2017-08

Bioleaching of Pyrite by Iron-Oxidizing Acidophiles under the Influence of Reactive Oxygen Species

Huynh, D

<http://hdl.handle.net/10026.1/10068>

10.4028/www.scientific.net/SSP.262.372

Solid State Phenomena

Trans Tech Publications

All content in PEARL is protected by copyright law. Author manuscripts are made available in accordance with publisher policies. Please cite only the published version using the details provided on the item record or document. In the absence of an open licence (e.g. Creative Commons), permissions for further reuse of content should be sought from the publisher or author.

Bioleaching of pyrite by iron - oxidizing acidophiles under the influence of reactive oxygen species

Dieu Huynh^{1,a*}, Sören Bellenberg^{2,b}, Mario Vera^{3,c}, Ansgar Poetsch^{4,d},
and Wolfgang Sand^{1,5,e}.

¹Institute of Biosciences, Leipziger Str.29, TU Bergakademie Freiberg, Germany

²Biofilm Centre, Universität Duisburg-Essen, Universitätsstraße 5, 45141, Essen, Germany

³Institute for Biological and Medical Engineering. Schools of Engineering, Medicine and
Biological Sciences, Department of Hydraulic and Environmental Engineering. School of
Engineering, Pontificia Universidad Católica de Chile

⁴Ansgar Poetsch, School of Biomedical and Healthcare Sciences, Plymouth University PL4
8AA, United Kingdom

⁵Donghua University, College of Environmental Engineering, Songjiang, Shanghai, PR China

^angoc-dieu.huynh@ioez.tu-freiberg.de, ^bsoeren.bellenberg@uni-due.de,
^cmaverav@uc.cl, ^dansgar.poetsch@ruhr-uni-bochum.de, ^ewolfgang.sand@uni-due.de

Keywords: bioleaching, oxidative stress, iron-oxidizing bacteria, proteomics.

Abstract

After 24h of exposure to acidic media, pyrite generates reactive oxygen species (ROS). Freshly-crushed pyrite with grain sizes between 50-100 µm at 5 % (w/v) pulp density generated 0.17 ± 0.01 mM H₂O₂, while 10% pyrite generated 0.29 ± 0.01 mM and 30 % pyrite generated 0.83 ± 0.06 mM. These levels of H₂O₂ probably inhibited iron oxidation in iron-grown cells of *Acidithiobacillus ferrooxidans*^T but not in pyrite-grown cells. ROS originating from pyrite, which was incubated for 24 h in acidic medium, likely prohibited pyrite dissolution by iron-grown cells, while pyrite-grown cells were probably adapted to these concentrations of ROS. Periodical addition of 100 µM H₂O₂ to pyrite cultures inoculated with pyrite-grown cells did not decrease iron dissolution. By high throughput proteomics analysis, an increased expression of proteins related to oxidative stress management, iron- and sulfur oxidation systems, carbon fixation and biofilm formation was observed in biofilm cells grown on pyrite compared to iron-grown cells.

Introduction

In acidic conditions, sulfide minerals, especially pyrite, generate toxic ROS, resulting in oxidative stress of acidophilic metal oxidizing microorganisms [1, 2]. Pyrite spontaneously generated H₂O₂, (O₂•⁻) and OH• radicals in aqueous solution either in the presence or in the absence of oxygen [3, 4]. As a result, bioleaching bacteria are likely to be confronted with high levels of oxidative stress. It is indicated that in comparison with iron-grown cells, pyrite- grown cells of *A. ferrooxidans*^T are better adapted to H₂O₂ [5]. Studies on the oxidative stress response of acidophiles have been carried

out [6, 7], though the influence of ROS on bioleaching and strategies to withstand ROS of *A. ferrooxidans*^T are still inadequately understood. The aim of this study was to characterize the effect of H₂O on *A. ferrooxidans*^T grown with iron or pyrite as growth substrate and subsequently on pyrite bioleaching efficiency. High throughput proteomics analysis was used to get insights into the mechanisms for ROS tolerance in *A. ferrooxidans*^T.

Materials and Methods

Bacteria and cultivation conditions. *A. ferrooxidans*^T was grown in Mackintosh medium [8] adjusted to pH 1.8, supplied either with ferrous iron 3 gL⁻¹ or pyrite at 5% w/v (50-100 µm grain size); cultures were incubated at 28°C and shaking at 120 rpm.

Pyrite grains. Pyrite was prepared as described [9] and sterilized by autoclaving at 120°C for 4h under N₂ atmosphere.

