Chapter

Acidithiobacillus Its Application in Biomining Using a Quorum Sensing Modulation Approach

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Abstract

A group of particular acidophiles microorganisms (bacteria and archaea) known as chemolithoautotrophs are capable of using minerals as fuel. Its oxidation generates electrons to obtain energy and carbon that is obtained by fixing CO_2 from the air. During this aerobic mineral oxidation, metals are solubilized or biodegraded. Metal bioleaching usually is used in biomining and urban biomining approaches to recovery metals such as copper, gold and zinc. Several species of bacterial genus *Acidithiobacillus* display a great bioleaching activity. Bacterial attachment and biofilm formation are the initial requirements to begin a successful bioleaching process. Biofilm formation in *Acidithiobacillus* bacteria is strongly regulated by cell to cell communication system called Quorum Sensing. The goal of this chapter is to review the Quorum Sensing system mediated by the autoinducer N-acyl- homoserine-lactones in the Bacterium *Acidiothiobacillus ferroxidans*, in order to enhance and to boost the bioleaching technologies based in the use of this bacterium. The main applications of the cell-to-cell communication system concepts in *A. ferrooxidans* are reviewed in this chapter. It is that the addition of synthetic autoinducers molecules, which act as agonist of quorum sensing system, especially those with long acyl chains, both as single molecules (C_{12}-AHL, 3-hydroxy-C_{12}-AHL, C_{14}-AHL, and 3-hydroxy-C_{14}-AHL) or as a mixture (C_{14}-AHL/3- hydroxy-C_{14}-AHL/3-oxo-C14-AHL) increased the adhesion to sulfur and pyrite and enhance the metal bioleaching in urban biomining approaches.

Keywords: AfeI/R, Biofilm, Bioleaching, EPS, Autoinducer, Synthetic Agonist

1. Introduction

As the world population grows, the reduction of several natural sources becomes more evident. One of the major concerns is the fastest decrease of metals ores. Currently, the hyper-technological society demands several metals in order to attend the rising request for electrical and electronical devices. The fact of the production of these devices is gradually cheapest and its useful life is increasingly shorter. Equally, the advertising campaigns increase the people desires to renew the older devices by a brand-new much faster and modern. It has been triggering the generation of waste of electrical and electronical devices (E-waste). It has been rising 5 folds in the last 20 years [1]. The organic and inorganic fractions of E-waste could be a serious threat to environment and public health if its final disposition is not done accurately. The incineration and landfill are the global strategies frequently applied in a whole world for final disposal of E-waste [2]. Although of its highly toxic nature, E-waste particularly, printed circuit boards (PCBs) are a promising secondary source of metals. The concentrations of copper (Cu) and precious metals, such as gold (Au), platinum (Pt) and palladium (Pd), are high compared to natural mines [3]. Urban E-waste mining knowing as Biomining is expected to be an important secondary source of metals in the near future. PCBs are electronic components whose most abundant metal is Cu (roughly 20–25% by weight). PCBs are also composed of a substantial amount of precious metals. Precious metals constitute the largest fraction of the value of discarded PCBs and are the main economic driver of metal recovery [4]. In the last 20 years, great efforts have been focused in the recovery of these metals from the E-waste, mainly based in traditional approaches such as pyrometallurgy and hydrometallurgy. However, these two approaches have been inconvenient, due to the high consumption of energy and the high emission of polluting gases in the first one approach and in the second one the high generation of acid leachates, these leachates could reach easily the subterranean and surface water bodies [5].

