pH, high temperature, and high heavy metal concentration. The most common bacterial species used in industry are Acidithiobacillus caldus (A. caldus), Acidithiobacillus ferrooxidans (A. ferrooxidans), Acidithiobacillus ferrivorans (A. ferrivorans), Leptospirillum ferrooxidans (L. ferrooxidans), Acidithiobacillus ferrooxidans (A. ferrooxidans) and Acidithiobacillus thiooxidans (A. thiooxidans). Bacterial activity promotes metal solubilization by two main mechanisms: the contact mechanism that is the oxidation of minerals by attachment of microorganism on the surface of the metal substrate; and the non-contact mechanism that is the oxidation of the minerals carried out by the oxidizing agent, generally ferric ion Fe(III), produced by microorganisms in the bulk solution [14,15]. The biological oxidation of ferrous iron to ferric iron and the chemical oxidation of metal sulfides with the ferric iron are shown in Reactions (1) and (2).

\[
2\text{Fe}^{2+} + \frac{1}{2}\text{O}_2 + 2\text{H}^+ \rightarrow 2\text{Fe}^{3+} + \text{H}_2\text{O} \quad (1)
\]

\[
\text{MS} + 2\text{Fe}^{3+} \rightarrow \text{M}^{2+} + \text{S}^0 + 2\text{Fe}^{2+} \quad (2)
\]

Fungal species are also used for leaching purposes. These microorganisms are capable of oxidizing siliceous oxide or carbonaceous material by excreting leaching agents, such as organic acids [16]. Unlike chemolithotrophic, these microorganisms require organic supplements as an energy source. Acidolysis is the governing mechanism in metal leaching by fungi. The rapid protonation of oxygen atoms covering the metal compound surface and its interaction with water promote metal detachment and metabolite production; protons catalyze solubilization reactions without neutralizing them. Even though bioleaching with fungi has been successfully achieved in laboratory scale [17–19], there are no reports of industrial operations that would utilize fungi.

Heap and continuous bioleaching are applied at the industrial scale; both contact and non-contact mechanisms govern these techniques. However, specific parameters are considered during the application of each technique: for bio-heap leaching operations, cell irrigation, gas and heat flows, as well as heap dimensions and particle characteristics are among the most highlighted parameters considered to obtain maximum extraction efficiencies [20]. Similarly, in continuous bioleaching, the most relevant parameters are gas transfer, particle size, solid concentration, and residence time [18,21]. As convergent points, the characteristics of the microorganisms play a fundamental role in both techniques; these characteristics may include: heavy metal tolerance and nutrients, temperature, and pH requirements.

The effectiveness of bioleaching has been compared with that of chemical leaching. Figure 1 presents the leaching efficiencies achieved in a comparative study. Bayat and Sari [22] proved that bioleaching was more effective than chemical leaching by Fe(III) or sulfuric acid. Escobar et al. [23] also reported higher removal efficiencies by biological means than that of chemical leaching with inorganic acids (i.e., sulfuric).

These results demonstrate the effectiveness of bioleaching to recover heavy metals. However, as a disadvantage, bioleaching may require longer reaction times compared with chemical leaching and produce the undesirable acid mine drainage. As a benchmark, Table 1 presents metal recoveries achieved through biological means using various species of microorganisms. Overall, high extraction efficiencies were reached; the values varied depending on the material and metal to be leached as well as the operating conditions.
mine tailings, mineral ores and concentrates, contaminated soil, electronic waste, spent catalysts, and other materials. Besides those categories, studies on genetics or at the molecular level were grouped in

3. Methodology

3.1. Compilation

We compiled bioleaching-related research articles stored in the Korean Journal Database using the Thomson Reuters ISI Web of Science. This database is linked to the National Research Foundation of Korea, which became a primary source of information. A search on “bioleaching” as a keyword returned a total of 63 papers up to 30 March 2016 as cutting-off date without including dissertations, conference proceedings, or partitioned publications. The papers were written in English and Korean languages, published by local and international research teams, and covered different purposes. Every paper was stored, classified, and systematically analyzed to address the current production of bioleaching published in Korean journals.

3.2. Classification

To analyze the available information, we classified the papers according to the target material: mine tailings, mineral ores and concentrates, contaminated soil, electronic waste, spent catalysts,
and other materials. Besides those categories, studies on genetics or at the molecular level were grouped in an independent section. Our database contains qualitative data, such as year of publication, journal, target metals, variables, and authors’ remarks, and quantitative data such as metal conversions.

After analyzing the information, we addressed the current tendencies of bioleaching studies in South Korea according to target materials. The percentage of publications related to each material prior to the cut-off date was: 17%, mine tailings; 5%, e-waste; 30%, mineral ores and metal concentrates; 10%, spent catalysts; 11%, contaminated soils; and, 21% corresponded to other materials (i.e., contaminated seawater, metal concentrates, and spent batteries). The remaining 6% addressed molecular studies.

3.3. Publications of Bioleaching Over Time

Our search showed an increase in bioleaching research over time. The annual average of papers published in Korean journals has increased from one to five papers per year over the last decade, reaching its highest point in 2010; at the cut-off date, no papers published before 2002 were registered. Figure 2 presents the number of publications published in Korean journals over time. However, the number of publications present no trend, making it difficult to draw proper conclusions. A total of 57% of the articles were written in English.

3.4. Authors’ Highlights

In order to investigate the main attributes of the authors who published articles in the field of bioleaching in Korean journals, we determined their nationality, publication motivations, and affiliation. Our research team identified over 100 researchers who submitted their findings to journals linked to the Korean Journal Database. Of note was the input of research articles written by foreign researchers in local journals that included China (7%), India (10%), and Iran (3%). The authors’ motivations corresponded to environmental and mining-related activities and most of the authors had academic and research affiliations.

