Review Article

Biochemical Engineering Parameters for Hydrometallurgical Processes: Steps towards a Deeper Understanding

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Increasing interest in biomining process and the demand for better performance of the process has led to a new insight toward the mining technologies. From an engineering point of view, the complex network of biochemical reactions encompassed in biomining would best be performed in reactors which allow a good control of the significant variables, resulting in a better performance. The subprocesses are in equilibrium when the rate of particular metal ion; for example, iron turnover between the mineral and the bacteria, is balanced. The primary focus is directed towards improved bioprocess kinetics of the first two subprocesses of chemical reaction of the metal ion with the mineral and later bacterial oxidation. These subprocesses are linked by the redox potential and controlled by maintenance of an adequate solids suspension, dilution rate, and uniform mixing which are optimised in bioreactors during mining operations. Rate equations based on redox potential such as ferric/ferrous-iron ratio have been used to describe the kinetics of these subprocesses. This paper reviews the basis of process design for biomining process with emphasis on engineering parameters. It is concluded that the better understanding of these engineering parameters will make biomining processes more robust and further help in establishing it as a promising and economically feasible option over other hydrometallurgical processes worldwide.

1. Introduction

Biomining is gaining importance as a unit process which involves biological organisms in mineral extraction industries worldwide. With the decreasing high grade ore reserves and increased concern regarding the effect of mining on the environment, biomining technology, which was nevertheless age old deserted technique, is now being developed as a main process in the mining industry to meet the demand [1]. Another important aspect is the feasibility of biomining technologies to treat ores deposits with complex mineralogy, which could be difficult to treat by conventional methods [2]. Besides, the most attractive feature of biomining is economic feasibility compared to other competitive techniques [2]. Gahan et al. [2] comparatively analysed how gold and copper biomining operations played a role with the increase or

decrease in metal pricing over time. Their analysis indicated that most biohydrometallurgical innovations have been commercially implemented during leaner times [3]. Economic factors such as eliminations of net Smelter Royalties associated with smelting and refining and possibility of the use bioleaching for on-site acid production to eliminate or reduce acid purchases are the reasons behind this observation. This has made us look toward the biomining with a broader insight into performance and profit oriented research to meet the commercial requirements. For this purpose, the process stoichiometry, kinetics, and the key parameters involved in the process have been studied [4–7] and the promising concepts like design and control of reactors for mining operations are yet to be practised [8].

Biomining in general is used to describe the exploitation of chemolithotrophic microorganisms to assist the extraction
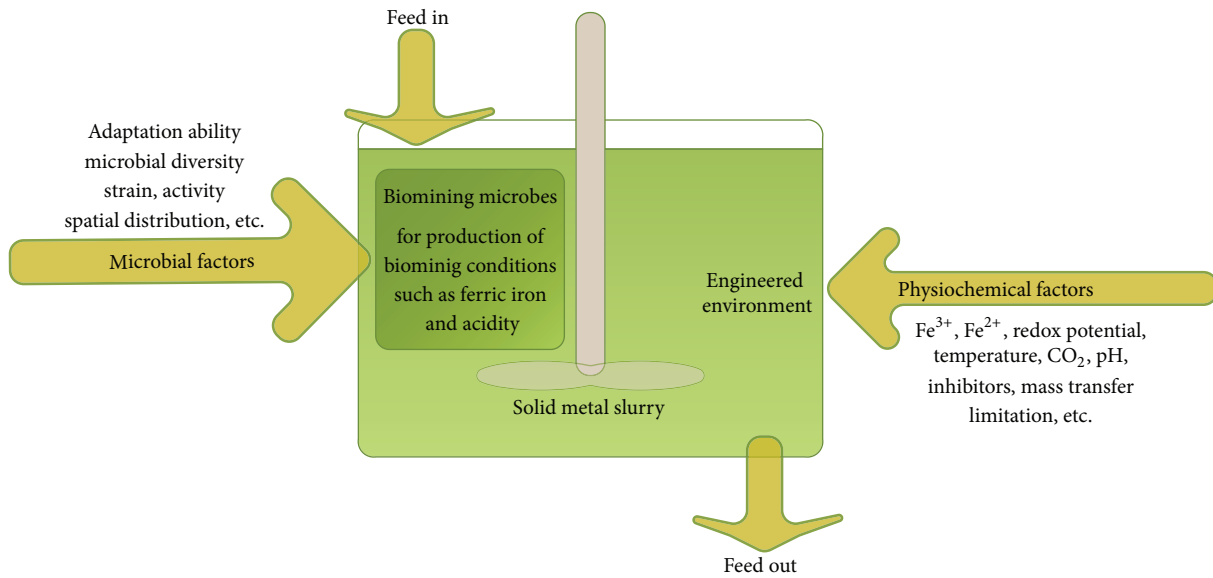
FIGURE 1: Factors involved in application of bioreactors in biomining.