Recent biotechnological developments have made possible the adaptation of various microorganisms to hostile bioleaching environments. Thus, emerging a new approach for the metal recovery from E-waste called biohydrometallurgy. This process involve microorganism in order to make use of metal elements for their structural and metabolic functions. This process based on the use of biomass has the advantages over pyrometallurgy and hydrometallurgy processes such as: it does not require the construction of a huge infrastructure, it is not necessary to deal with a large amount of harmful residual pollutants generated in the process. However, the main bottleneck that prevents this process from being attractive at an industrial level despite its high efficiency, it is the drop in yield of metal recovery when using large amounts of E-waste. However, the main bottleneck that prevents this process from being attractive at an industrial level despite its high efficiency, it is the drop in yield of metal recovery when using large amounts of E-waste. The cell-to-cell communication system known as Quorum sensing, it allows bacteria to colonize new ecological niches, to resist environmental changes and toxic substances, enhances its competitiveness and to resist host defenses [6]. As this quorum sensing system regulates near to 25% of non-essential genes, its knowledge, understanding and modulation could help to enhance the bioleaching reactions at industrial level. In this chapter, we focused to review the quorum sensing system in the bacterium *Acidithiobacillus ferrooxidans*, which bacterium is widely recognized by its tremendous bioleaching ability, and discussed the application potential of QS of this bacterium in bioleaching.

2. *Acidiothibacillus ferroxidans*

The gram-negative bacterium *A. ferrooxidans* belongs to a gamma-proteobacterium group. *A. ferrooxidans* is a facultative anaerobe bacterium, which is able to grow from oxic to anoxic environments. *A. ferrooxidans* is acidophile, mesophilic and chemolithoatotrophic, that obtain energy mainly by the oxidation of ferrous iron (Fe^{2+}), elemental and inorganic sulfur and other sulfur compounds [7]. *A. ferrooxidans* grows optimally at temperature of around 30°C and pH below 2. This bacterium is a natural inhabitant of ecological niches associated to pyritic ores, coal deposits and acid drainages [8]. Another exceptional aspect of A. *ferrooxidans* is its outstanding ability to thrive in environments with higher concentration of dissolved Fe^{2+} around of 10^{16} folds superior that neutral environments. Opposing to what would expect that this massive soluble Fe concentration leads to bacterial DNA and protein damage, *A. ferrooxidans* uses Fe as micronutrient and energy source, which makes it an exceptional model microorganism for the homeostasis and assimilation of Fe [9].

The genome size of *A. ferrooxidans* is 2.98 MB with 58.7% of G + C, a total of 3217 protein encoding genes of which 2070 have a putative function [10]. Like others bioleaching bacteria, *A. ferrooxidans* must face with huge heavy metal concentration in their natural niches. Diverse gene cluster have been related with the tolerance to mercury, arsenic and copper. These genes including: copper extrusion system, resistance-nodulation cell division family transporters and encoding copper translocating ATPases [10]. Two Quorum Sensing systems mediated by the autoinducer acyl homoserine lactones molecules (AHLs) have been described in *A. ferrooxidans,* the first one AfeI/R resembling to classical *LuxI/R* system presents in numerous gram-negative bacteria [11] and the second one called *act* for de acyl transfer function also produces AHL autoinducer molecules [12]. The review of these quorum sensing systems in *A. ferrooxidans*, its conformation likewise, the biological traits regulated by these communication systems such as: energy metabolism, attachment, biofilm production and toxic tolerance are the main purpose of this chapter.

3. Bioleaching reaction by *A. ferrooxidanss*

In the bacterium *Acidithiobacillus ferroxidans*, bioleaching is carried out by three types of mechanisms: (i) contact, (ii) independent contact and (iii) cooperative. These three mechanisms are not mutually exclusive and generally work synergistically. The contact mechanism is characterized by the imperative need for the bacterial production of Extracellular Polymeric Substances (EPS). This EPS is essential for the subsequent formation of biofilms that will facilitate the contact between the bacterial wall and the surface of the E-waste and thus achieve the oxidation of the metal.

