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Potential application of a knowledgebase of iron metabolism of *Acidithiobacillus ferrooxidans* as an alternative platform ☆

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ABSTRACT

Background: *Acidithiobacillus ferrooxidans* is a facultative anaerobe that depends on ferrous ion oxidation as well as reduced sulfur oxidation to obtain energy and is widely applied in metallurgy, environmental protection, and soil remediation. With the accumulation of experimental data, metabolic mechanisms, kinetic models, and several databases have been established. However, scattered data are not conducive to understanding *A. ferrooxidans* that necessitates updated information informed by systems biology.
Results: Here, we constructed a knowledgebase of iron metabolism of *A. ferrooxidans* (KIMAf) system by integrating public databases and reviewing the literature, including the database of bioleaching substrates (DBS), the database of bioleaching metallic ion-related proteins (MIRP), the *A. ferrooxidans* bioinformation database (Af-info), and the database for dynamics model of bioleaching (DDMB). The DBS and MIRP incorporate common bioleaching substrates and metal ion-related proteins. Af-info and DDMB integrate nucleotide, gene, protein, and kinetic model information. Statistical analysis was performed to elucidate the distribution of isolated *A. ferrooxidans* strains, evolutionary and metabolic advances, and the development of bioleaching models.
Conclusions: This comprehensive system provides researchers with a platform of available iron metabolism-related resources of *A. ferrooxidans* and facilitates its application.
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1. Introduction

Acidithiobacillus ferrooxidans, a branch of the genus *Acidithiobacillus* that contains 11 species [1], is a facultative anaerobe that can obtain energy from respiration and depends on ferrous ion oxidation as well as reduced sulfur oxidation. Since Colmer and Hinkle [2] discovered *A. ferrooxidans* in 1947, a large number of *A. ferrooxidans* strains have been isolated [1]. As the most important member of this genus, this microorganism has been employed in various fields such as bioleaching of metal sulfides and desulfurization of coal and gas by accelerating the oxidative dissolution of sulfide minerals, thus facilitating the recovery of base and precious metals from mineral leachates [3,4]. Additionally, it plays an important role in biogeochemical nutrient and metal cycling in acidic environments [5].

Although *A. ferrooxidans* can use both sulfur elements and ferrous ions during bioleaching, the ferrous iron oxidation system is less complex and more indispensable. On the one hand, iron metabolism is essential for *A. ferrooxidans,* and it participates in energy metabolism, bioleaching, and biosynthesis of biochemical products. On the other hand, the dissimilatory oxidation of iron and the reduction of iron catalyzed by *A. ferrooxidans* have a major impact on the biogeochemical cycling of metals in the environment. Previous research found that a medium that contains a ferrous iron product could promote the detachment of cell metabolites from the mineral surface and was more suitable for bacterial growth. In contrast, pH of the medium was too low and metabolites could cover the surface of the ore while the bacterium used elemental sulfur as an energy source [6]. Moreover, studies on the kinetics of the bioleaching process indicated that the growth and leaching rate were related to the presence and concentration

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of iron ions [7,8]. Taking the energy of carbon fixation, ATP synthesis, and conversion efficiency into consideration, a considerable amount of ferrous iron is required for oxidization by *A. ferrooxidans* [9]. To date, bioinformatics-based multiomics analysis has revealed that two electron transfer pathways participate in Fe^{2+} oxidation, which include the "downhill electron pathway" through c-cytochrome Cyc1 to aa3 cytochrome oxidase and the "uphill electron pathway" through c-cytochrome CycA1 > bc1 complex > ubiquinone pool > NADH dehydrogenase [5,10]. Thus, we focused on the iron metabolism of *A. ferrooxidans* in this study.