of metals from sulphide or iron-containing ores or concentrates [9, 10]. It is a combination of two major operations bioleaching and biooxidation. The metal-solubilisation process is a blend of microbiology and chemistry. Knowledge in microbiology is required as specific microorganisms are responsible for producing the ferric iron and acid leading biooxidation; while strong base on chemistry is equally necessary since the solubilisation of the metal is a result of the action of ferric iron and/or acid on the mineral further known as bioleaching [6, 11, 12]. Conventional biomining is usually performed in heaps of ground ore or in the dumps of waste or spent material. Though this process offers several advantages such as simple equipment and operation, low investment, operational costs, and acceptable yields, it is beset with severe operational limitations, such as high heterogeneity of piled material and practically no close process control. Moreover, the low oxygen and carbon dioxide transfer rate and extended periods of operation to achieve sufficient conversions are very challenging [8]. From the process engineering point of view, bioreactors are the best choice for regulating the complex network of biochemical reactions comprehended in biomining as they allow for a close control of the variables involved, rendering significantly better performances. The reactors are usually arranged in series, with a continuous flow of material into the first, which overflows to the next, and so on for reduction of reaction volume required [13]. Therefore, process designing approach along with the defined application and monitoring of the abundance and activity of the metal sulfide oxidizing microorganisms will make the biomining process more industrially popular and as a portfolio of flexible techniques to provide a way of recovering metal (Figure 1).
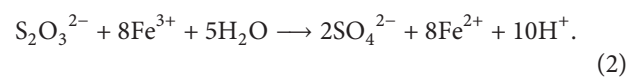
In this paper we discuss many of the theoretical considerations regarding the development of process for application of continuous-flow stirred-tank reactors in biomining with focus on different engineering parameters which should be taken into consideration for better control and design of biomining processes.

2. Mining Mechanisms and Stoichiometry

The most important mineral degrading microorganisms in biomining applications are the iron and sulphide oxidizing chemolithotrophs which grow autotrophically by fixation of atmospheric CO_2. However, the mineral dissolution mechanism is not the same for all metal sulphides. Schippers and Sand [14] reported that the oxidation of different metal sulphides proceeds via different intermediates: (1) thiosulphate mechanism for the oxidation of acid-insoluble metal sulphides such as pyrite (FeS_2) and molybdenite (MoS_2) and (2) polysulphide mechanism for acid-soluble metal sulphides such as sphalerite (ZnS), chalcopyrite ($CuFeS_2$), or galena (PbS) (Figure 2) [1].

In the thiosulphate mechanism, solubilisation is through ferric iron attack on the acid-insoluble metal sulphides, with thiosulphate being the main intermediate and sulphates the main end-product. Schippers and Sand [14] proposed the reactions using pyrite as an example:

$$FeS_2 + 6Fe^{3+} + 3H_2O \longrightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+ \quad (1)$$

$$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \longrightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+. \quad (2)$$