Previous studies have shown that *A. ferroxidans* bacteria do not adhere randomly to solid surfaces, but there is still a fine chemotaxis mechanism involved in preferential adherence to metals [13]. In the independent contact mechanism, the bacteria are not attached to the surface and the oxidation of Fe^{2+} in solution to Fe^{3+} occurs. Fe will subsequently act as an oxidizing agent, solubilizing the metals contained in the E-waste particles [14]. The cooperative mechanism is a combination of the two previous mechanisms in which the attached bacteria and the oxidizing agent Fe^{3+} in solution cooperate to oxidize the metals present in the E-waste particles. In the E-waste, specifically in PCI, metals are not present in the form of metallic sulphides. These are present as zero valence metals such as Cu^0, Zn^0, Ni^0, etc. Thus, the ferric ions and/or protons produced biologically from ferrous ions or the reduced sulfur compounds are responsible for the conversion of insoluble neutral metals to water soluble metallic ions.

4. Quorum sensing systems in extremophiles and its biotechnological applications

Cooperative bacterial behavior is one of the most intensively studied topics of microbial ecology in recent decades. The understanding of this lifestyle allows us to revel the fitness strategies of bacteria to thrive in very different environmental niches. Quorum sensing (QS) is a system of bacterial cell–cell communication that enables the microorganism to sense a minimum number of cells (quorum) in order to respond to external stimuli in a concerted fashion. QS system is based on the production, releasing to the extracellular environment and perception of small diffusible molecules known as auto-inducers (AI) [15]. Thus, this communication allows the bacteria to detect changes in the density of the population, which leading to generate variations in the concentration of nutrients, oxygen, inorganic molecules, pH and osmolarity in the extracellular environment. In this way, an increase in the bacterial population causes the accumulation of the AI molecule in the medium, which, when detected by the bacteria, generates changes in the expression of several target genes in order to regulate phenotypes traits in pathogenic or environmental bacteria. The main traits regulated are: attachment, virulence, resistance and bioluminescence among others [16].

In Gram-negative bacteria including the extremophiles (i.e. thermophiles, halophiles, psychrophiles and acidophiles) the most studied system involves AI molecules of the N-acyl homoserine lactones (AHLs) or autoinducer-1 (AI-1) type. This QS system is involved in intra-species and inter-species communications [17]. It was first described in the bioluminescent marine bacterium *Vibrio fischeri*. This system is considered as the QS paradigm in Gram-negative bacteria, which includes at least four elements: (i) AHLs as signal molecule or AI-1, (ii) an AHL synthase protein responsible for the synthesis of AI-1, (iii) a transcriptional regulator (belonging to the R protein family) and (iv) a cis-active palindromic DNA sequence that is the target of the R-AHL binary complex [18]. The general mechanism of action of AHLs molecules is to diffuse into the target cell and bind to an R-type transcriptional regulator to generate its dimerization (**Figure 1**). This occurs when the concentration of AHLs in the extracellular medium is adequate to generate a gradient towards the intracellular space [19]. The concentration of AHL molecules in the extracellular environment not only depends on their synthesis, but also on their diffusion, transport and degradation. The diffusion of AHL molecules depends on their size.

