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SULFUR BLISS OR CURSE FOR ENVIRONMENT

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ABSTRACT

Life on earth evolved because of Sulfur as debated by many scholars. The anthropogenic sulfur released in the environment has started a visious cycle in the nature. Reckoned Sulfur is a yellow crystalline solid that occurs in nature as a pure element or as sulfide and sulfate minerals. It is a multivalent non-metal that is abundant, tasteless and odorless. The major derivative of sulfur is sulfuric acid (H_2SO_4), one of the most important elements used as an industrial raw material, it is of central importance to the world's economies, its production and consumption is an indicator of a nation's industrial development. But when sulfur burns in air, it produces sulfur dioxide, the emissions of which cause adverse impacts to vegetation, including forests and agricultural crops. Hence a careful extraction of sulfur from the natural bodies is necessary. *Acidithiobacillus ferrooxidans* is a Gram-negative bacterium which is abundant in natural environments associated with pyritic ore bodies, coal deposits and their acidified drainages. It is an important member of microbial consortia used to recover copper via a process known as bioleaching or biomining. It is capable of anaerobic growth on elemental sulfur, using ferric iron as an electron acceptor. This ability of *A.ferrooxidans* to grow under anaerobic conditions is relevant to its application in biohydrometallurgy. For very low-grade metal sulfide ores that cannot be economically recovered using traditional metallurgical methods, the use of bioleaching is a environmental friendly alternative for the metallurgical industry. In this review, the sulfur production, its effects on the environment and the role *Acidithiobacillus ferrooxidans* in sulfur bioleaching is discussed. Furthermore, the closely related species of *Acidithiobacillus ferrooxidans* are also considered.

KEYWORDS: *Acidithiobacillus ferrooxidans*, Sulfur, Bioleaching

1. INTRODUCTION

Evolution of life on earth is an unsolved mystery. Penetration of UV radiation, due to the absence of atmospheric absorption the radiation range of 200 – 300 nm must have made it impossible for the life to originate on earth (Sagan 1973; Ratner and Walker 1972). Unknown circumstances prevailed during the evolution, scientists have argued various possibilities out of which most accepted one is the development of UV protection mechanism (Rambler and Margulis 1980). Kasting et al (1989) proposed that sulfur vapor having S8 with other sulfur molecules (Sn, $2 < n < 12$) to provide UV protection for organisms to evolve.

According to Kasting and Ackerman (1986), free O_2 should have been poison to earlier life. The first photosynthetic oxidation of ancient environment was elemental sulfur and oxidized sulfur compounds rather than molecular oxygen, with sulphate as a mild oxidant (Broda, 1975). Starting from Precambrian seas to modern day earth, Sulfur is an integral part of the biosphere. As one of the key elements of life that accounts for some 0.5 to 1.5% (dry weight) of biological matter (Schidlowski 1989). Proteins, Iron – Sulfur Cluster (Felipe et al 2004), enzymes (Rahlfs *et al.*, 2001;), metabolites are enriched by sulfur. The study of Sulfur in old rocks and sulfur isotopes reveal earth's history (Farquhar, 2000). Volcanic horizons in ice cores (Savarino, 2002). This century is witnessing the large variation in sulfur production due to modern sulfate aerolsols (Romero et al 2000; Romero et al 2002). Large release of Sulfur in the environment is from metallurgical process. The thermodynamic equilibrium of sulfur and sulfur bearing compounds in environment has presently become a threat to the environment as well as living organism. We have reviewed modern global scenario of sulfur production and microbial avenue to resolve the sulfur problem.

2. Sulfur

Sulfur is a multivalent non-metal, abundant, tasteless and odorless. In its native form sulfur is a yellow crystalline solid. In nature it occurs as the pure element or as sulfide and sulfate minerals. Although sulfur is infamous for its smell, frequently compare to rotten eggs, that odor is actually characteristic of hydrogen sulphide (H_2S). The crystallography of sulfur is complex. Depending on the specific conditions, sulfur allotropes form several distinct crystal structures.

Sulfur can also forms polyatomic molecules with different chemical formulas, with the best-known allotrope being octasulfur, cyclo-S_8. Octasulfur is a soft, bright-yellow solid with only a faint odor, similar to that ofmatches (Greenwood et al. 1997). It melts at 115.21 °C, boils at 444.6 °C and sublimes easily. At 95.2°C, below its melting temperature, cyclo-octasulfur changes from α-octasulfur to the β-polymorph. The structure of the S_8 ring is virtually unchanged by this phase change, which affects the intermolecular interactions. Between its melting and boiling temperatures, octasulfur changes its allotrope again, turning from β-octasulfur to γ-sulfur, again accompanied by a lower density but increased viscosity due to the formation of polymers (Greenwood et al. 1997). At even higher temperatures, however, the viscosity decreases as depolymerization occurs. Molten sulfur assumes a dark red color above 200 °C. The density of sulfur is about 2 $g \cdot cm^{-3}$, depending on the allotrope; all of its stable allotropes are excellent electrical insulators (Steudel et al. 2003)**.**