In the polysulphide mechanism, solubilisation of the acid-soluble metal sulphide is through a combined attack by ferric iron and protons, with elemental sulphur as the main intermediate. This elemental sulphur is relatively stable but
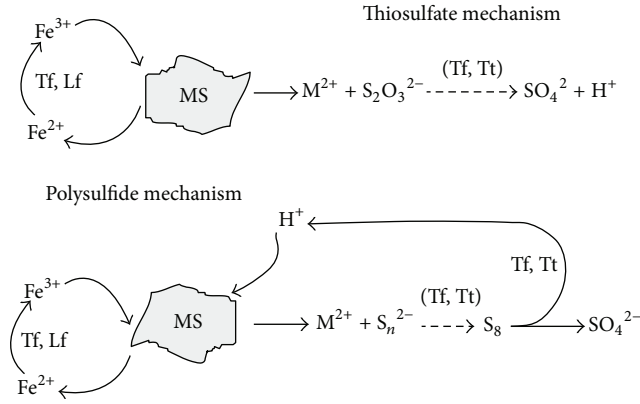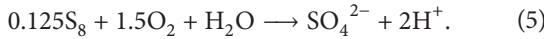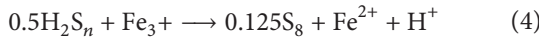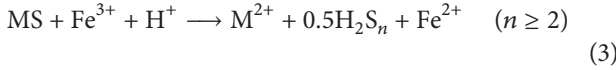
FIGURE 2: Scheme of the two metal sulphide oxidation pathways (mechanisms) via thiosulphate or via polysulphides and sulphur based on the properties of metal sulphides (MS).

can be oxidized to sulphate by sulphur-oxidizing microbes [14] (3)–(5):

$$MS + Fe^{3+} + H^+ \longrightarrow M^{2+} + 0.5H_2S_n + Fe^{2+} \quad (n \geq 2)$$
(3)

$$0.5H_2S_n + Fe_3+ \longrightarrow 0.125S_8 + Fe^{2+} + H^+$$
(4)

$$0.125S_8 + 1.5O_2 + H_2O \longrightarrow SO_4^{2-} + 2H^+.$$
(5)

This explains why strictly sulphur-oxidizing bacteria, such as *A. thiooxidans* or *A. caldus*, are able to leach some metal sulphides but not others. The ferrous iron produced during metal dissolution and biomining might also be reoxidised by iron-oxidizing organisms to ferric iron:

$$2Fe^{2+} + 0.5O_2 + 2H^+ \longrightarrow 2Fe^{3+} + H_2O.$$
(6)
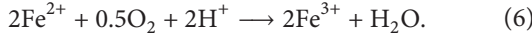
The role of the microorganisms in the solubilisation of metal sulphides is, therefore, to provide sulphuric acid (5) for a proton attack and to keep the iron in the oxidized ferric state (6) for an oxidative attack on the mineral.

In another methodology proposed by Byrne and Luo [15], it was experimentally shown that the ferric iron precipitation process in the basal medium could be described by the following stoichiometry:

$$X^+ + 3Fe^{3+} + 2SO_4^{2-} + 6H_2O$$
$$\longrightarrow XFe_3(SO_4)_2(OH)_6 + 6H^+,$$
(7)
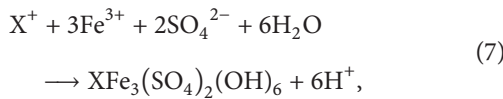
where X^+ corresponds to a monovalent cation. This stoichiometry provides the numeric relation between moles of precipitated iron and moles of released protons to be used in the calculations.

For the formulation of microbial metabolic reaction occurring during this course, the three half reactions for cell synthesis (R_c), electron donor (R_d), and electron acceptor (R_a) should be combined. So the overall metabolic reaction (R) is the sum of the half reactions [15] given as

$$R = f_e^0 (R_a - R_d) + f_s^0 (R_c - R_d)$$
$$= f_e^0 R_a - f_s^0 R_c - R_d.$$
(8)

The negative sign of an electron donor reaction means that this reaction should be reversed before adding it to the others.