In recent decades, despite a large volume of experimental data based on *A. ferrooxidans* bioleaching, including mineral leaching information, genome and nucleotide sequences, protein and enzyme structures, and proteomics and kinetic models, scattered data have not been effectively integrated and are not beneficial for a comprehensive understanding of *A. ferrooxidans*. For example, owing to its advantages in metallurgy, environmental protection, and soil remediation, many researchers have studied *A. ferrooxidans* kinetics and tried to maximize its efficiency; however, the last systematic review was almost 20 years ago [7]. Furthermore, more than 500 isolates of *A. ferrooxidans* sensu stricto hitherto have been recovered, yet the taxonomic assignment of some isolates or clones remains elusive, and systematic analysis may be helpful to resolve evolutionary relationships [11,12]. With the advance of powerful tools such as omics, massive amounts of data have been generated, resulting in gradual mechanistic profiling [13,14,15]. Integration of findings may give insights and hasten progress. For example, metabolic flux analysis (MFA) has been improved with the availability of more gene and protein data, providing knowledge of internal cellular processes by showing internal reaction rates (fluxes) at steady state [16]. Meanwhile, various iron-based bioleaching substrates of minerals also deserve integration [17]. Therefore, it is necessary to comprehensively study the iron metabolism system of *A. ferrooxidans*.

With this background, the existing research data on iron metabolism of *A. ferrooxidans* were collected systematically by investigating public and private databases as well as the literature. Next, a web-based platform using structured query language (SQL) was constructed, and statistical analysis was performed. The knowledgebase of iron metabolism of *A. ferrooxidans* (KIMAf, http://gsbios.com/index/experimental/kimafen?id=9) provides an alternative platform to facilitate its potential application.

2. Materials and methods

2.1. Design and construction of the KIMAf system

The *KIMAf* was designed to integrate current resources and study results. It currently contains four databases: the database of bioleaching substrates (DBS), the database of bioleaching metallic ion-related proteins (MIRP), the *A. ferrooxidans* bioinformation database (Af-info), and the database for dynamics model of bioleaching (DDMB). DBS contains three classes of Fe-based, As-based, and Cu-based databases (db-Fe, db-As, and db-Cu). MIRP comprises two public databases named DICP and DCCP and one self-built database named Database of Metal-related Proteins in *A. ferrooxidans* (DMRP, http://gsbios.com/index/experimental/dmrpen?id=8). Af-info integrates cultured strains (DAf), nucleotide sequences (Af-NSD), protein sequences (Af-PSD), protein 3D structures (Af-PSD-3D), and protein–protein interaction information (Af-PPID) of *A. ferrooxidans*. DDMB includes kinetic models of the bioleaching process, which involve iron, copper, arsenic, uranium, and zinc ores (Fig. 1a).

The database array is listed in Fig. 1b, which shows the construction process and framework of KIMAf. The integration process

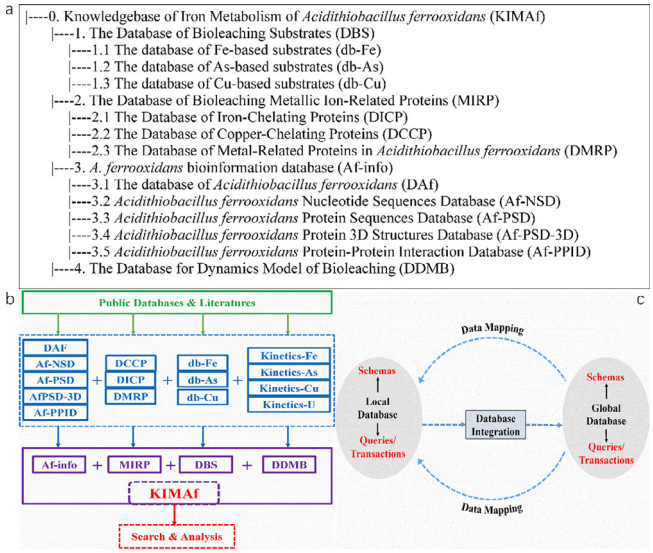

Fig. 1. Composition frame and workflow of KIMAf. (a) Dataset of the integration system of ion metabolism of *Acidithiobacillus ferrooxidans*, (b) Framework and relationships between different databases, and (c) Working principle of data input and output.

for local data and network resources is depicted in Fig. 1c. Scattered data of interest were queried and obtained from global databases, then summarized into corresponding local databases. Finally, other information was added by reviewing the literature.