4. Bioleaching Research in South Korean Journals

4.1. Microorganisms

According to our findings, the microorganisms used for the bioleaching of different materials were chemolithotrophic bacteria and fungi. Most researchers used bacteria—mainly from the genus Acidithiobacillus. Several research teams claimed bacterial isolation from different sources: hot springs, mine drainages, pond water, and commercial tanks. However, most of them used bacteria purchased from biotechnological institutes. Also, several articles documented the use of a bacterial consortia composed of two [34,35] to five [36] different species. Scholars preferred flask experiments to study bioleaching. Studies related to bioleaching by fungi also received special attention from the scholars; our study found that A. niger was the most-used species.
4.2. Bioleaching of Mine Tailings

This section addresses the advances in bioleaching of mine tailings from published research in South Korean journals considering the main discussion points. Most mine tailings were composed of sulfide minerals containing heavy metals such as arsenic (As), chromium (Cr), cadmium (Cd), lead (Pb), mercury (Hg), Cu, Zn, and iron (Fe). The weathering vulnerability of mine tailings is enhanced by the intrinsic mining processes, such as milling and grinding, that enhance the surface exposure [37]. These characteristics, in cooperation with the catalytic activity of naturally occurring microorganisms, facilitate the spread of heavy metals into the environment causing soil and water pollution and, subsequently, provoking ecosystem destruction and affecting human health [38,39]. In addition to these issues, the ever-increasing global demand of mineral resources creates a perfect business opportunity for recovering precious metals from deposited tailings. The challenging aspects of bioleaching mine tailings are mainly related to the presence of high concentrations of heavy metals and/or other reagents which may inhibit bacterial activity reducing the extraction efficiency and enhancing the operation time. Other relevant factors, such as temperature, pH, and gas production have to be considered. Table 2 summarizes the relevant aspects of the research conducted in this area.

The influence of bacterial attachment onto the mine tailing surface over the bioleaching process has received much support to date. Kim et al. [40] studied the attachment behavior of indigenous acidophilic bacteria with different shapes onto the mine tailings surface. They found that rod-shaped bacteria formed linear chains attached to mineral surfaces. Similarly, Cho et al. [41] determined that rod-shaped bacteria tended to be attached onto chalcopyrite in groups, whereas filament-shaped bacteria attached individually; the scholars used bacterial strains isolated from hot springs living at temperatures higher than hyperthermophilic ranges. In the same context, Park et al. [42] observed that filament-shaped indigenous acidophilic bacteria tended to attach onto the internal face of pyrite cracks, and rod-shaped onto the external wall of the mineral. The findings presented by these scholars underline that the characteristics of bacterial attachment onto mineral surfaces are influenced by the bacterial shape. Bacterial attachment plays a fundamental role in leaching by biological means as it influences extraction efficiency depending on the target metal and parameters, such as pulp density [43].

The impact of other parameters has also been examined from different perspectives. Ko et al. [44] found that bioleaching from mine tailings obtained from different sources had different extraction rates due to the different metal ion concentrations in the samples. Their results highlight the importance of a bacterial adaptation to target minerals prior to the bioleaching process. The influence of bacterial adaptation on bioleaching kinetics was further analyzed by Kim et al. [45,46]. They compared the extraction efficiencies achieved by bacteria fully and partially adapted to Cu ion. Later, Panda et al. [47] studied the effect of cell irrigation rates on Cu recovery in column experiments and determined that jarosite formation was lower at higher flow rates (i.e., 2.0 and 2.5 L/h). Similarly, our research team observed the relevance of bacterial adaptation for the bioleaching process [48]. In long-term column experiments (i.e., 436 days) we obtained up to 70% as removal efficiency. However, we speculate that a higher efficiency would have been obtained if the bacterial culture (i.e., A. thiooxidans and A. ferrooxidans) had passed through an adaptation process prior to the core experiment: we observed a series of recovery rate flaws during the experiment. This phenomenon was attributed to the increase of arsenic concentration in the pregnant leaching solution (PLA), which subsequently became inhibitory to the strain. Similarly, Park and Cho [49] leached valuable metals from mine waste by column experiments at room temperature. They satisfactorily leached Cu and iron from the ore. Also, the synergy between bioleaching and electrochemical processes has been proven to be successful to recover metals from mine tailings. Lee et al. [50] revealed that the removal speed of an integrated process of biological leaching and electokinetics was around 2.5 times faster than that obtained in independent processes. Similarly, Park et al. [51] obtained Cu recoveries of approximately 98% after applying electrowinning to the PLA obtained by biological means which is significantly higher than the 78% obtained by acid leaching.
The research within bioleaching of mine tailings demonstrated that this technology is an actual alternative to traditional methods, such as cyanide leaching, to recover metals from mine tailings and mitigate implicit environmental issues. Our research shows that the studies are mainly focused on determining the optimum operating conditions for the process in flask and column experiments. The importance and influence of bacterial shape and adaptation in the process were also underlined by the scholars who presented their findings in Korean journals.

### Table 2. Bioleaching research of mine tailings according to the parameters studied and recoveries achieved as well as microorganisms used in the studies. The articles appear in chronological order.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Microorganism(s)</th>
<th>Parameters</th>
<th>pH</th>
<th>Particle Size</th>
<th>Time (Day)</th>
<th>Leaching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[50]</td>
<td>Ind. bacteria ^a^</td>
<td>Bioleaching/electrokinetics</td>
<td>10-12</td>
<td>−2000 µm</td>
<td>29</td>
<td>As: 64.5</td>
</tr>
<tr>
<td>[44]</td>
<td><em>A. thiooxidans</em></td>
<td>Mineral source</td>
<td>2.0</td>
<td>−177 µm</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>[42]</td>
<td>Ind. bacteria ^a^</td>
<td>Bacterial attachment</td>
<td>3.5</td>
<td>−841 µm</td>
<td>20</td>
<td>-</td>
</tr>
<tr>
<td>[49]</td>
<td>Ind. bacteria ^a^</td>
<td>Leaching feasibility</td>
<td>4.2</td>
<td>+2000 µm</td>
<td>22</td>
<td>-</td>
</tr>
<tr>
<td>[40]</td>
<td>Ind. bacteria ^a^</td>
<td>Attachment</td>
<td>3.5</td>
<td>−841 µm</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>[41]</td>
<td>Ind. bacteria ^a^</td>
<td>Attachment</td>
<td>3.5</td>
<td>−841 µm</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>[45]</td>
<td>Ind. bacteria ^a^</td>
<td>Bacterial adaptation</td>
<td>2.82</td>
<td>1 mm</td>
<td>42</td>
<td>-</td>
</tr>
<tr>
<td>[47]</td>
<td><em>A. ferrooxidans</em></td>
<td>Surface pretreatment</td>
<td>1.5</td>
<td>10-10 mm</td>
<td>20</td>
<td>Cu: 72</td>
</tr>
<tr>
<td>[51]</td>
<td><em>A. ferrooxidans</em></td>
<td>Bioleaching/electrokinetics</td>
<td>−2</td>
<td>4 cm</td>
<td>13</td>
<td>Cu: 76</td>
</tr>
<tr>
<td>[48]</td>
<td><em>A. ferrooxidans</em></td>
<td>Removal rates in long-term experiments</td>
<td>1.8</td>
<td>+4000 µm</td>
<td>450</td>
<td>As: 70</td>
</tr>
<tr>
<td>[46]</td>
<td>Ind. bacteria ^a^</td>
<td>Effect of bacterial adaptation</td>
<td>2.6 and 2.8</td>
<td>−2380 µm</td>
<td>43</td>
<td>Cu: 92.79</td>
</tr>
</tbody>
</table>