Quantification of H₂O₂: H₂O₂ was quantified using the spectrophotometric determination method of Baga [10].

Iron determination. Pyrite dissolution was assessed by quantification of ferrous and total iron concentration using the 1,10-phenanthroline method according to German standards [5].

Proteomics analysis. Proteins from biofilm cells grown on pyrite surfaces and cells grown on ferrous iron after 5 days of incubation were extracted, purified, and analyzed as described [11].

Results and Discussion

Generation of H₂O₂ in pyrite-containing acidic MAC media. Table 1 summarizes the concentrations of H₂O₂ produced by pyrite after 24 h incubation in MAC media. Obviously, pyrite generated H₂O₂ in dependence of pyrite grain size and pulp density. Pyrite with the grain size of 50-100 µm generated higher concentrations of H₂O₂ than the fraction with 100-200 µm grain size. Also, pulp densities of 30% (w/v) generated H₂O₂ concentrations 5 times higher than that of 5% (w/v).

Tab. 1. H₂O₂ generation (mM) by pyrite of different grain size and pulp density after 24h in MAC medium.

Grain sizes (µm)	Pulp density % (w/v)		
	5	10	30
50-100	0.17 ± 0.01	0.29 ± 0.01	0.83 ± 0.06
100-200	0.05 ± 0.00	0.09 ± 0.01	0.25 ± 0.01

About 0.9 mM H₂O₂ is formed by a pyrite load of 10% (pyrite sizes <106 µm) at pH 4.5 and more H₂O₂ is formed at lower pH [12]. The H₂O₂ generated in this study was lower than in the study by Nooshabadi et al [12]. However, H₂O₂ concentrations of ≥ 50µM have significant toxic effects on *A.ferrooxidans* [13, 14]. Therefore, 5% (w/v) pyrite loading, a size of 50-100, generates toxic levels of H₂O₂ for *A.ferrooxidans*.

Pyrite dissolution. Figure 1 shows the dissolution of pyrite by *A.ferrooxidans*^T. Total concentration of iron in solution without the preincubation of pyrite was nearly two times the total iron concentration in solution with pyrite-generated H₂O₂ in the case of iron-grown cells (**a**). In contrast, there was no significant reduction in pyrite dissolution when pyrite-grown cells were used (**b**).

Also, in case of pyrite-grown cells no significant differences in pyrite dissolution assays with the periodical additions of external H₂O₂ concentrations occurred (Figure 2). In abiotic assays, with and without addition of H₂O₂, the total iron ions remained relatively constant at low concentration.

In biotic assays, regardless of the addition of external H_2O_2 , total iron concentrations increased steadily and reached approximately 45 mM after 28 days of incubation.

Fig. 1. Pyrite dissolution with a) iron-grown cells and b) pyrite-grown cells of *A.ferrooxidans*^T. Control without preincubation of pyrite (*circles*) and assay with 24h preincubation of pyrite in MAC media (*squares*) were compared. Iron ion concentrations include ferrous iron (*dashed line*) and total iron (*solid line*). The initial cell inoculation was 5×10^7 cells/mL. All assays in experiment were performed in triplicate; error bars show standard deviation of the mean as indicated.

Fig. 2. Effect of external addition of H_2O_2 on pyrite dissolution using pyrite grown-cells of *A.ferrooxidans*^T. The additions of H_2O_2 were 100 μM at day 0, 6, 12 and 18 (*black arrows*), 500 μM at day 22, 24 (*dotted black arrows*) and 1 mM at day 26 (*grey arrows*). Total iron concentrations were measured in sterile (*circles*) and inoculated (*squares*) pyrite dissolution assays, with (*empty symbols*) and without (*filled symbols*) periodic addition. The initial cell inoculum was 5×10^7 cells/mL. All assays in experiment were performed in triplicate; error bars show standard deviation of the mean as indicated.

Those results are similar to previous findings, indicating that compared to iron-grown cells, pyrite -grown cells of *A.ferrooxidans*^T were probably adapted to the presence of elevated levels of ROS, which are generated on metal sulfide surfaces [5]. Additionally, although H_2O_2 is commonly known as a toxic agent, at low levels it may also function as signaling agent [15]. Also, pre-exposure to low concentrations of H_2O_2 allows microorganisms to resist efficiently detrimental effects of oxidative stress [16]. Genetic response is of importance among strategies to protect enzymes and DNA against oxidative stress damage [16]. Bioleaching acidophiles possess several proteins responsible for coping with oxidative stress, involving several oxygen detoxification and repair

systems [6]. Proteomic data showed that during the growth with pyrite as energy source, cells of *A.ferrooxidans*^T utilized ROS degradation, redox balance, macromolecule repair mechanisms, metal and oxygen homeostasis as fundamental adaptation strategies to the elevated presence of ROS (data not shown). Also, our shot-gun proteomics analysis indicated that there was a remarkable increase in the expression of proteins related to oxidative stress response in biofilm cells grown on pyrite surfaces compared to iron-grown cells of *A.ferrooxidans*^T.