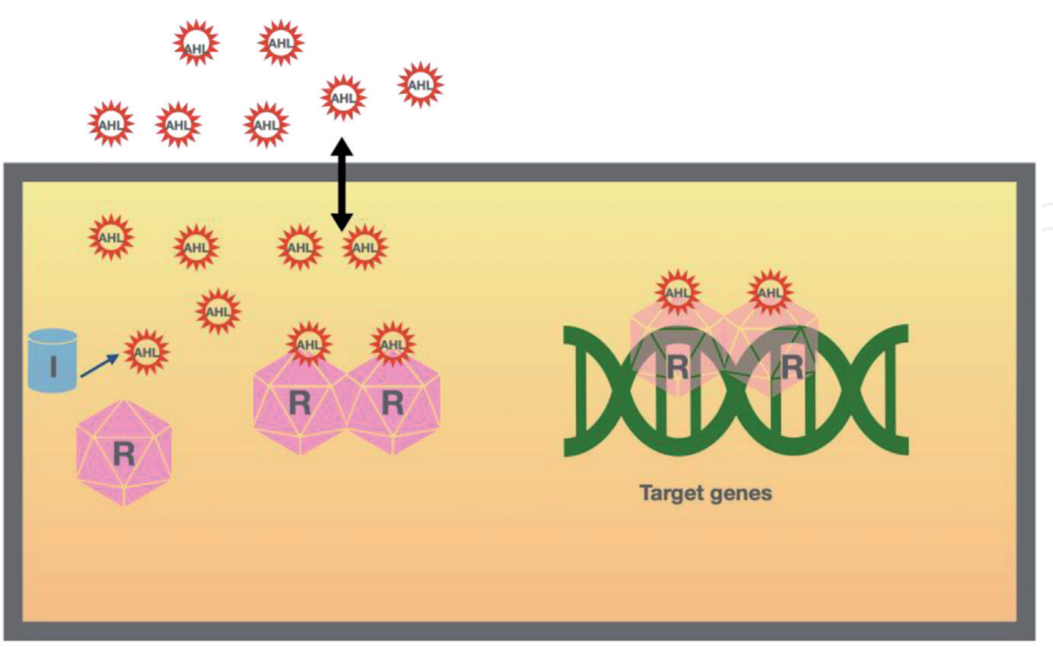

Figure 1.
Schematic representation of Quorum Sensing system type I AI-1. AHLs are synthesized inside the bacteria by protein I. The AHLs molecules diffuse towards the extracellular environment, increasing their concentration both in the internal and external environment of the cell in proportion to the increase in the cell population. Upon reaching the threshold concentration, the AHLs molecules bind with the R protein, which dimerizes and promotes or represses the transcription of target genes. Some of these target genes have a binding site for the R/AHL complex in their promoter region.

Short acyl chain AHLs diffuse freely across the cell membrane [20], while long chain AHLs are excreted by transporters [21].

The biological meaning of cell to cell communication in extreme environments is not completely comprehended, however, this communication systems play a pivotal role in the survival to fitness to these restrictive habitats. However, a better understanding about the adaptation strategies regulated by quorum sensing in these extreme ecological niches, could possibly contribute to the design and development of innovative approaches for the biotechnological solutions with industrial, medical and research applications.

Most of the Quorum sensing systems present in mesophyll bacteria are shared by thermophilic bacteria. Bioinformatic analysis revealed the existence of complete AI-2 systems in 17 thermophilic bacteria from phyla Deinococcus-Thermus.

For other hand, 18 show incomplete QS systems having only LuxS [22]. Particularly interesting is the AI-2 production in the bacteria *Thermotoga maritima* and *Pyrococcus furiosus.* These two hyperthermophiles bacteria produce AI-2 signals despite lacking of synthase enzyme LuxS [23]. *T. maritima* and *P. furiosus* have genes that encode for the SAH (S-adenosylhomocysteine) hydrolase, which catalyzes the cleavage of SAH producing adenosine and homocysteine. Then, the adenosine produced is transformed to AI-2. This production of AI-2 in independent fashion of luxS could be explained by the action of the rearrangement of phosphorylated ribose, which rearrangement seems to be modulated by temperature depending mechanism [24].

Psychrophile bacteria produce DiKetoPiperazine (DKPs) molecules, which can activate the LuxR circuit. *Pseudoalteromonas haloplanktis* bacterium produce eight different types of DKPs [25]. DKPs shows a great potential for disruption of the AHLs quorum sensing system in the pathogenic bacteria *Burkholderia cenocepacia* and *Pseudomonas aeuroginosa.* It results from the direct inhibition of AHL synthase. This fact represents a great potential for the alternative treatment of disease such as cystic fibrosis [26]. Another industrial application mediated by QS in psychrophile bacteria is the alginate overproduction at low temperature by the *Pseudomonas mandelii*. Pseudomonads bacteria produce alginate by the expression of genes *algU* and *mucA*. These genes expression are QS regulated [27]. In the biofilm formation process at low temperature (4–15°C) the alginate operon transcription is keeping active because its repressor is downregulated. Biofilm formation shows to be an adaptation strategy for survival at low temperatures [28].