2.1. Sulfur in The Environment

Life on Earth may have been possible because of sulfur. Conditions in the early seas were such that simple chemical reactions could have generated the range of amino acids that are the building blocks of life. Sulfur occurs naturally near volcanoes. Many sulphide minerals are known: pyrite and marcaiste are iron sulphide; stibnite is antimony sulphide; galena is lead sulphide; cinnabar is mercury sulphide and sphalerite is zinc sulphide. Other more important, sulphide ores are chalcopyrite, bornite, penlandite, millerite and molybdenite (Gonzalez-Toril et al. 2003)**.**

2.2. Uses of Sulfur

The major derivative of sulfur is sulfuric acid (H_2SO_4), one of the most important elements used as an industrial raw material. Sulfur is also used in batteries, detergents, fungicides, manufacture of fertilizers, gun power, matches and fireworks. Other applications are making corrosion-resistant concrete which has great strength and is forst resistant, for solvents and in a host of other products of the chemical and pharmaceutical industries (Heal et al. 1980).

The principal ores of copper, zinc, nickel, cobalt, molybdenum, and other metals are sulfides. These materials tend to be dark-colored semiconductors that are not readily attacked by water or even many acids. They are formed, both geochemically and in the laboratory, by the reaction of hydrogen sulfide with metal salts. The mineral galena (PbS) was the first demonstrated semiconductor and found a use as a signal rectifier in the cat's whiskers of early crystal radios. The iron sulfide called pyrite, the so-called "fool's gold," has the formula FeS_2 (Vaughan et al. 1978).The upgrading of these ores, usually by roasting, is costly and environmentally hazardous. Sulfur corrodes many metals via the process called tarnishing.

Elemental sulfur is mainly used as a precursor to other chemicals. Approximately 85% is converted to sulfuric acid (H_2SO_4):

$2\ S + 3\ O_2 + 2\ H_2O \rightarrow 2\ H_2SO_4$

With sulfuric acid being of central importance to the world's economies, its production and consumption is an indicator of a nation's industrial development. For example with 32.5 million tonnes in 2010, the United States produces more sulfuric acid every year than any other inorganic industrial chemical (Apodaca et al. 2010). The principal use for the acid is the extraction of phosphate ores for the production of fertilizer manufacturing. Other applications of sulfuric acid include oil refining, wastewater processing, and mineral extraction (Nehb et al. 2006).

2.3. Sulfur Producing Industries

In 1900, Herman Frasch was trying to perfect his hot water melting process for producing sulfur. Domestic production was about 3,200 metric tons of sulfur valued at $88,100. Native sulfur deposits in Louisiana, Nevada, Texas, and Utah were mined with conventional mining methods. Domestic sulfur production, including mined elemental sulfur and pyrites, supplied about one-quarter of the U.S. sulfur demand of about 415,000 tons. Most sulfur and pyrites, domestic and imported, were used to produce sulfuric acid that was consumed in many different industries. Virtually all elemental sulfur imports came from the Italian island of Sicily, and pyrite imports were from unspecified locations. Pyrites remained a significant raw material for sulfuric acid until 1982. When the Frasch process was successfully commercialized in 1903, the U.S. sulfur industry took a turn for the better. By about 1915, the United States surpassed Italy as the world’s leading producer of sulfur, a situation that continued throughout the century, during which eight companies produced nearly 340 million tons of sulfur from 36 mines in Louisiana and Texas. Frasch sulfur production hit its peak in 1974 when 12 mines produced 8 million tons.