^a^ Indigenous bacteria.

### 4.3. Bioleaching of Electronic Waste

Discarded cell phones, appliances, and printed circuit boards constitute the majority of e-wastes that may contain precious metals (Cu and Au), heavy metals (Pb, Sb and Hg), and other compounds (polybrominated diphenyl ethers and polychlorinated biphenyls) [52,53]. The constant growing demand for precious metals to satisfy industrial and population growth-related necessities has added to the strengthening and implementation of environmental policies, such as extended product responsibility, thereby highlighting the importance of treating e-waste. Bioleaching is gaining ground as an effective pathway for metal recovery from this source. Nevertheless, its application in this field still requires further research, especially to determine optimum operational conditions and to find a feasible process that may combine other technologies. The aim of this section is to address the work to date in the bioleaching of e-waste from electronic wastes found in Korean journals. Table 3 presents the relevant highlights of the research produced on the bioleaching of electronic waste.

### Table 3. Bioleaching research of electronic waste according to the parameters studied and recoveries achieved as well as microorganisms used in the studies. The articles appear in chronological order.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Microorganism(s)</th>
<th>Parameters</th>
<th>pH</th>
<th>Particle Size</th>
<th>Time (Day)</th>
<th>Leaching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[54]</td>
<td><em>T. ferrooxidans</em></td>
<td>Solid concentration</td>
<td>2.0</td>
<td>−149 µm</td>
<td>7</td>
<td>Cu and Co: 90; Al, Zn, and Ni: 40</td>
</tr>
<tr>
<td>[55]</td>
<td><em>A. niger</em></td>
<td>Chemical vs. biological leaching</td>
<td>5.5-6.0</td>
<td>−500 µm</td>
<td>72</td>
<td>Cu and Co: 95; Al, Zn and Pb: 15–35</td>
</tr>
<tr>
<td>[56]</td>
<td><em>A. niger</em></td>
<td>Biodegrading and solvent extraction combination</td>
<td>2.5</td>
<td>−500 µm</td>
<td>-</td>
<td>Cu: 99.9</td>
</tr>
</tbody>
</table>

Several scholars reported their findings in metal recovery from electronic scrap by microbiological means. In flask experiments, Ahn et al. [54] used *T. ferrooxidans* to leach heavy metals from e-waste and they focused on determining the optimum pulp density for the process. They achieved a 90% leaching efficiency of Cu and Co and a 40% efficiency of Al, Zn, and Ni at 10% of the solid concentration. However, the precipitation into lead (II) sulfate (PbSO₄) and stannous oxide reduced Pb and tin (Sn) leaching, respectively. As a reference, the scholars compared these results with those obtained from the
bioleaching of metal powders containing the target metals; bioleaching of electronic scrap presented higher efficiencies.

The use of fungi to recover minerals from electronic waste was also reported in the Korean Journal Database. The impact of reaction time and concentration of organic acids on the leaching efficiency by *A. niger* was emphasized by Ahn et al. [55]; the researchers obtained 95% leaching efficiency of Cu and Co at an electronic scrap concentration of 50 g/L. Using the same fungal species, Ahn et al. [56] conducted a combined process of bioleaching, solvent extraction (with LIX84), and electrowinning to recover Cu and Sn from a solution of electronic scrap. They reported a 99% recovery leaching rate that was directly proportional to the concentration of LIX84, considering 20% (*v*/v) of LIX84 as the concentration limit.

The results obtained using microorganisms to recover metals from e-waste are encouraging. Unfortunately, the currently scarce amount of literature from Korean journals makes it difficult to determine the trends and future directions. Similar to the research approach used to treat other materials, the findings presented in Korean journals were related to the determination of basic operating conditions. Nonetheless, this area, due to its relevance from an environmental and economic perspective, has high potential and an intensive cooperation between academia and industry have to meet to exploit the opportunities.

### 4.4. Bioleaching of Ores and Metal Concentrates

Metal recovery from ores and concentrates constitute the main application of bioleaching worldwide. The proof of this fact is reflected in the 33 commissioned plants worldwide since 1986 (17 heap leaching and 16 stirred tank reactors) [57]. The main constraints that the bioleaching of mineral ores and concentrates face are mainly related to the long extraction times, the necessity to further process the generated by-products, and the metal toxicity to the biomining microorganisms. This section addresses the relevant findings to process these materials published in Korean journals. Table 4 summarizes the research conducted in this area.