2.2. Sources of the bioleaching substrates database

Resources in this study were derived from various public databases, the literature, and authoritative professional websites such as Google Scholar and MinDAT. To obtain comprehensive information, iron-related, copper-related, and arsenic-related minerals were summarized by searching MinDAT, Rruff.Info, Am.Min, Crystal Structure Database, etc. To obtain other information, Google Scholar, QUT Mineral Atlas, and the Handbook of Mineralogy were referenced (Table 1).

2.3. Database of bioleaching metallic ion-related protein sources

MIRP comprises DICP, DCCP, and DMRP. DICP and DCCP were published previously and include two types of proteins (1D and 3D). The database contains 21,656 entries, most of which were obtained from Protein Data Bank (PDB), GenBank (Release 141.0),

Table 1
The data sources of bioleaching substrate information.

Information sources	Websites
Am. Min. Crystal Structure Database	http://rruff.geo.arizona.edu
Amethyst Galleries' Mineral Gallery	http://mineral.galleries.com
EUROmin Project	http://euromin.w3sites.net
Ecole des Minerals de Paris	http://cri.ensmp.fr
Google Images	http://images.google.com
Google Scholar	http://scholar.google.com/
Handbook of Mineralogy (MinSocAm)	http://www.handbookofmineralogy.com
Handbook of Mineralogy (UofA)	http://rruff.geo.arizona.edu
MinDAT	http://www.mindat.org
Mineralienatlas (Deutsch)	http://www.mineralienatlas.de
Philatelic Mineralogy	http://stampmin.home.att.net
QUT Mineral Atlas	http://www.mineralatlas.com
Rruff.Info	http://rruff.info
WWW-MINCRYST	http://database.iem.ac.ru

SWISS-PROT, and Protein Information Resource [15]. DMRP is a database published by our lab previously, which is accessible through a web interface that indirectly generates MySQL queries. The data were retrieved from the National Center for Biotechnology Information (NCBI) and supplementary materials in the literature by searching key words such as copper, mercury, nickel, arsenic, zinc, and other element names. We finally obtained 2138 entries of protein sequence information, which include protein registration numbers, species names, the number of amino acids, amino acid sequences, and protein structures [18]. The construction steps were as follows: collect data by using an automated retrieval program and building the initial data set, then apply secondary analysis with analysis software. Finally, corresponding databases were established by adding comments manually and excluding redundant data through secondary analysis [19]. Fig. 2 shows the construction process using protein sequences as basic data.

2.4. Bioinformation data sources of A. ferrooxidans

Bioinformation data sources of *A. ferrooxidans* were derived from a search of the NCBI and manual screening. The key word "*A. ferrooxidans*" was used in different modules, and a summary of these data is listed in Table 2 and updated on the KIMAf website, including 15 features of this microbe. Nucleotide sequences, protein sequences, and protein 3D structures were added to Af-NSD, Af-PSD, Af-PSD-3D, and Af-PPID. For DAf, the literature in PubMed and Google Scholar was reviewed, and related information on the cultured strains was collected. Furthermore, the ATCC, CMCC, and CICC websites were searched.

2.5. Bioleaching kinetic model database sources

The DDMB aimed to integrate kinetic models of *A. ferrooxidans* (also called *Thiobacillus ferrooxidans*) that were related to ferrous/-ferric ions during bioleaching. The literature as well as supplementary materials were retrieved from PubMed and Google Scholar after applying the following search criteria. Two keyword groups were used, and the first group was "*Acidithiobacillus ferrooxidans*" or "*Thiobacillus ferrooxidans*" while the second group comprised "kinetic model," "iron and kinetics," "equation," "formula," "bioleaching," "model," and "kinetics." Related information was collected and added into Excel tables and classified into five parts that include the kinetic model, kinetic constants, experimental conditions, computing methods, and comments [7].