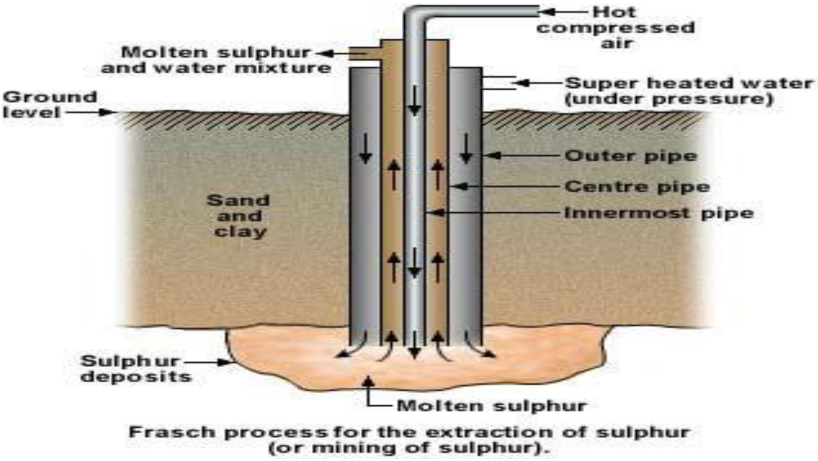

Figure: 1- Frasch Process for the Extraction of Sulfur

["Crude" sulfur is produced from the Frasch process or recovered from "sour" natural gas or petroleum. Although termed "crude", this sulfur possesses a minimum purity of 99.5 percent and is suitable for a majority of uses. The impurities consist primarily of trapped organic matter**]**.

Domestic production of elemental sulfur recovered as a byproduct of natural gas processing and oil refining was first reported in 1938 and grew steadily from that time. Recovered sulfur overtook Frasch as the primary domestic sulfur source in 1982. Sulfur also was recovered in the form of byproduct sulfuric acid from nonferrous metal smelters; production data were published for the first time in 1911. In 2000, domestic sulfur production totaled 10.3 million tons, 81% from recovered operations, 9% from one Frasch mine, and 10% as byproduct acid from nonferrous metal smelters. Consumption was 12.5 million tons, including 2.8 million tons of imported sulfur and sulfur contained in sulfuric acid. The end uses did not change significantly during the century, although consumption in 2000 was nearly 4,000 times higher than it was in 1900. About 90% of domestic sulfur consumption was converted to sulfuric acid and then used, in decreasing order, in phosphate fertilizer and other agricultural chemical production, in oil refining, in copper ore leaching, and in many other industrial uses. Elemental sulfur was used as a plant nutrient, in synthetic rubber production, in pulp and paper products, and in other inorganic chemicals (Robert H 1938)**.** The global sulfur industry remained divided into two sectors—discretionary and nondiscretionary. In the discretionary sector, the mining of sulfur or pyrites is the sole objective; this voluntary production of native sulfur or pyrites is based on the orderly mining of discrete deposits, with the objective of obtaining as nearly a complete recovery of the resource as economic conditions permit.

In the nondiscretionary sector, sulfur or sulfuric acid is recovered as an involuntary byproduct, the quantity of output subject to demand for the primary product irrespective of sulfur demand. Nondiscretionary sources represented nearly 87% of the sulfur in all forms produced worldwide as listed in table 1.

Table 1 Sulfur: World Production in All Forms, By Country And Source

(Thousand metric tons)

Country And Source 3/	**1996**	**1997**	**1998**	**1999**	**2000e/**
Australia, byproduct: e/					
Metallurgy	327	474	507	441	654
Petroleum	35	35	35	34/4	35
Total	362	509	542	475	689
Belgium, byproduct, all sources e/	406	430	428	408	410
Canada, byproduct:					
Metallurgy	1,044	1,072	1,153	1,156	1,200 p/
Natural gas, petroleum, tar sands	8,446	8,408	8,541	8,960	8,700 p/
Total	9,490	9,480	9,694	10,116	9,900 p/
Chile, byproduct, metallurgy e/	587	768	899	1,040	1,100
China: e/					
Elemental	170	200	210	250	250
Pyrites	5,990	6,040	4,490	3,860	3,370
Byproduct, metallurgy	1,100	1,400	1,450	1,580	1,600
Total	7,260	7,640	6,150	5,690	5,220
Finland:					
Pyrites	425	373	430 r/ e/	500 r/ e/	500
Byproduct:					
Metallurgy	291	307	296 e/	300 e/	300
Petroleum	38	50	40 r/ e/	45 e/	50
Total	754	730	766 r/ e/	845 r/ e/	850
France, byproduct: e/					
Natural gas	755	697	600	600	600
Petroleum	235	263	245	250	250
Unspecified	99	100	261	250	260
Total	1,090	1,060	1,110	1,100	1,110