**Table 4.** Bioleaching research of mineral ores and metal concentrates research according to the parameters studied and recoveries achieved as well as microorganisms used in the studies. The articles appear in chronological order.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Microorganism(s)</th>
<th>Parameters</th>
<th>pH</th>
<th>Particle Size (µm)</th>
<th>Time (Day)</th>
<th>Leaching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[58]</td>
<td><em>T. ferrooxidans</em></td>
<td>Particle size, pulp density, and Fe concentration</td>
<td>2.0</td>
<td>210–250</td>
<td>18</td>
<td>Cu: 78</td>
</tr>
<tr>
<td>[59]</td>
<td>-</td>
<td>Review</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[60]</td>
<td><em>A. ferrooxidans</em></td>
<td>Energy source, initial pH, pulp density, and temperature</td>
<td>1.0–2.5</td>
<td>~74</td>
<td>30</td>
<td>Cu: 80</td>
</tr>
<tr>
<td>[61]</td>
<td><em>A. ferrooxidans</em></td>
<td>Thermal pretreatment</td>
<td>1.5</td>
<td>-</td>
<td>33</td>
<td>Ni: 59.18 Co: 65.09</td>
</tr>
<tr>
<td>[62]</td>
<td>-</td>
<td>Review</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[63]</td>
<td><em>A. ferrooxidans</em></td>
<td>Feasibility assessment</td>
<td>1.5</td>
<td>1–9.5</td>
<td>100</td>
<td>Co: 10</td>
</tr>
<tr>
<td>[64]</td>
<td>Ind. bacteria <em>a</em></td>
<td>Chemical vs. biological leaching</td>
<td>4.0</td>
<td>~841</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>[65]</td>
<td>Ind. bacteria <em>a</em></td>
<td>Initial pH and temperature</td>
<td>4.0, 7.0, 9.0</td>
<td>~74</td>
<td>19</td>
<td>-</td>
</tr>
<tr>
<td>[66]</td>
<td>Ind. bacteria <em>a</em></td>
<td>Pulp density</td>
<td>4.4</td>
<td>~841</td>
<td>84</td>
<td>-</td>
</tr>
<tr>
<td>[67]</td>
<td>-</td>
<td>Review</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[68]</td>
<td>Ind. bacteria <em>a</em></td>
<td>Bacterial attachment</td>
<td>4.20</td>
<td>~74</td>
<td>45</td>
<td>-</td>
</tr>
<tr>
<td>[69]</td>
<td><em>L. ferriphilum, Acidithiobacillus caldus</em></td>
<td>Bacterial attachment</td>
<td>2.0</td>
<td>~149</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[70]</td>
<td>Ind. Bacteria <em>a</em></td>
<td>Feasibility</td>
<td>3.2</td>
<td>-</td>
<td>28</td>
<td>-</td>
</tr>
<tr>
<td>[71]</td>
<td>-</td>
<td>Review</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[72]</td>
<td>Ind. bacteria <em>a</em></td>
<td>Temperature</td>
<td>2.43</td>
<td>~841</td>
<td>16</td>
<td>-</td>
</tr>
<tr>
<td>[73]</td>
<td><em>A. ferrooxidans</em></td>
<td>Feasibility assessment</td>
<td>1.75</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>[74]</td>
<td><em>A. niger</em></td>
<td>Strain variations</td>
<td>3.5</td>
<td>-</td>
<td>23</td>
<td>Cu: 98</td>
</tr>
<tr>
<td>[75]</td>
<td><em>A. niger</em></td>
<td>Manganese supplement</td>
<td>6.8</td>
<td>~74</td>
<td>27</td>
<td>Ni: 38.6</td>
</tr>
<tr>
<td>[76]</td>
<td><em>A. niger</em></td>
<td>Growth medium</td>
<td>3.5</td>
<td>-</td>
<td>30</td>
<td>Co: 90 Co: 70</td>
</tr>
</tbody>
</table>

*Indigenous bacteria.*
Bioleaching of mineral concentrates has received attention to date. In flask experiments, Song et al. [69] analyzed the attachment characteristics of *L. ferrirphilum* and *A. caldus* on pyrite. They observed that the prevalence of *L. ferrirphilum* on the mineral surface was associated with the bacterial surface area and amount of organic functional groups produced; both were higher than that of *A. caldus*. The bacterial distributions were determined by real-time quantitative polymerase chain reaction (PCR). Jin et al. [58], on the other hand, used chalcopyrite concentrates to study the impacts of contact and non-contact mechanisms on the presence of *A. ferrooxidans*. The scholars reported a higher leaching rate with the non-contact mechanisms and claimed slow efficiency with the contact one. In addition, Han et al. [70] demonstrated the feasibility of bioleaching of Fe, Cu, As and Zn from chalcopyrite concentrates in column experiments without adding sulfuric acid to the system. Furthermore, using fungi species Ahn et al. [74] carried out several experiments to determine the optimum parameters of Cu and Co extraction from metal concentrates by *A. niger* cultured at different concentrations of citric, oxalic, and malic acid. They observed the highest extraction rates using the following concentrations: 227.06 ppm of citric acid, 1017 ppm of oxalic acid, and 38.82 ppm of malic acid; and at a 5% pulp density. Similar results were reported by the same research team after further studies [76]. Also, within 27 days of leaching time, Sukla et al. [75] obtained a 38.6% Ni recovery from a chromite overburden using *A. niger* supplemented with 80 ppm of manganese, which was 14.6% higher than that obtained with no manganese.

Multiple authors studied bioleaching of precious metals from chalcopyrite ores. Sukla et al. [63] and Panda et al. [73] documented the process flow of Cu recovery from chalcopyrite (0.3% Cu) from the Malanjkhand mine, India. The process included the extraction, purification, and final recovery of the metal mainly using bioleaching, solvent extraction, and electrowinning, respectively. They also calculated the energy demand of the process. A deep insight into bacterial attachment onto chalcopyrite surface was provided by Park and Kim [68]. They carried out mineralogical analyses, isoelectric point measurements, and visual observations to study indigenous bacterial attachment onto a mineral surface without adding sulfuric acid. The authors underlined the selective adhesion of vibrio-shaped bacteria onto the crystal phase of chalcopyrite regardless of sulfuric acid addition. Park et al. [66] and Han et al. [70] provided further evidence of bacterial attachment to a pyritic mineral surface through the characterization of EPS formation and bacterial attachment patterns of bacteria with different shapes. The scholars observed higher attachment trends by rod-shaped bacteria.