Acidophiles bacteria have endowed with Quorum Sensing systems such as: AI-1, AI-2 and CAI-1. The genome of acidophilic iron oxidizing bacterium *Ferrovum* sp. harbors a thermophilic ene-reductase (ERs). The ERs Family enzymes has shown that it could play a role in the Quorum Sensing mediation of oxidative stress response [29]. *Ferrovum* sp. lacks of encoding gene for the AHL synthase LuxI. The gene *foye-1* in the genome of *Ferrovum* is located directly downstream of the LuxR. A previous study suggests that ERs FOYE-1 in this bacterium could be responsible for the production of HLA and triggers the LuxR perception [30]. The ene-reductase enzymes have increasing relevance in the field of bio-catalysis because of its superior stability. This fact rises the biotechnological potential of these ERs.

Enteric pathogenic bacterium *Vibrium cholerae* produces and senses three AIs such as: AI-1, DPO and CAI-1. The first two are recognized for the interspecies communication role, while the last one is employed as intra-genus communication AI. These all three QS systems work together for the transition from an aerobic to an anaerobic environment. This QS represses the virulence and biofilm formation in the *Vibrium cholerae* bacterium at high cell density [31].

The bacterium *Acidithiobacilus ferrooxidans* widely recognized by its bioleaching ability displays two quorum sensing systems, (i) a completely functional

LuxI/R like, encoding the gene cluster *afeI-orf3-afeR* [32]. *At. ferrooxidans* is able to produce nine types of AHLs with acyl chains, whose length ranges between 8 and 16 carbons. A recent study shows that depending on energy substrate, the protein AfeI synthetizes different types of acyl-HSLs, similarly, the regulation of different metabolic systems also depends on substrate energy [33]. (ii) The second Quorum Sensing system in *A. ferrooxidans* has been identified using a reporter strain strategy. This QS system was termed *act* QS system. This reporter strain evidenced the production of HLA molecule e.g. C_{14} acyl-HSL by *E. coli* cloned with the *act* gene [34]. The quorum sensing system *act* is mainly expressed when *A. ferrooxidans* cells are grown in culture medium enriched with Fe^{+2} than when the cells are cultivated in microbiological medium enriched with sulfur. The role of *act* Quorum Sensing system of *A. ferrooxidans* remains uncertain.

Synthetic biology is based on the use of engineering principles to design and apply new biological components, besides to integrate functions and traits into the present ones in order to standardize or modulate their behavior. The completely understanding of a particular Quorum sensing circuit enable to design tools to precisely and predictably manipulate cell responses under quorum sensing regulation. That is, QS and synthetic biology research have been highly complementary, with QS research expanding the synthetic biology toolkit and synthetic biology providing new tools for investigating QS [35]. The HLA QS system is a relatively simple system to synthetic biology approaches, due to few components to comprise the circuit. Several studies using synthetic biology to engineer cell were performed to modulate behavior when the phenotype is depending on cell density. One of the most usual approaches in this case is the manipulation of the regulator protein LuxR i.e. modifying its affinity to is cognate or not cognate AHL signals, regulating the transcription to control the number of copies of LuxR and by modifying the AHL binding site [36, 37]. Some of the above mentioned approaches were used with success in the biomining sewage bioremediation [38].