Germany, byproduct: e/					
Metallurgy	20	25	25	25	30
Natural gas and petroleum	1,000	1,085	1,100	1,100	1,110
nspecified	90	50	50	60	100
Total	1,110	1,160	1,180	1,190	1,240
Iran, byproduct: e/					
Metallurgy	50	50	50	48 r/	50
Natural gas and petroleum	840	850	1,280 r/	1,302 r/	1,300
Total	890	900	1,330 r/	1,350 r/	1,350
Italy, byproduct: e/					
Metallurgy	216	229	199	193	203
Petroleum	335	380	425	485	490
Total	551	609	624	678	693
Japan:					
Pyrites e/	45	39	23	41	30
Byproduct:					
Metallurgy	1,314	1,331	1,322	1,363	1,400
Petroleum e/	1,790	2,010	2,080	2,060	2,070
Total e/	3,150	3,380	3,430	3,460	3,500
Kazakhstan: e/					
Pyrites	71	----	----	----	----
Byproduct:					
Metallurgy	139	139	212	245	300
Natural gas and petroleum	515	778	933	1,070	1,200
Total	725	917	1,150	1,320	1,500
Korea, Republic of, byproduct: e/					
Metallurgy	260	265	270	280	290
Petroleum	200	200	200	200	200
Total	460	465	470	480	490
Kuwait, byproduct, natural gas and petroleum e/	595	675 4/	665	675	675
Mexico, byproduct:	359	417	474	450	450
Metallurgy	921	923	913	860	860
Natural gas and petroleum					
Total	1,280	1,340	1,387	1,310	1,310

Netherlands, byproduct: e/					
Metallurgy	119	127	131	129	84
Petroleum	380	450	432	445	428
Total	499	577	563	574	512
Poland: 4/					
Frasch	1,745 r/	1,673 r/	1,348	1,175	1,369
Byproduct: e/					
Metallurgy	200 r/	256	260 r/	260	260
Petroleum	25	44	57	74	70
Gypsum e/	12 r/	12 r/	10 r/	----	----
Total	1,982 r/	1,985 r/	1,675 r/	1,509 r/	1,700
Russia: e/ 5/					
Native	70	50	50	50	50
Pyrites	400	400	254	300	350
Byproduct, natural gas	3,000	2,950	3,940	4,410	4,900
Other	325	350	411	510	600
Total	3,800	3,750	4,650	5,270	5,900
Saudi Arabia, byproduct, all sources e/	2,300	2,400	2,300	2,400	2,400
South Africa:					
Pyrites	184	167	152 e/	141	146
Byproduct:					
Metallurgy	91	37	100 e/	100 e/	100
Petroleum 6/	232	256	178	164	203
Total	507	460	430	406	448
Spain:					
Pyrites	438	424	430	388	115
Byproduct: e/					
Coal (lignite) gasification	2	2	2	2	1
Metallurgy	428	456	461	455	454
Petroleum	75	85	100	110	115
Total e/	943	967	993	955	685
United Arab Emirates, byproducts, natural gas and petroleum e/	780	967	967	1,089 r/	1,120
United States:					
Frasch e/	2,900	2,820	1,800	1,780	900
Byproduct:					

Metallurgy	1,430	1,550	1,610	1,320	1,030
Natural gas	2,100	2,420	2,160	2,010	2,020
Petroleum	5,370	5,230	6,060	6,210	6,360
Total e/	11,800	12,000	11,600	11,300	10,300
Uzbekistan, byproduct:					
Metallurgy	145	165 e/	170	175 e/	180
Natural gas and petroleum	250	250 e/	275	280 e/	280
Total	395	415 e/	445	455 e/	460
Venezuela, byproduct, natural gas and petroleum	250	319	425	450	450
Other: e/ 7/					
Frasch	25	20	25	23 r/	24
Native	485	471	463	446	398
Pyrites	552 r/	421 r/	290 r/	227 r/	257
Byproduct:					
Metallurgy	661 r/	606 r/	649 r/	617 r/	677
Natural gas	150	130	206	215	255
Natural gas, petroleum, tar sands, undifferentiated	136 r/	148 r/	156 r/	153 r/	156
Petroleum	490 r/	569 r/	587 r/	540 r/	550
Unspecified	750	810	879	887 r/	888
Total	3,250 r/	3,180 r/	3,250 r/	3,110	3,200
Grand total of which:	55,200 r/	57,100	57,100 r/	57,700 r/	57,200
Frasch	4670 r/	4,510 r/	3,170		2,290
Native 8/	725	721	723	2,980	698
Pyrites	8,100 r/	7,860 r/	6,070 r/	746	4,770
Byproduct:				5,460 r/	
Coal (lignite) gasification e/	2	2	2		1
Metallurgy	8,780 r/	9,670 r/	10,200 r/	2	10,400
Natural gas	6,000	6,200	6,900	10,200	7,780
Natural gas, petroleum, tar sands, undifferentiated	13,700 r/	14,400	15,300 r/	7,230 15,900	15,900
Petroleum	9,210 r/	9,580 r/	10,400 r/	r/	10,800
Unspecified	3,970	4,140	4,330		4,660
Gypsum e/	12 r/	12 r/	10 r/	10,600 4,510 ----	----
Notes:					

e/ Estimated. p/ Preliminary. r/ Revised. -- Zero.

1/ World totals, U.S. data, and estimated data are rounded to no more than three significant digits; may not add to totals shown.

2/ Table includes data available through July 20, 2001.