Mineral processing by bacteria from other ores has also attracted the attention of researchers. Metal recovery from galena was addressed by Park et al. [65,72] who studied the optimum operation conditions for mesophilic and thermophilic bacteria. They reported that the mesophilic strain was capable of oxidizing the mineral at room temperature while the optimum operating temperature for the thermophilic strain was 52 °C. Similarly, Park et al. [64] focused on sphalerite ore and extracted Pb and Zn using indigenous acidophilic bacteria. The highest extraction efficiency was obtained at 62 °C and a pH ranging from 2.40 to 2.55. The effect of a high temperature pretreatment on the bioleaching of laterite was addressed by Mohapatra et al. [61]. Using a consortium composed mainly of *A. ferrooxidans*, they found that the highest Ni and Co leaching efficiencies were achieved when the mineral was heated at 600 °C prior to the leaching experiments. At this temperature, according to the XRD results presented, goethite was converted to hematite, which reacts with sulfuric acid to produce ferric sulfate. The latter reacts with NiO, whereby its exposure, at the same time, is enhanced by the heating process. Thus, the leaching efficiency increases. Aiming to recover Cu from lagoon material, Sukla et al. [60] underlined the importance of bacterial adaptation to reach high leaching efficiencies; they also evaluated the role of solution pH, ferrous ion (Fe(II)) dosage, pulp density, and temperature. To identify the adequate leaching conditions of Manganese(II) from nodules, Choi et al. [62] combined isolated heterotrophic bacteria fed with corn starch, an economical carbon source. Some review articles on bioleaching from mineral concentrates have provided additional relevant information [59,67,71].

Overall, the research related to the bioleaching of mineral ores and concentrates mainly supports the importance of studying the influence of bacterial shape on the surface of the target mineral.
Such research also enhances the importance of the contribution of the contact mechanism on the overall process. Additionally, the study of other parameters, such as type of medium and temperature, received attention from researchers. As noted above, the application of bioleaching at an industrial scale to recover metals from these materials was limited to one article. Studies in this area are still in an elementary phase, if considering the techniques used for the experiments.

4.5. Bioleaching of Spent Catalysts

Certain solid catalysts used for industrial processes have to be replaced on a regular basis. The disposal of spent catalysts is a major issue due to its high content of metals, in oxide or sulfide forms, such as Co, Cr and Zn. However, at the same time, spent catalysts represent a secondary ore of precious metals. Therefore, both environmental and economic reasons have motivated the research and industry sectors to develop economical and environmentally friendly technologies to treat spent catalysts. Currently, hydrometallurgy and pyrometallurgy, besides landfilling, constitute the alternatives to treat and manage spent catalysts [77]. Nevertheless, these techniques demand a high economic investment and technicality. Consequently, other technologies are being developed, such as microbial leaching. The necessity of developing strains capable of tolerating high concentrations of heavy metals and other compounds present in spent catalysts constrain the application of bioleaching. Table 5 shows relevant details of the current research on the bioleaching of spent catalysts.

Table 5. Bioleaching research of spent catalysts according to the parameters studied and recoveries achieved as well as microorganisms used in the studies. The articles appear in chronological order.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Microorganism(s)</th>
<th>Parameters</th>
<th>pH</th>
<th>Particle Size</th>
<th>Time (Day)</th>
<th>Leaching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[78] A. ferrooxidans</td>
<td>Metal concentration</td>
<td>2.0</td>
<td>-</td>
<td>10</td>
<td>Ni: 97, V: 98, Mo: 18</td>
<td></td>
</tr>
<tr>
<td>[80] A. ferrooxidans</td>
<td>Pulp density, particle size, and temperature</td>
<td>1.8</td>
<td>44-105 µm</td>
<td>10</td>
<td>Ni: 95, V: 95</td>
<td></td>
</tr>
<tr>
<td>[77] -</td>
<td>Review</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Pradhan et al. [80] conducted a comparative analysis of the leaching efficiencies achieved using cultured and uncultured bacteria under different experimental conditions: contact time, particle size, pulp density, and temperature. The authors used spent catalysts samples obtained from the petrochemical industry in South Korea. They confirmed that the use of cultured bacteria produced faster leaching rates, agreeing with the findings presented by Pradhan et al. [78]. However, the bioleaching of cultured and uncultured bacteria presented similar responses based on changes to the above mentioned parameters. Particle size (45–212 µm) and pulp density (5%–25% w/v) variations provoked a considerable reduction of leaching efficiency—up to 15%. Similarly, Srichandan et al. [36] observed higher extraction efficiencies using size fraction 45 to 106 µm. The leaching efficiency decreased as particle size was increased. Also, Pradhan et al. [80] examined the influence of temperature during bioleaching experiments and found a higher leaching efficiency at 35 °C of operation. The results support those obtained by Pradhan et al. in an earlier study related to bioleaching kinetics of spent catalysts using Acidithiobacillus-type microorganisms; they added the influence of Fe(II) concentration on the bioleaching efficiency and leaching kinetics [79]. Inhibition of A. ferrooxidans by metal ion concentrations Ni(II), V(IV), and Mo(VI) was individually and synergistically studied.
by Pradhan et al. [78]. They determined that the tolerance of *A. ferrooxidans* to heavy metal ions was Mo(VI) 0.03 g/L, V(IV) 5 g/L, and Ni(II) up to 25 g/L.

Bioleaching reports of spent catalysts by fungi were scarce in the South Korean Journal Database. Gholami et al. [81] modeled the parameters involved in the bioleaching of spent catalysts by *A. niger*—acetic acids producers—using response surface methodology. They found that pH and pulp density constituted the most relevant factors in the process. To obtain a leaching efficiency of Co (71%), Mo (69%), and Ni (46%), the authors determined a pH of 5.0 and pulp density of 2% as optimum operating parameters. Although the scholars detected that an increased extraction efficiency was directly proportional to the concentration of gluconate, its dosage represented a constraint from the economic point of view. Several other aspects in the bioleaching of spent catalysts were presented by Mousavi et al. [77] in their review paper.

The authors who published in this category mainly investigated the influence of several parameters, such as pulp density, pH, and solid concentrations over the bioleaching kinetics. The feasibility of applying bacteria and fungi for the bioleaching of spent catalysts was proven by the researchers. Nevertheless, the current knowledge presented in the journals related to the treatment and beneficiation of these materials was scarce.