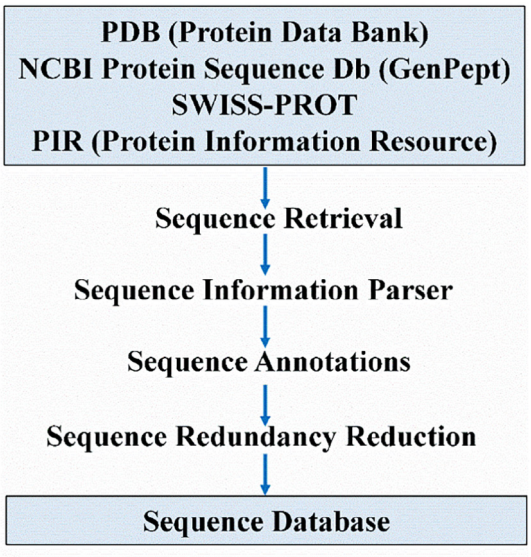

Fig. 2. Construction process of MIRP using public databases.

Table 2
The data sources of *A. ferrooxidans* bioinformation.

Bioinformation sources	Description
Nucleotide	4,703
EST: Expressed Sequence Tag	2
Protein sequence	32,305
Genome (whole genome sequences)	11
Structure: three-dimensional macromolecular structures	44
Taxonomy: organisms in GenBank	1
Gene: gene-centered information	3
BioSystems: pathways and systems of interacting molecules	562
SRA: Sequence Read Archive	9
BioProject: genome project information	23
CDD: conserved protein domain database	16
PopSet: population study data sets	50
GEO DataSets: experimental sets of GEO data	20
PubChem Substance: deposited chemical substance records	91
Protein Clusters: a collection of related protein sequences	344

3. Results and discussion

3.1. Relationship between A. ferrooxidans and bioleaching substrates

DBS integrates common ores, and they are classified by element, referring to public databases and the literature [17,20]. DBS has ten categories that include elemental iron, sulfides and sulfosalts, halides, oxides, carbonates, borates, sulfates, (phosphates, arsenates, and vanadates), silicates, and organic compounds. There were 1994 different kinds of minerals in total, which include 860 kinds of iron minerals, 519 kinds of arsenic minerals, and 565 kinds of copper minerals (Table 3). According to the composition groups, arsenic minerals can be further divided into 6 categories that include elemental arsenic, arsenic (III) oxides, arsenic (V) oxides, arsenic sulfides, arsenic sulfosalts, and arsenides [17].

Minerals are not only important metal resources but also provide energy sources and growth environments to microbes such as *A. ferrooxidans*. Deeper insight into ores will be beneficial to the bioleaching process. Here, db-As was taken as an example. Under fully oxidized conditions, arsenate can bind strongly to Fe^{3+} oxide minerals, whereas the relationship between arsenic and redox-active substrates is less clear when environmental conditions are less oxidized and microorganisms participate. During the processes of uptake and release from solution, adsorption, and precipitation, the rate was mediated by microbial activities [21], which were reflected by the bioleaching mechanism of realgar. On the one hand, *A. ferrooxidans* can participate in the biodissolution of realgar directly. On the other hand, in the presence of Fe (II), *A. ferrooxidans* oxidizes Fe(II) in the system to Fe(III). As a strong oxidant, Fe(III) can oxidize realgar to As(V) and be reduced to Fe(II) [6]. Meanwhile, Fe(III) and Fe(III) complexes produced in this process eventually form a chemical adsorbent, which cooperates with the biosorption of *A. ferrooxidans*, causing the removal of soluble arsenic from the entire leaching system [22]. Therefore, broadening the knowledge of mineral composition and basic properties can provide guidance for experiments.