References

- [1] G.C. Jones, K.C. Corin, R.P. van Hille, and S.T.L. Harrison, The generation of toxic reactive oxygen species (ROS) from mechanically activated sulphide concentrates and its effect on thermophilic bioleaching. *Minerals Engineering*, 24 (2011) 1198-1208.
- [2] G.C. Jones, R.P. van Hille, and S.T. Harrison, Reactive oxygen species generated in the presence of fine pyrite particles and its implication in thermophilic mineral bioleaching. *Appl Microbiol Biotechnol*, 97 (2013) 2735-42.
- [3] C.A. Cohn, S. Mueller, E. Wimmer, N. Leifer, S. Greenbaum, D.R. Strongin, and M.A.A. Schoonen, Pyrite-induced hydroxyl radical formation and its effect on nucleic acids. *Geochemical Transactions*, 7 (2006) 3-3.
- [4] M.J. Borda, A.R. Elsetinow, M.A. Schoonen, and D.R. Strongin, Pyrite-induced hydrogen peroxide formation as a driving force in the evolution of photosynthetic organisms on an early earth. *Astrobiology*, 1 (2001) 283-8.
- [5] S. Bellenberg, R. Barthen, M. Boretska, R. Zhang, W. Sand, and M. Vera, Manipulation of pyrite colonization and leaching by iron-oxidizing *Acidithiobacillus* species. *Appl Microbiol Biotechnol*, 99 (2015) 1435-49.
- [6] J.P. Cárdenas, F. Moya, P. Covarrubias, A. Shmaryahu, G. Levicán, D.S. Holmes, and R. Quatrini, Comparative genomics of the oxidative stress response in bioleaching microorganisms. *Hydrometallurgy*, 127–128 (2012) 162-167.
- [7] L. Dekker, F. Arsene-Ploetze, and J.M. Santini, Comparative proteomics of *Acidithiobacillus ferrooxidans* grown in the presence and absence of uranium. *Res Microbiol.*, 167 (2016) 234-9.
- [8] M.E. Mackintosh, , Nitrogen Fixation by *Thiobacillus ferrooxidans*. *Microbiology*, 105 (1978) 215-218.
- [9] A. Schippers, P. Jozsa, and W. Sand, Sulfur chemistry in bacterial leaching of pyrite. *Appl Environ Microbiol*, 62 (1996) 3424-31.
- [10] A.N. Baga, G.R.A. Johnson, N.B. Nazhat, and R.A. Saadalla-Nazhat, A simple spectrophotometric determination of hydrogen peroxide at low concentrations in aqueous solution. *Analytica Chimica Acta*, 204 (1988) 349-353.
- [11] M. Vera, B. Krok, S. Bellenberg, W. Sand, and A. Poetsch, Shotgun proteomics study of early biofilm formation process of *Acidithiobacillus ferrooxidans* ATCC 23270 on pyrite. *Proteomics*, 13 (2013) 1133-44.
- [12] A. Javadi Nooshabadi. and K. Hanumantha Rao, Formation of hydrogen peroxide by sulphide minerals. *Hydrometallurgy*, 141 (2014) 82-88.
- [13] S. Bellenberg, D.H. Ngoc, L. Castro, M. Boretska, W. Sand, and M. Vera, Reactive Oxygen Species Influence Biofilm Formation of Acidophilic Mineral-Oxidizing Bacteria on Pyrite, in *Advanced Materials Research* (2015) 118-122.
- [14] Y. Ma, and C. Lin, Microbial Oxidation of Fe²⁺ and Pyrite Exposed to Flux of Micromolar H₂O₂ in Acidic Media. *Scientific Reports*, 3 (2013) 1979.

- [15] J.R. Stone and S. Yang, Hydrogen peroxide: a signaling messenger. *Antioxid Redox Signal*, 8 (2006) 243-70.
- [16] D.R. Crawford and K.J. Davies, Adaptive response and oxidative stress. *Environmental Health Perspectives*, 102 (1994) 25-28.