### 4.6. Bioleaching of Contaminated Soils

Dust, military, and refinery by-products have degraded the soil quality of urban and country side areas and have become a major concern to the environmental authorities as well as to the scientific community. This fact, added to the continuous enforcement of environmental regulations, boosts the upgrade of soil-treatment technologies; according to the current tendencies, bioleaching appears as an effective candidate. Nevertheless, its application for soil treatment is restricted to those soils contaminated with relatively low concentrations of heavy metals and is restricted by the presence of other pollutants that may inhibit bacterial activity. This section examines the literature produced considering the main findings on the bioleaching of mine tailings. Table 6 shows the highlights of these studies.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Microorganism(s)</th>
<th>Parameters</th>
<th>pH</th>
<th>Particle Size</th>
<th>Time (Day)</th>
<th>Leaching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[82]</td>
<td><em>A. ferrooxidans</em></td>
<td>pH</td>
<td>1.8-2.5</td>
<td>−210 µm</td>
<td>10</td>
<td>U: 82</td>
</tr>
<tr>
<td>[83]</td>
<td>-</td>
<td>Review</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[34]</td>
<td><em>Shewanella oneidensis</em> and <em>Shewella algae</em></td>
<td>Operation time, carbon source</td>
<td>7.2</td>
<td>−2000 µm</td>
<td>28</td>
<td>As: 63</td>
</tr>
<tr>
<td>[86]</td>
<td><em>A. thiooxidans</em></td>
<td>Sulfur supplementation</td>
<td>2.3</td>
<td>−2000 µm</td>
<td>26</td>
<td>Cu: 67.6, Pb: 25.8, As: 53.3</td>
</tr>
</tbody>
</table>

Past and present intensive military activities provoked the spread of heavy metals in soil, especially in shooting range areas. Han et al. [84] dealt with high concentrations of Pb, Zn, Cu and Cr during their experiments using *A. thiooxidans*; they studied the effect of temperature, sulfur concentration, and amount of initial bacterial concentration. The highest conversion for every metal was achieved at 26 °C. The influence of sulfur concentration had a low impact on bioleaching: the higher leaching efficiency was achieved at a 2% sulfur concentration for Pb and 5% for Zn and Cu. The leaching
efficiencies differed from metal to metal, Pb being the least extracted. Scholars attributed the low extraction efficiency to the formation of precipitates in the form of PbSO\(_4\). The results demonstrate the applicability of bioleaching to treat soils contaminated with heavy metals.

Soil pollution from refining activities also presents several challenges to recover metals by biological means. Heavy metals, such as, Cu, Pb and Zn, are commonly found in soils located in the vicinities of refineries or disposal zones, and bioleaching has been studied as a potential remediation technology. Han et al. [35] studied the differences of bioleaching using \textit{A. thiooxidans} and \textit{A. ferrooxidans} to treat contaminated soils from the vicinity of a refinery. According to the article, the authors observed the highest removal of heavy metals using \textit{A. ferrooxidans}. They attributed this result to the adsorption of Pb and As onto colloidal Fe(III) suspensions, produced by iron-oxidizing bacteria, and the negative impact on the production of PbSO\(_4\), by the sulfur-oxidizing bacteria. The same research team determined the leaching efficiency of \textit{A. thiooxidans} under various factors: stirring, bacterial inoculation, temperature, the solid-liquid ratio, and sulfur concentration [85]. The influence of stirring was mainly addressed as a means to improve oxygen transfer in the system; the beneficial effect of stirring was reflected in a lower pH. In regard to other parameters, the highest extraction efficiency was reached at 28 °C, a 6%-36% bacterial inoculation, 1.5 solid-liquid ratio, and a 3 g/L (w/w) sulfur concentration. The optimum sulfur concentration was in agreement with that suggested by Han and Lee [86]. Lee et al. [34] studied biostimulation and bioaugmentation phenomena supplying glucose or lactate as carbon sources to \textit{S. oneidensis} and \textit{S. algae} bacteria, respectively. Bioaugmentation induced the highest dissolved As concentration of 24.4 mg/L during 2 days of experiments. They spotlighted the impact of reaction time in the bioleaching process to maximize efficiency. Earlier, the bioleaching of polluted soil had been discussed by Kim et al. [82] who determined the optimum operating conditions of pH and temperature using \textit{A. ferrooxidans}.

Similar to the findings presented in other categories of this review, the articles found in the Korean Journals Database mainly discussed determining the optimum conditions for the bioleaching process. Several authors addressed the impact of factors such as solid concentration, pH, and energy supply. Certain scholars documented a high extraction efficiency of Cu, As and Zn. Nevertheless, the extraction efficiency of Pb was limited due to the formation of precipitates, which was promoted by the addition of sulfur as an energy source.

### 4.7. Other Applications

The advantages of bioleaching have been studied for purposes other than those addressed in previous sections. Contaminated wastewater, industrial wastes, spent batteries, as well as medical and ecosystem design studies through bacterial means have received some attention; published articles in Korean journals dealt with different challenges depending on the material. Therefore, the aim of this section is to address the literature production related to the aforementioned materials. Table 7 shows the highlights of the bioleaching research of these materials.

Biodegrading of wastewater from different activities has received outstanding support. In flask experiments, Song et al. [87] performed a comparative study of the adsorption time of nitrate onto various materials, such as dredged sediments and yellow clays, in a polluted seawater environment. These materials were activated by bioleaching for heavy metal removal and other methods (i.e., heat, bioleaching, and neutralization) applied in a different sequential order. According to the article, the adsorption equilibrium for sediments treated by bioleaching followed by heat was reached faster (17 min) than that observed by neutralization and a heat treatment. Leaching of mine drainage by biological means was also reported by Park et al. [88]. Using rod-shape indigenous bacteria, they focused on determining the influence of bacterial attachment with a special emphasis on EPS production. SEM studies showed the affinity of bacteria to attach onto straight-line fractures to obtain nutrients from a pyrite surface and subsequently form EPS. After 111 d of experiments, the scientists supported the feasibility of bioleaching to treat mine drainage.
We also found research articles on the bioleaching of spent batteries and Cu smelter dusts. The Chinese research team Li et al. [89] studied the impact of solution pH and the oxidation-reduction potential (ORP) on the bacterial leaching of lithium cobalt oxide (LiCoO$_2$) from spent batteries. By varying initial pH and redox potential (controlling Fe$^{2+}$ addition), the scholars determined that the dissolution of LiCoO$_2$ was mainly affected by solution redox potential. Even though the Eh-pH diagram of Li-Co-H$_2$O shows that LiCoO$_2$ is transformed into Co$^{2+}$ and Li$^+$ at solution redox potential ranging from $-300$ to $+1800$ mV and solution pH below 7.8, the highest removal efficiency in this study was obtained at 525 mV of solution redox potential (using Ag/AgCl as reference electrode), which was obtained at 1.5 of initial pH. In other words, the higher extraction efficiency was achieved at higher solution redox potential; they reported a 47.6% recovery of Co. In resembling studies, Mishra et al. [90] carried out flask experiments to observe the influence of elemental sulfur inputs in bioleaching. They suggested that 5–15 g/L of elemental sulfur was the optimum range. Their results were encouraging: they obtained around 80% of Co and 20% of Li conversions.