3.2. Data analysis of MIRP

MIRP comprises various metal ion-related protein sequences of *A. ferrooxidans*, whose abnormal metabolism, particularly with transition metal ions such as copper and iron, is associated with

Table 3
Summary and examples of the bioleaching substrates database.

Category	Elements	Sulfides/ Sulfosalts	Halides	Oxides	Carbonates	Borates	Sulfates	Phosphates Arsenates Vanadates	Silicates	Organic Compounds
db-Fe	34 species Iron	97 species Cubanite	11 species Molysite	113 species Ferberite	10 species Siderite	10 species Ericaite	91 species Mikasaite	231 species Maricite	260 species Fayalite	3 species Minguzzite
db-As	5 species Arsenic	146 species Enargite	2 species Ecdemite	37 species Arsenolite	1 species Claraite	2 species Cahnite	5 species Fornacite	312 species Alarsite	9 species Nelenite	NA NA
db-Cu	18 species Copper	206 species Anilite	34 species Marshite	45 species Crednerite	19 species Azurite	5 species Bandylite	82 species Bonattite	126 species Blossite	25 species Kinoite	5 species Antipinite

References: Handbook of Mineralogy (Anthony et al., 2000); the Mineral Database (http://www.mindat.org).
Abbreviations: NA, non-available.

many human diseases [23]. MIRP contains more than 29,000 protein entries that involve 16 kinds of elements (Fig. 3). Keyword queries such as protein sequence number (GI), GenBank (code), element name, PDB ID, common protein name, and ion valence are available. The protein serial number, registration number, number of amino acids, sources of primary structure, species, and protein sequences are in the results.

Metalloproteins play a significant role in organisms, performing functions in metal binding, sensing, transporting, and metabolism [24]. With advances in various technologies, such as proteomics, more structures of metal-related proteins will be available, and the importance of systematic integration will be highlighted. DCCP and DICP were constructed by Shandong Normal University [13], allowing primary sequence comparison, secondary structure prediction, and SCOP structure classification, and revealing the relation between amino acid differences and structure domains. For DMRP, 63 *A. ferrooxidans* arsenic-responsive protein sequences were selected to predict and analyze the physicochemical properties, hydrophobicity, and transmembrane regions by using TMHMM2.0, Protscale, and Protgaram software, which provide guidance for the isolation and purification of these proteins. Finally, the arsenate membrane protein and the arsenate resistance protein were selected to construct corresponding protein phylogenetic trees, which could facilitate the understanding of similarity among different species [18]. Furthermore, applications of such databases can be validated by experiments. Recently, liquid chromatography, mass spectrometry, NMR, and proteomic analysis

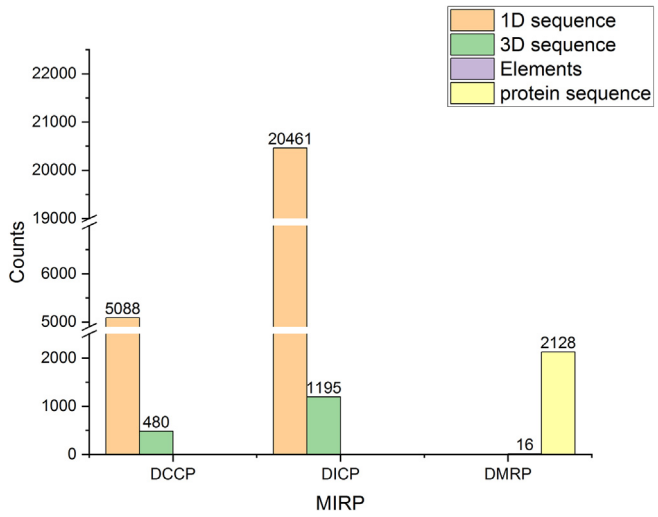

Fig. 3. Statistics of MIRP, including DCCP, DICP, and DMRP, by the number of 1D sequences, 3D sequences, elements, and protein sequences.

were combined to identify the growth of *A. ferrooxidans* on realgar nanoparticles. Twenty-four differentially expressed proteins were identified and purified, then protein pathways were constructed (Table S1), and PI values were similar to previous predictions. In addition to the information in this protein database, the roles of these pathways in the processes of energy metabolism and transport reactions should be traced back to genes and metabolic mechanisms.