3/ The term "Source" reflects the means of collecting sulfur and the type of raw material. Sources listed include the following: (1) Frasch recovery, (2) native, comprising all production of elemental sulfur by traditional mining methods (thereby excluding Frasch), (3) pyrites (whether or not the sulfur is recovered in the elemental form or as acid), (4) byproduct recovery, either as elemental sulfur or as sulfur compounds from coal gasification, metallurgical operations including associated coal processing, crude oil and natural gas extraction, petroleum refining, tar sand cleaning, and processing of spent oxide from stack-gas scrubbers, and (5) recovery from the processing mined gypsum. Recovery of sulfur in the form of sulfuric acid from artificial gypsum produced as a byproduct of phosphatic fertilizer production is excluded, because to include it would result in double counting. It should be noted that production of Frasch sulfur, other native sulfur, pyrites-derived sulfur, mined gypsum-derived sulfur, byproduct sulfur from extraction of crude oil and natural gas, and recovery from tar sands are all credited to the country of origin of the extracted raw materials. In contrast, byproduct recovery from metallurgical operations, petroleum refineries, and spent oxides are credited to the nation, where the recovery takes place, which is not the original source country of the crude product from which the sulfur is extracted.

4/ Official Polish sources report total Frasch and native mined elemental sulfur output annually, undifferentiated; this figure has been divided between Frasch and other native sulfur on the basis of information obtained from supplementary sources.

5/ Sulfur is believed to be produced from Frasch and as a petroleum byproduct; however, information is inadequate to formulate estimates.
6/ Includes by product production from synthetic fuels.

7/ "Other" includes all countries, except the above mentioned: Australia, Belgium, Canada, Chile, China, Finland, France, Germany, Iran, Italy, Japan, Kazakhstan Republic of Korea, Kuwait, Mexico, Netherlands, Poland, Russia, Saudi Arabia, South Africa, Spain, United Arab Emirates, United States, Uzbekistan, and Venezuela.

8/ Includes about "China, elemental" and "Iraq, elemental, all sources."

Of these 25 countries listed in table-1 that produced 400,000 t or more of sulfur, 18 obtained the majority of their production as recovered elemental sulfur. These 25 countries produced 94% of the total sulfur produced worldwide. The international sulfur trade was dominated by a limited number of exporting countries, which, in descending order of importance, were Canada, Russia, Saudi Arabia, Japan, and the United Arab Emirates; these countries exported more than 1 Mt of elemental sulfur each and accounted for 64% of total sulfur trade. Major sulfur importers, in descending order, were China, Morocco, the United States, India, Tunisia, and Brazil, all with imports of more than 1 Mt according to the US Geological Survey Minerals Year Book—2000.

3.1. Sulfur As An Industrial Pollutant

Sulfur is currently produced from major sources such as elemental sulfur (brimstone) from mined deposits by the Frasch process; elemental sulfur recovered from oil and gas production; sulfuric acid from smelting of non ferrous metals (sulfur in other forms or SOF); sulfuric acid from pyrites etc.

When this sulfur burns in air, it produces sulfur dioxide. In water, this gas produces sulfurous acid and sulfites, which are antioxidants that inhibit growth of aerobic bacteria and allow its use as a food additive in small amounts. However, at high concentrations these acids harm the lungs, eyes or other tissues. In organisms without lungs such as insects or plants, it otherwise prevents respiration in high concentrations. Sulfur trioxide (made by catalysis from sulfur dioxide) and sulfuric acid are similarly highly corrosive, due to the strong acids that form on contact with water (Zhao et al. 1999)**.**

Sulfur oxide emissions cause adverse impacts to vegetation, including forests and agricultural crops. Studies in the United States and elsewhere have shown that plants exposed to high ambient concentrations of sulfur dioxide may lose their foliage, become less productive, or die prematurely. Some species are much more sensitive to exposure than others. Plants in the immediate vicinity of emissions sources are more vulnerable. Studies have shown that the most sensitive species of plants begin to demonstrate visible signs of injury at concentrations of about 1,850 µg for 1 hour, 500 µg for 8 hours, and 40 µg for the growing season (NAPAP 1990).

Agricultural crops may also be injured by exposure to depositions. Alfalfa and rye grass are especially sensitive. It appears that leaf damage must be extensive before exposure affects the yields of most crops. It is possible that over the long-term, sulfur input to soils will affect yields (OECD 1981). However, sulfur dioxide may not be the primary cause of plant injury, and other pollutants such as ozone may have a greater impact.