Table 7. Bioleaching research of other materials’ research according to the parameters and materials studied as well as recoveries achieved. The table presents the microorganisms used for the studies.

The articles appear in chronological order.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Material</th>
<th>Microorganism(s)</th>
<th>Parameters</th>
<th>pH</th>
<th>Particle Size</th>
<th>Time (Day)</th>
<th>Leaching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[91]</td>
<td>Metal sulfides</td>
<td><em>A. ferrooxidans</em></td>
<td>Energy source</td>
<td>1.8</td>
<td>-</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>[92]</td>
<td>Metal sulfides</td>
<td><em>A. ferrooxidans</em></td>
<td>Energy source</td>
<td>1.8</td>
<td>$841 \text{ } \mu \text{m}$</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>[87]</td>
<td>Dredged sediments</td>
<td>-</td>
<td>Adsorption</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[93]</td>
<td>-</td>
<td><em>Ind. bacteria</em></td>
<td>Review</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[90]</td>
<td>Lithium batteries</td>
<td><em>Ind. bacteria</em></td>
<td>Energy source</td>
<td>2.5</td>
<td>$106 \text{ } \mu \text{m}$</td>
<td>12</td>
<td>Co: 80 Li: 20</td>
</tr>
<tr>
<td>[94]</td>
<td>Industrial waste</td>
<td><em>A. ferrooxidans</em>, <em>A. thiooxidans</em>, <em>L. ferrooxidans</em></td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[88]</td>
<td>Mine drainage</td>
<td><em>Ind. bacteria</em></td>
<td><em>Ind. bacteria</em></td>
<td>Initial pH</td>
<td>3.16</td>
<td>$841 \text{ } \mu \text{m}$</td>
<td>110</td>
</tr>
<tr>
<td>[95]</td>
<td>Copper smelters dust</td>
<td><em>A. ferrooxidans</em>, <em>A. thiooxidans</em>, <em>L. ferrooxidans</em></td>
<td>Pulp density, nutrients, temperature, and amount of pyrite</td>
<td>1.8</td>
<td>$74 \text{ } \mu \text{m}$</td>
<td>35</td>
<td>Cu: 89.2</td>
</tr>
<tr>
<td>[96]</td>
<td>Artificial ecology</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[89]</td>
<td>Lithium-ion batteries</td>
<td><em>A. ferrooxidans</em></td>
<td>pH and Energy source</td>
<td>2.0</td>
<td>$707 \text{ } \mu \text{m}$</td>
<td>7</td>
<td>Co: 47.6</td>
</tr>
<tr>
<td>[47]</td>
<td>Industrial wastes</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[97]</td>
<td>Realgar-bioleaching solution</td>
<td><em>Caenorhabditis elegans</em></td>
<td>Reactive oxygen species</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>[98]</td>
<td>Pyrite</td>
<td><em>A. ferrooxidans</em></td>
<td>Mineralization potentials</td>
<td>1.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

$^a$ Indigenous bacteria.

Studies on the bioleaching of industrial wastes were mainly from critical reviews of the technology. Panda et al. [47] documented the acidophilic microbial diversity and its application to solid industrial wastes determining the state of the technology for different type of wastes and prospective. Earlier, Ahn et al. [93] provided general concepts, analyzed the feasibility of bioleaching, including the advantages and current applications, and finally drew general projections. Pradhan et al. [94] also devoted a whole section of their review article to address bioleaching of industrial wastes focused on fly ash and mine tailings.

Isolation of bacterial cultures and their subsequent activity assessment was presented. Won et al. [91] studied the leaching characteristics of metal sulfide using *A. ferrooxidans* isolated from acid mine drainage depending on an initial Fe(II) concentration over various solution mixtures of ZnS, CuS, and PbS. They reported that ZnS presented the highest affinity as an energy source. In contemporary studies, the same team gave deeper insight into the leaching characteristics of metal sulfides (i.e., CuS and ZnS) from wastewater. They emphasized the importance of particle size and the amount of Fe(II) supplementation [92].
Some uncommon applications of bioleaching were reported according to our search. In the area of ecosystem design, Kim [96] studied bacterial activity and the phenomena involved in mineral production by bioleaching. Zhi et al. [97] evaluated realgar bioleaching solutions for cancer treatment using C. elengans, thereby highlighting the capacity of these microorganisms to thrive at high arsenic concentration. Similarly, Zhou et al. [98] studied pyrite bioleaching to increase osteoblast formation and activity in rats. This article supports bioleaching as a valid pathway to improve pyrite bioavailability, which is traditionally used in Chinese medicine.

The studies presented in this section show the diversification of bioleaching applications in different fields. The articles found in the Korean Journal Database presented some applications of bioleaching related to the treatment of waste materials, such as spent batteries and industrial wastes. The researchers also contributed to knowledge in the medical sciences.

4.8. Molecular Studies

Understanding the response(s) of biomining microorganisms to certain changes in the environment and/or the presence of substances at the molecular level would certainly improve the current recovery efficiencies. Experts on biotechnology are currently focused on identifying and understanding genes, proteins, macro, and small molecules and their responses to fixed parameters, such as temperature, pH, and nutrient fixation [99–101]. For instance, N₂ fixation, essential for nitrogen assimilation in biomining microorganisms, has been predicted in A. ferrooxidans and L. ferrphilum strains. Bacteria fix nitrogen through the action of nitrogenase complex [102,103]. Similarly, genome sequencing was applied to understand, among others, the metabolic characteristics of ferrous and sulfur oxidation of acidophilic bacteria in different environmental conditions [104]. These studies are relevant to determine the optimum dosage of those elements to the bioleaching system. Although the genetic study of biomining microorganisms has not received major support to date in South Korean journals, several findings ought to be taken into account considering the relevant literature input from Chinese institutions. Table 8 presents relevant aspects of the research conducted in this area.

Table 8. Molecular studies research according to the parameters studied and recoveries achieved as well as microorganisms used. The articles appear in chronological order.