3.3. Evolutionary and metabolic analysis

Af-info consists of DAf, Af-PSD, Af-PSD-3D, and Af-PPID, which include 4,703 nucleotide sequences, 2 expressed sequence tags (EST), 32,305 protein sequences, 11 genomes, 44 three-dimensional macromolecular structures, 3 genes, 562 BioSystems, 9 sequence read archive (SRA) datasets, 23 BioProject datasets, 16 conserved protein domains, 50 population study datasets, 20 GEO datasets, 91 PubChem substances, and 344 protein clusters (Table 2). In particular, DAf-integrated isolated *A. ferrooxidans* strains that comprise more than 300 strains and their initial origin information plus molecular feature information (Table S2). Based on their geographical features, their distribution is shown in Fig. 4, which suggests that most of the cultured *A. ferrooxidans* strain reports were from China, America, India, Russia, Peru, and Canada.

With the improvement of classification tools and accumulation of sequencing data, integration of such information can provide insights related to geobiology and evolution. Recently, 11 genomes and corresponding annotations of *A. ferrooxidans* were made available in NCBI. Clusters of Orthologous Groups analysis found that energy production and conversion as well as inorganic ion transport and metabolism category accounted for a large portion, which indicated their adaptability to grow in environments with high concentrations of metal ions [13,25]. When these data are collected and analyzed together, differences among them are helpful to define conserved gene clusters and the prediction of metabolic pathways of proteins encoded by conserved genes. Moreover, various molecular tools for the typing and identification of bacteria have been used, changing their classification based on morphological and physiological characteristics. As a result, polyphasic taxonomy was proposed [5]. 16S rRNA gene-based ribotyping, oligotyping, and multilocus sequencing analysis were applied to improve the phylogenetic resolution and better define the species boundaries, which is useful information for isolation and evolutionary studies [12]. Therefore, DAf can be used to interpret geographical diversity or specific environmental cues and discover evolutionary trees [26].

This data integration can also be applied to metabolic mechanisms. *A. ferrooxidans* obtains energy by oxidizing ferrous ions or

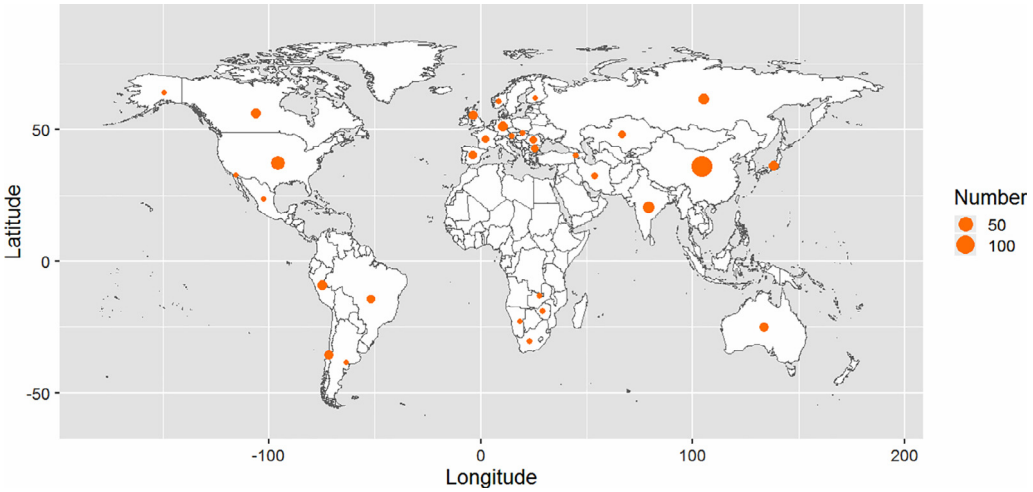

Fig. 4. Distribution of isolated *Acidithiobacillus ferrooxidans* strains across the world. The size of the points is positively correlated with the number of isolated strains.