The burning of coal and/or petroleum by industry and power plants generates sulfur dioxide (SO_2), which reacts with atmospheric water and oxygen to produce sulfuric acid (H_2SO_4) and sulfurous acid (H_2SO_3). These acids are components of acid rain, which lower the pH of soil and freshwater bodies, sometimes resulting in substantial damage to the environment and chemical weathering of statues and structures (NAPAP 1990). Fuel standards increasingly require that fuel producers extract sulfur from fossil fuels to prevent acid rain formation. This extracted and refined sulfur represents a large portion of sulfur production. In coal-fired power plants, flue gases are sometimes purified. More modern power plants that use synthesis gas extract the sulfur before they burn the gas.

4. Bioagumentation of Sulfur Using Microorganisms

Bioleaching is the use of bacterial microorganisms to extract precious metals, such as gold, from ore in which it is embedded. As an alternative to smelting or roasting, miners use bioleaching when there are lower concentrations of metal in ore and they need an efficient, environmentally responsible method to extract it (Devasia et al. 1993). The bacteria feed on nutrients in minerals, thereby separating the metal from the ore. Other metals that are commonly extracted via bioleaching include silver, zinc, copper, lead and uranium (Corbett et al. 1987**).** The major types of bacteria most often used in this process of sulfur bioleaching include *A. ferrooxidans*, *A.thiooxidans*, *A.caldus* and *A.albertensis* and certain species of *Sulfobacillus.* At present bioleaching mechanisms are based more or less exclusively on the activity of *T.ferrooxidans*, *L.ferrooxidans*, *T.thiooxidans* which convert heavily soluable metal sulfides via bio chemical oxidation reactions into water soluble metal sulphates (London et al. 1964). These microorganisms are catalysts to speed up natural processes in the ore. These constituent parts of ore are separated into valuable metal and leftover sulfur and other acidic chemicals. Eventually, enough material builds up in the waste solution to filter and concentrate it into recoverable metal (Devasia et al. 1993). The sulfur oxidations of these microorganisms are described below and a few other species assigned to new genera are tabulated.

4.1. *Acidithiobacillus caldus*

Acidithiobacillus caldus (*A.caldus),* is a gram-negative, acidophilic, obligately chemolithotrophic, moderately thermophilic bacterium (Hallberg et al. 1994, 1996). It is an important member of proteobacterium that along with iron oxidizing bacteria plays a key role in bio-leaching (Rawlings 1998; Dopson 1999). It uses energy and electrons derived from sulfur oxidation for carbon dioxide fixation and other anabolic Processes (Hallberg et al. 1994, 1996; Kamimura et al. 1999; Edwards et al. 2000). An unique sulfur oxidation systen exists in caldus that is quite different of *A.ferrooxidans* according to comparitive genome analysis (Sugio et al. 2000). It was shown that *A. caldus* could metabolise S^{2-}, S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$ and SO_3^{2-} and an increase in phosphorylation potential was observed during oxidation of all these compounds (Corbett et al. 1987). The sulfur oxidation in *A.caldus* can be classified into three subsystems:the truncated sulfur oxidation (Sox) system,non-Sox sulfur oxidation system and sulfur oxygenase reductase (SOR) (Vaughan et al. 1978). No substrate level phosphorylation was detected and it was concluded that all ATP is synthesised by membrane bound ATPase driven by a proton gradient (Heal et al. 1980). Sulfur atoms (S) produced from other sulfur oxidation pathways in the periplasm can be accumulated in form of polymeric sulfur (Sn), then S or Sn are transported via an unknown mechanism into the cytoplasm where they are immediately oxidized by SOR (Linxu et al. 2012). The location of SOR in cytoplasm is in agreement with the reports that there are no intracellular sulfur globules in *A. caldus*, whereas sulfur globules are accumulated in *A. ferrooxidans* which lacks sor gene (Huan et al. 2009).

4.2. *Acidithiobacillus thiooxidans*

The description of *Acidithiobacillus thiooxidans* is given by Waksman and Joffe (1922), Kelly and Harrison (1989) and Kelly and Wood (2000). It grows in liquid medium on elemental sulfur, thiosulfate or tetrathionate; it cannot oxidize iron or pyrite but has been shown to grow on sulfur from pyrite in co-culture with '*Leptospirillum ferrooxidans*' (Kelly and Wood 2000*).* It was shown not to oxidise Fe^{2+} but was able to metabolise various RISCs such as thiosulphate ($S_2O_3^{2-}$), tetrathionate ($S_4O_6^{2-}$) and sulphite (SO_3^{2-}) with different pH optimums for certain compounds (Masau et al. 2001; Suzuki 1965a; Suzuki 1965b; Suzuki et al. 1992). Oxidation of thiosulphate by *A. thiooxidans* follows two different pathways depending on the pH (Masau et al. 2001). At pH 5, the intermediate products are sulphite and sulfur and at pH 2.3 they are tetrathionate and sulfur. In both cases sulfur and sulphite are finally oxidised to sulphate. The main product of tetrathionate metabolism in *A. thiooxidans* ON 107 was thiosulphate. Sulphite oxidation in *A. thiooxidans* JCM 7814 was studied by Nakamura et al.(Nakamura et al. 1992, 1995) and proven to be catalysed by an adenosine

5-monophosphate independent membrane bound sulphite: cytochrome *c* oxidoreductase (sulphite dehydrogenase).