<table>
<thead>
<tr>
<th>Author(s)</th>
<th>Microorganism(s)</th>
<th>Parameters</th>
<th>Leaching (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[105]</td>
<td>T. ferrooxidans</td>
<td>Proteomic analysis</td>
<td>-</td>
</tr>
<tr>
<td>[106]</td>
<td>A. ferrooxidans</td>
<td>Gene expression</td>
<td>-</td>
</tr>
<tr>
<td>[107]</td>
<td>A. caldus</td>
<td>Electrotransformation</td>
<td>Cu: 92</td>
</tr>
<tr>
<td>[108]</td>
<td>L. ferrphilum</td>
<td>Genome</td>
<td>-</td>
</tr>
</tbody>
</table>

Findings related to protein and gene expression studies have been published in biotechnology related journals. Hu et al. [105] conducted a proteomic analysis of T. ferrooxidans comparing the bacterial growth on elemental sulfur with that in Fe(II). The logarithmic phase lasted 10 to 32 h when the microorganisms were cultivated in elemental sulfur and 4 to 12 in Fe(II). They found different protein patterns related to each energy source identifying 17 protein spots. Every spot was abundant when Fe(II) was used and only 11 using elemental sulfur. These proteins were characterized and compared with those present in other biomining bacteria. The results allow to properly understand the effects of different energy sources on bioleaching by T. ferrooxidans. Later, using real-time PCR, Wang et al. [106] studied the expression of CO₂ fixation genes in the presence of lix984n, a solvent extraction reagent, during various exposure times. The response of the microorganisms differed at different exposure times. In particular, the Calvin cycle, responsible for CO₂ fixation, was affected when the strain was exposed to the reagent for 15 min as every cbb gene expression was upregulated. However, only 10 min of exposure were necessary to affect cbb gene expression. Transferring exogenous DNA is also a challenge within biotechnology and it is certainly a key factor to improve the bioleaching process. Linxu et al. [107] provided a deep insight into the topic by developing a novel method to
carry out electrotransformation of *A. caldus*. They claimed to be the first research team to achieve the electrotransformation of this biomining species. One year later, the Chinese team Mi et al. [108] sequenced and annotated the complete genome of *L. ferriphilum* in the Journal of Microbiology. They studied the genes responsible for iron oxidation, pH and metal tolerance, as well as carbon fixation.

Contributions to molecular studies of biomining microorganisms published in Korean journals mainly focused on understanding the genetic response to environmental factors such as energy source. Publications of the complete *L. ferriphilum* genome along with a novel method to perform electrotransformation of *A. caldus* are relevant articles published in the Korean Journal Database. Nevertheless, the state of the art within this category is still scarce.

5. Challenges and Prospective of Biohydrometallurgy

Bioleaching technologies are developing rapidly and the efforts are mainly dedicated at the laboratory scale with some studies focused on pilotscale development. From the last specialized conferences in the area, we observed a major focus on the improvement of industrial reactors of the BIOX™ processes, especially the blowing system [109]. The current continuous bioleaching operations are also being challenged by the presence of hydrocarbons in the bioreactors. These compounds may accidentally enter into the production chain and inhibit bacterial activity (unpublished data). Similarly, several scholars are using cutting-edge technology to understand the irrigation and colonization of bio-heaps [110,111]. On the other hand, studies on the bioleaching from polymetallic sources and of rare elements are also part of the scientific discussion [112,113].

While technological advances have been made, mainly in the study of sulfide mineral interactions with microbes, research should focus on the bioleaching of silicate, carbonate, and oxide ores. A few research studies at the laboratory scale were focused on studying these minerals [114,115], and a systematic study is highly desired to understand the fundamentals of microbe-mineral interactions.

Our mineral reserves are getting scarce, are of a low grade, and difficult to mine and process. A significant amount of energy is directed just to liberate the mineral grains. Bioleaching, being an environmentally benign process, will play a major role in the near future for making mineral extraction sustainable by selectively attacking the exposed mineral grains. Research has started at some private institutions to develop specific enzymes for bioleaching applications. This may provide better opportunities as these specific enzymes target specific minerals and solubilize them in the liquid environment from which the metal can be extracted. The research direction on the development of bioleaching with enzymes is secretive, and although data are available, not much information has been published. The evolution of the technology towards these sources is expected in the short term.

The limitation of bioleaching is the slow kinetics of mineral extractions utilizing microbes. New techniques should target developing new catalysts that can improve the interactions of microbes with minerals while accelerating the kinetics. The catalyst should provide three critical functions: (1) activation of the mineral surface for faster interactions with microbes; (2) prevent passivation of the mineral surfaces during the leaching process; and (3) provide a continuous supply of the nutrients or electrons for the microbes. Those functions might effectively accelerate the kinetics.

Technological advancement should progress in both fronts—process and equipment development. While the above discusses the process development, equipment developments should be made during staged reactor design. In one reactor, microbe conditioning and production can take place while another reactor should focus on essential leaching conditions. Still, significant testing has to be done to make biohydrometallurgy amenable to mineral extractions.

6. Conclusions

In the present article, we addressed the current status of bioleaching research presented in South Korean journals to provide scholars with a body of knowledge that allows them to identify the gaps and demands of the technology. We identified an increasing literature production related to bioleaching in Korean and international journals registered in the Korean Journal Database. Such a work further highlights the opportunities for improvement.
According to the present body of knowledge, bioleaching is mainly applied for environmental reclamation and precious metal recovery processes. Metal recovery from mine tailings, mineral ores, and metal concentrates have caught the attention of researchers. Nevertheless, relevant gaps should be underlined, especially regarding the scale application. Despite our search to identify industrial applications of the technology, these studies were carried out abroad. This shows a successful response to the call for publications, but a weakness in the local development of the technology. Encouragingly, we can perceive the commercialization in the next years based on the research presented in different papers—in which the interest of companies in the technology is reflected in the acknowledgments section. According to the literature published in South Korean journals, we consider mine tailings and electronic waste as potential sources of precious metals. To face and overcome this challenge, deeper studies in heap and tank bioleaching are necessary. Most importantly, from our perspective, international collaboration from research institutions, experts, and mining-related companies as well as economic and political support will constitute tipping points for the technological development of bioleaching. Nevertheless, the current prices of metals worldwide may challenge the sector.

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