elemental sulfur; therefore, they are widely used in bioleaching processes. However, a high concentration of soluble iron in an acidic environment raises questions about the mechanisms of iron assimilation and homeostatic control of internal iron concentrations. Problems were resolved by genome sequencing for nearly 10 years, revealing diverse standard iron uptake mechanisms. Kyoto Encyclopedia of Genes and Genomes (KEEG) analysis showed that genes encoding iron oxidation functions of *A. ferrooxidans* were organized into two transcriptional units, the petI and rus operons, which are involved in electron transfer of the downhill electron pathway [5]. The operons are highly essential for *A. ferrooxidans* that an organism can contain up to 5% of its total cell protein in the form of Rus while surviving on a substrate with little energy [27]. Additionally, when energy is derived from the oxidation of iron, NADPH and ATP are produced, and then *A. ferrooxidans* fixes CO_2 through the Calvin-Benson-Bassham reductive pentose phosphate cycle (Calvin cycle) [28,29]. In addition to the applications mentioned above, iron-related genes and proteins are closely related to quorum sensing, the extrusion of toxic organic compounds and attachment during bioleaching [30,31]. After the elucidation of gene annotation and metabolic pathways, construction of the MFA was possible, which was accomplished in 2008 based on a combined pathway/genome database. A mathematical model involving 138 pathways with 658 enzymes catalyzing 752 enzymatic reactions with 631 compounds was derived and parameterized by the balancing of metabolites. Verified experimental data of catabolism and transport processes were reproduced well by this model [16,32]. These studies further illustrate the importance of the integration of bioinformatics data to gain a holistic view of the organism.

3.4. Progress with bioleaching kinetics

DDMB contains 50 kinetic models of *A. ferrooxidans* that are associated with ferrous/ferric ions. Kinetic models, kinetic constants, experimental conditions, computing methods, and comments are shown in Table S3. The development of leaching kinetics usually follows the elucidation of the leaching mechanism. Thus, a summary of these kinetic models is beneficial to the design, optimization, and control of the bioleaching process.

Bioleaching is defined as the mobilization of metal cations from often almost insoluble ores by biological oxidation and complexation processes [30]. During this process, *A. ferrooxidans* converts ferrous iron to ferric iron, thus it maintains a high redox potential. Therefore, kinetic models were always described as functions of the ferric-ferrous-iron ratio and fit the experimental data well. With progress made in elucidating the bioleaching mechanism, some alterations took place in the dynamic model, changing from empirical equations to mechanism equations [33]. In the beginning, the logistic equation was successful in predicting the rate of bioleaching, whereas it did not work so well for different size fractions. Consequently, the parameters for the different size fractions were introduced through a shrinking particle process [34]. Lacey and Lawson found that the growth rate decreased because of increase in iron concentrations, which could be fitted to the Monod equation [35]. Then, many complicated equations were generated by improvement of the Monod equation, such as the introduction of the concept of a threshold ferrous iron concentration [33]. Taking the chemiosmotic theory and electrochemical theory into consideration, the rate expressions were rather complex. A simplified form of the Michaelis–Menten kinetic equation was proposed to model the rate, which incorporated the specific oxygen utilization rate and the ferrous/ferric-iron ratio [36]. The kinetics could also be expressed with the Nernst equation, including the effects of temperature, substrate, and cell inhibition [37]. Furthermore, Boon tried to determine the kinetics in terms of a specific oxygen utilization rate using the Pirt equation. Some others also proposed models according to the Langmuir adsorption isotherm based on the direct mechanism. May and Hansford used the Butler–Volmer equation to model the kinetics of the two subprocess mechanisms [33]. Currently, “contact” and “noncontact” leaching models have been widely accepted. Recent studies involving surface science, biochemistry of iron and sulfur metabolism, attachment, and biofilm formation such as the dissolution process occurring in the extracellular polymeric substances layer and the good performance of metabolic models for predicting reaction rates, deepen insights into bioleaching, and provide novel perspectives [32,38]. However, comprehensive models to define the complex process are lacking. By integrating related models, more potential biotechnological applications will become available.