4.3. *Acidithiobacillus albertensis*

The description of *Acidithiobacillus albertensis* is given by Bryant *et al*. (1983, 1988), Kelly and Harrison (1989) and Kelly and Wood (2000). *A.albertensis* is the least studied species of all acidithiobacilli. DSM 14366 and BY-05 are the only two strains known and both were demonstrated to metabolise S^0, $S_2O_3^{2-}$, $S_4O_6^{2-}$ and ZnS (Kelly 1989; Xia et al. 2007). The details of S^0 oxidation in this bacterium are unknown. *A.albertenis* was shown to colonise elemental sulfur particles by micro coloniesand it was suggested that glycocalyx plays an important role in the attachment (Bryant et al. 1984).

Table 2: Key Features of The Type Strains of The Obligately Chemolithoautotrophic *Thiobacillus* Species Assigned To The New Genera *Acidithiobacillus*, *Halothiobacillus* And *Thermithiobacillus* (Kelly, D.P. And Wood, A.P., 2000).

Organisms	Lowest Ph After Growth On Sulfur Compounds	Other Special Properties
Acidithiobacillus ferrooxidans	1.5	Growth with Fe[II] as sole energy substrate
Acidithiobacillus thiooxidans	0.5-0.8	Grows in liquid medium on elemental sulfur, thiosulfate or tetrathionate, cannot oxidize iron or pyrite
Acidithiobacillus caldus	NA	Oxidize elemental sulfur and a wide range of reduced inorganic sulfur compounds (RISCs) but could not oxidize ferrous iron
Acidithiobacillus albertensis	2.0	Glycocalyx and tuft of flagella
Halothiobacillus halophilus	5.5-6.0	Obligate halophile
Halothiobacillus hydrothermalis	4.8	Poor growth without Nacl
Thermithiobacillus tepidarius	4.5-5.0	Can grow on RISCs,Unable to oxidize ferrous iron

NA: Not available

5. Role of *Acidithiobacillus* Species in Sulfur Bioleaching

Bioleaching can involve numerous ferrous iron and sulfur oxidizing bacteria, including

Acidithiobacillus ferrooxidans and *Acidithiobacillus caldus* (formerly known as *Thiobacillus*) (Charls and Suzukii 1996). As a general principle, Fe^{3+} ions are used to oxidize the ore. This step is entirely independent of microbes. The role of the bacteria is the further oxidation of the ore, but also the regeneration of the chemical oxidant Fe^{3+} from Fe^{2+}. For example, bacteria catalyse the breakdown of the mineral pyrite (FeS_2) by oxidising the sulfur and metal [in this case ferrous iron, (Fe^{2+})] using oxygen. This yields soluble products that can be further purified and refined to yield the desired metal (Quatrini et al. 2005)**.**

5.1. Mechanism of *Acidithiobacillus ferrooxidans*

A.ferrooxidans is an obligate chemolithoautotroph obtains its energy mainly from the oxidation of ferrous iron, Fe(II), or various reduced sulfur compounds (Leduc et al. 1994) it can also use hydrogen or formate in oxic conditions (Drobner et al. 1990). Moreover, under anoxic conditions, it is able to reduce ferric iron, Fe (III) with sulfur (S^0) or hydrogen as electron donors (Das et al. 1992; Ohmura et al. 2002).

Since the discovery of bacterial leaching, two different mechanisms have been proposed to explain bacterial attack by *A. ferrooxidans*: a direct one and an indirect one. The direct mechanism is based on catalytic sulfide oxidation, while the indirect one implies sulfide oxidation by ferric ions producing sulfur and ferrous ions. These products are oxidized by the microorganisms allowing the iron redox cycle to be repeated (Rawlings et al.1997; Donati et al. 1996)**.** Two indirect leaching mechanisms have also been proposed to explain degradation of sulfides. Both mechanisms combine characteristics of the former direct and indirect mechanisms. One is based on the oxidative attack of ferric iron on acid-insoluble metal sulfides involving thiosulfate as the main intermediate (Scippers et al. 1996)**.** The other mechanism is started by proton and/or ferric iron attack on acid-soluble metal sulfides with polysulfides and sulfur as intermediates (Schippers et al. 1999). It is well documented that the physical attachment of thiobacilli to sulfur particles is necessary and plays an important role in the microbial oxidation rate of sulfur showed the necessity for direct contact in sulfur oxidation of *Thiobacillus thiooxidans*. Schaeffer and Umbreit identified phosphatidyl inositol as a "wetting agent" of sulfur in *T. thiooxidans*. Takakuwa showed that thiol groups may be essential for the adhesion process because adhesion was inhibited by sulfhydryl binding reagents and this inhibition was released by sulfhydryl donors.They also showed that adhesion ability seemed energy dependent.