5. Improving of bioleaching ability through EPS regulation synthesis in *A. ferrooxidans*

Secretion of EPS (Extracellular Polymeric Substances) by *A. ferrooxidans* is a determinant factor for biofilm formation and an essential feature for mineral dissolution in bioleaching process [39]. Bioleaching denotes direct and indirect actions exerted by microorganisms that lead to the dissolution of metals in ores or urban biomining strategies. It has been widely demonstrated that bacterial attachment to various mineral surfaces and the formation of a well-established biofilm, contribute significantly to enhancing bioleaching activity, since, the biofilm allows the formation of reaction space identified as “surface conditioning layer” between the ores or E-waste and bacteria [40]. A previous urban bioleaching study was done using a partition system based on a semipermeable layer, in order to avoid the contact between bacteria and E-waste. This study showed that the lack of bacterial attachment reduced 25% the recovery of copper [41]. These physiological steps are mediated by extracellular polymeric substances (EPS), which are composed of polysaccharides, proteins, and lipids. Furthermore, by increasing EPS secretion and biofilms formation on mineral surfaces, attached cells extend their reactive space and obtain significantly higher amounts of substrate than planktonic cells [42]. Thus, the increase of EPS secretion could increase bioerosion capacity of the overexpression strain on the substrates and enhance the efficiency of the bioleaching process.

Early studies showed that it is feasible to modulate *A. ferrooxidans* adhesion to pyrite particles by addition of synthetic AHL and HLSs analogues. Nevertheless, the questionable point of this study was that the results were obtained using an indirect approach, i.e. the number of the residual planktonic cells was employed in order to calculate the number of cells attached to the surface of pyrite [43]. Subsequent studies based on the use of different microscopy techniques provided conclusive evidence on the effect of bacterial adhesion on bioleaching efficiency [44]. In the bioleaching process, a bacterial attachment to copper sulfide minerals occurs selectively [45]. Thus, in the cell population that interacts electrostatically or hydrophobically with pyrite or sulfur surfaces, an increase in cell densities is observed on these surfaces. It triggers the activation of Quorum sensing regulon in those cells that remaine attached to solid surfaces much faster than in planktonic cells. This activation could contribute to favor the biofilm producer phenotype at the cell population. As it was mentioned above, the AfeI/R Quorum Sensing system promotes the EPS production and consequently contributes to the biofilm formation. Based on these observations, several studies have been implemented the addition of synthetic auto-inducers in order to make the bioleaching reaction more efficient and get a higher yield of metals. The QS agonists employed were principally those with Carbon long length e.g. C_{12}-C_{14} [46]. In a previous study using direct microscopy technics, the researchers reported that AHLs with long acyl chains used as single molecules (C_{12}-AHL, 3-hydroxy-C_{12}-AHL, C_{14}-AHL, and 3-hydroxy-C_{14}-AHL) or as a mixture (C_{14}-AHL/3- hydroxy-C_{14}-AHL/3-oxo-C14-AHL) increased the adhesion to sulfur and pyrite [47]. Though, studies focused on elucidating and clearly understating all molecular steps produced by the addition of different AHLs autoinducers, and its effects on biofilm formation are required.

6. Conclusions

The cell communication system Quorum sensing is a ubiquitous phenomenon in prokaryotes. This communication system modulates near to 25% of no essential genes. Induction or quenching of quorum sensing system could be a high valuable tool to select the desire trait in bacteria. The bacterium *A. ferrooxidans* is equipped with a fully functional Quorum Sensing system AfeI/R, which promotes the EPS secretion and biofilm formation. These traits are extremely desirable for the bioleaching reaction both for natural ores and urban biomining processes. However, special care must be taken in order to choose the energy source for the media culture where the bacteria will be grown and the bioleaching reaction will take place. As mentioned before, long chain synthetic agonists of AHL Quorum sensing system could favor the biofilm formation when the Sulfur is provided as energy source, otherwise, the addition of AHL synthetic agonist of long chain in microbiological media culture using iron as energy source, reduce drastically the bacterial growth and repress the genes responsible for the EPS and biofilm formation. Act, the second Quorum system in *A. ferrooxidans* has shown an over expression cultured in the media enriched with iron. Nevertheless, the mechanistic details about its encoding genes and their biological roles remain unclear. In addition to modulating the biofilm formation, AfeI/R Quorum Sensing system in *A. ferrooxidans* controls the grown rate, the metabolic systems and membrane permeability. Finally, the synergism between synthetic biology and Quorum sensing research enables a wide specter of approaches, in order to modulate the behavior of acidophile microorganisms with potential applications in the biomining industry.

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Conflict of interest

The authors declare no conflict of interest.

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