3.5. Prospects in bioleaching research based on KIMAf

Based on genomic data, previous studies revealed the oxidation process of ferrous ions and sulfur and reconstructed the metabolic system with sulfur as energy [39,40]. In addition, an iron ion absorption and metabolism model as well as a primary model of whole cell metabolism were established [10]. Meanwhile, intrinsic links between genomic data and life activities were clarified with powerful bioinformatics tools [5,31,40]. These explorations highlighted

that the integration of biological leaching information of *A. ferrooxidans* will continue to expand our knowledge of this field.

Here, the iron metabolism integration system of *A. ferrooxidans* was established for the first time. In DBS, a large number of compounds related to arsenic, copper, and iron were collected and further classified into 10 categories. These ores are mainly inorganic materials and come from mineralogical knowledge that involves the identification of materials and analytical methods from material science. In MIRP, focus was mainly placed on the establishment of the arsenic ion-related protein database. Current studies have confirmed that the metal tolerance of *A. ferrooxidans* strains is related to the roles of oxidases, reductases, and metal-binding proteins, which are involved in arsenic resistance mechanisms that include As(III) oxidation, cytoplasmic As(V) reduction, and As(III) methylation [41]. Further analysis revealed that these mechanisms were closely related to their molecular structures. Van Drie's research on the geometry of 3D molecular structures, atomic ranking search, computer-aided molecular design, and pharmacophore recognition provided an effective method to study the structure of a matrix from a protein [42]. Therefore, research on bioleaching mechanisms can benefit from the metal ion-related protein database and Af-info database. The DDMB will be gradually improved and biological dynamics network computer simulation (CSBDN) will be added into the KIMAf system to realize the *A. ferrooxidans* bioleaching simulation model [43]. Moreover, the composition of metabolites after leaching and the surface modification changes before and after leaching were explored [6] and it was also useful to add them into this system.

Previously, many studies addressed the topic of *A. ferrooxidans* and its functional implications for bioleaching as well as its metabolic mechanism, phylogenetic structure and diversification and kinetic models. However, the KIMAf system provides comprehensive information on the iron metabolism of *A. ferrooxidans* in a more convenient way by the collection of study results and scattered internet resources. In the future, the internal relationships among *A. ferrooxidans* biological information, molecular mechanisms ranging from metabolism to bioleaching, and computer simulation models of bioleaching and protein–protein interaction mechanisms will be explored, laying a solid theoretical foundation for application research.

4. Conclusions

In this study, a KIMAf was constructed, which comprised a bioleaching substrate database, a bioleaching metallic ion-related protein database, a Af-info database, and a kinetic model of the bioleaching database. Then, the information sources, framework, and functions of the KIMAf system were introduced. Furthermore, the relations between bioleaching substrates and *A. ferrooxidans*, the application of evolutionary analysis with MIRP, iron-related metabolic progress, and the development of leaching kinetics accompanied by advances in leaching mechanisms were discussed. Such integration and analyses provide researchers with a comprehensive platform of available resources and will facilitate applications of *A. ferrooxidans*. (available at http://www.gsbios.com/index/experimental/kimafen?id=9).

Conflict of interest

The authors declare that there are no conflicts of interest.

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Supplementary material

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