The abundance of soluble iron has the potential to pose severe oxidative stress that could lead to DNA and protein damage via the Fenton reaction. This prompts questions as to the mechanisms that *A. ferrooxidans* employs for iron assimilation and homeostasis (Quatrini et al.2009) and how it balances its use of iron as both a micronutrient and as a required energy source. In its natural environment, it must also confront unusually severe toxicity due to the high concentration of dissolved metals (e.g., copper, arsenic, mercury).

5.2. Iron and Sulfur Energetic Metabolism of *Acidithiobacillus ferrooxidans*

Genetic evidence has been presented for the existence of a cytochrome -containing complex functioning exclusively during iron oxidation (Cabrejos et al. 1999; Levica´n et al. 2002) and it has been reported that a bc1 complex functions only in reverse in iron-grown cells (Elbehti et al. 2000). In addition bc1 complex has been shown recently to function in direct mode in sulfur-grown cells (Brasseur et al. 2004). This raises the possibility that two operationally independent bc1 complexes are needed to cope with iron and sulfur oxidation, respectively: one for uphill flow during iron oxidation and the other for downhill flow during sulfur oxidation (Bruscella et al. 2007). A corollary of this is that neither of the two complexes can switch the direction of electron flow. Hence according to Patrice Bruscella (2007), the bc1 complex encoded by petI is the one that functions in reverse and transfers the electrons from ferrous iron to NAD(P). But the bc1 complex encoded by petII functions directly, transferring electrons from sulfur to oxygen and may be involved in the aerobic and anaerobic oxidation of sulfur and formate described (Pronk et al. 1991).

5.3. Reconstitution of Iron Oxidase From Sulfur Grown *Acidithiobacillus ferrooxidans*

Oxidation of ferrous iron produced from the reduction of ferric Iron by sulfide: ferric ion oxidoreductase ($H_2S + 6Fe^{3+} + 3H_2O \rightarrow H_2SO_3 + 6Fe^{2+} + 6H^+$) and sulfite: ferric ion oxido reductase ($H_2SO_3 + 2Fe^{3+} + H_2O \rightarrow H_2SO_4 + 2Fe^{2+} + 2H^+$) (Sugio et al.1985). sulfide: quinine oxidoreductase, from *A.ferrooxidans* NASF-1 (Wakai et al.2004), and thiosulfate:quinine oxidoreductase, from *A.ferrooxidans* ATCC19859, in electron transport from sulfide and thiosulfate, ,to the quinone pool, and then to bd quinol oxidase or to ba3 cytochrome c oxidase (terminal oxidase)via the bc1 II complex and c4 cytochrome (Brasseur et al. 2004). bd Quinol and ba3 cytochrome oxidase. Ferrous iron oxidation activity was measured by the o-phenanthroline method (Saywell et al. 1937; Sugio et al. 2007). High iron-oxidizing activity of the intact cells decreased when a cell-free iron oxidation was prepared (Taher et al. 2008).

6. CONCLUSION

The ability of *A. ferrooxidans* to use ferric iron as an electron acceptor for the oxidation of elemental sulfur was first demonstrated in 1976. However, so far it has remained unclear whether this process can support autotrophic growth under anaerobic conditions. *T.ferrooxidans* is capable of anaerobic growth on elemental sulfur, using ferric iron as an electron acceptor. In contrast, aerobic growth yields of *T. ferrooxidans* on inorganic sulfur compounds are over two fold higher than the growth yield on ferrous iron. Johnson and McGinness demonstrated that many heterotrophic acidophiles are capable of anaerobic ferric iron respiration and suggested that ferric iron may be an important electron acceptor under acidic conditions. Anaerobic, ferric iron-dependent autotrophic growth of *T. ferrooxidans* and possibly other acidophiles, on sulfur compounds may play an important role in the iron and sulfur cycles in acidic environments. In addition to this, the ability of *T. ferrooxidans* to grow under anaerobic conditions may be relevant to its application in biohydrometallurgy. For very low-grade metal sulfide ores containing valuable metals that cannot be economically recovered using traditional metallurgical methods, the use of bioleaching is a welcome environmentally bening processing alternative for the metallurgical industry and for diverse governments.

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