3. Process Kinetics

Kinetics of biomining process depends mainly on the overall sum of the kinetics of the two subprocesses of bioleaching and biooxidation in the mineral matrix. These subprocesses are balanced when the rate of ferrous/ferric iron turnover between them is balanced ($r_{Fe^{2+}}^{chem} = -r_{Fe^{2+}}^{bac}$) [16]. The ferric leach kinetics can be described by

$$\xi_{Fe^{2+}} = \frac{-r_{Fe^{2+}}}{\alpha [FeS_2]} = \frac{\xi_{Fe^{2+}}^{max}}{1 + A ([Fe^{2+}] / [Fe^{3+}])}$$
(9)

and the kinetics of the bacterial oxidation of ferrous iron is given by

$$q_{Fe^{2+}} = \frac{-r_{Fe^{2+}}}{X} = \frac{q_{Fe^{2+}}^{max}}{1 + K ([Fe^{3+}] / [Fe^{2+}])}.$$
(10)

According to the shrinking core model by Lizama et al. [6], leaching kinetics involves a surface reaction and pore diffusion transport which can be described by the following equation:

$$1 - \frac{2}{3}\alpha_M - (1 - \alpha_M)^{2/3} = k \cdot t.$$
(11)

The shrinking core rate constant "k" is related to the surface area of leaching samples and should be constant over time. If the particle shrinks at a uniform rate, the leaching rate is proportional to the surface area of the mineral particle.

Assuming that the ferrous oxidation kinetics follows a Monod dependence on ferrous iron and includes a term related to ferric inhibition, the rate of ferrous iron oxidation can be given [17] as

$$V_{oxi} = \frac{V_{max} [Fe^{2+}]}{[Fe^{2+}] + K_s (1 + K_I [Fe^{3+}])},$$
(12)

where $V_{max} = (\mu_{max}/Y)$ and Y is being the yield for ferrous iron oxidation. Using this expression, value of V_{max} can be obtained from the values of V at particular pH in a system operated under broad pH range.

The kinetics of the chemical reaction in the biomining can also be related to the chemiosmotic mechanism [17, 18] which can be given as V_{chem} where the diffusional process can be related to diffusion of protons across the ferric iron precipitates layer formed on the bacteria in the leaching sample. Thus, V_{max} can be expressed as

$$V_{max} = \frac{V_{chem}}{1 + k\Delta x},$$
(13)

where Δx represents the thickness of the precipitates layer and k is a constant directly proportional to the ratio between the diffusional and the kinetics constant.

In broad terms the overall microbial growth during biomining is modelled via Michaelis-Menten kinetics described as

$$\frac{dN}{dt} = Ck_g \left[\Pi f_M \left(c_{Fe^{2+}}, c_{O_2}, c_{H^+} \cdots \right) - k_d f_d \right], \quad (14)$$

where C is the microbial concentration, k_g and k_d are the specific growth and death rate, the function f_M represents a series of Monod terms to account for the effects of ferrous ion, and dissolved oxygen and acid concentrations and f_d represent death rate. The variable Π represents the product of growth/substrate limiting and inhibitory terms [19]. The two kinetics, namely, microbial oxidation (12) and the microbial growth (14) are linked via the Pirt equation given as

$$V_{oxi} = C \left(\frac{k_g f_g \Pi}{y_g} + k_m \right), \quad (15)$$

where y_g is the yield constant and k_m is the maintenance (nongrowth) coefficient. These formulations can be made for growth and functional kinetics of both sulphur and ferrous oxidizing microbes in the reactor.

4. Gas-Liquid Mass Transfer

Though some anaerobic processes for biomining have been proposed recently [1, 20, 21], the aerobic processes are more in practice, which should be designed to optimise the mass transfer of the gases from the sulphide gas phase to liquid phase via gas-liquid interface and allow the gas to diffuse through the culture medium to the cell surface and then into the cell. Additionally, the biooxidation processes rely on CO_2 as the key carbon source for microbial growth. Many industrial bioprocesses in fact use air enriched with CO_2 to ensure rapid biomass growth. However, this can also be the rate limiting due to high availability of more soluble carbon dioxide and hence causing inhibitory effects due to reduction in the solubility of oxygen. The principal resistance to gas mass transfer can be assumed to be in the liquid film at the gas-liquid interface for sparingly soluble gas-like oxygen and can be given by the following relation:

$$N_{transported} = \frac{K_L a}{H} \left(P_g - P_l \right). \quad (16)$$

From a process engineering standpoint, different mass transfer strategies have been modelled [8, 19]. Supply of oxygen which takes place through diffusion can be estimated as the rate of change of oxygen concentration in static medium layer given as

$$\frac{\partial C_R}{\partial t} = D_0 \left(\frac{\partial^2 C_R}{\partial x^2} \right), \quad (17)$$

with the assumption that mineral matrix is in contact with the gas phase via gas-liquid interface and oxygen concentration on the surface is in equilibrium with the gas phase. The oxygen concentration in the mineral matrix can be given as

$$\frac{dc_P}{dt} = D_{eff} \frac{d^2 c_P}{dx^2} - \left(\frac{X \cdot q_{max}}{V} \right) \left(\frac{c_P}{k_s + c_P} \right). \quad (18)$$
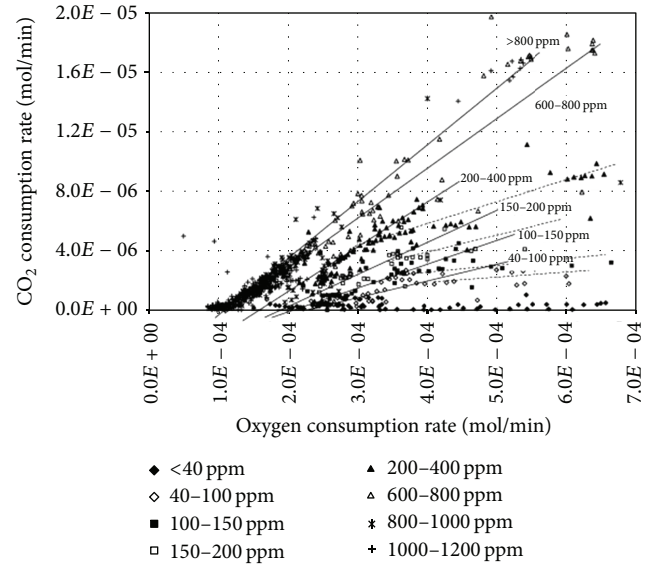

FIGURE 3: Plot of O_2 and CO_2 consumption rates in the various column segments over the length of the entire column experiment, grouped by residual CO_2 concentration [19].

Petersen et al. [22] demonstrated that the rates of O_2 and CO_2 consumption in the biomining are closely correlated (Figure 3). When grouping the data in ranges of CO_2 concentrations (average concentration in a segment between those measured at the bottom and top of the segment), it was shown that beyond a certain threshold value of oxygen consumption, linear trends between O_2 and CO_2 consumption rates (r_{O_2}, r_{CO_2}) exist. This shows that the rate of substrate (O_2) consumption r_{O_2} can be linked to biomass growth (proportional to CO_2 consumption) using a conventional substrate consumption model as follows:

$$r_{O_2} = \frac{r_{CO_2}}{Y_{O_2}} + m_{O_2}, \quad (19)$$

where Y_{O_2} is the carbon yield on O_2 (mol C·mol O_2^{-1}) and m_{O_2} is the maintenance rate of oxygen consumption (mol·min^{-1}).

The effects of dissolved oxygen and carbon dioxide concentration on microbial activity have been investigated by de Kock et al. [23] considering the microbial activity to be directly proportional to the Fe^{3+} ion concentration in the medium.

The relative activity data points were fitted using a four-parameter log normal fit function:

$$y = 0.1072 + 0.9029l \left[-0.5 \left(\frac{\ln(x/2.5077)}{0.9908} \right)^2 \right], \quad (20)$$

where y and x are the relative activity and dissolved oxygen (mg·l^{-1}), respectively. The relative activities of the Fe^{3+} production curves at different dissolved oxygen and gaseous CO_2 concentration concentrations are given in Figure 4. The effects of increased CO_2 concentrations on microbial activity
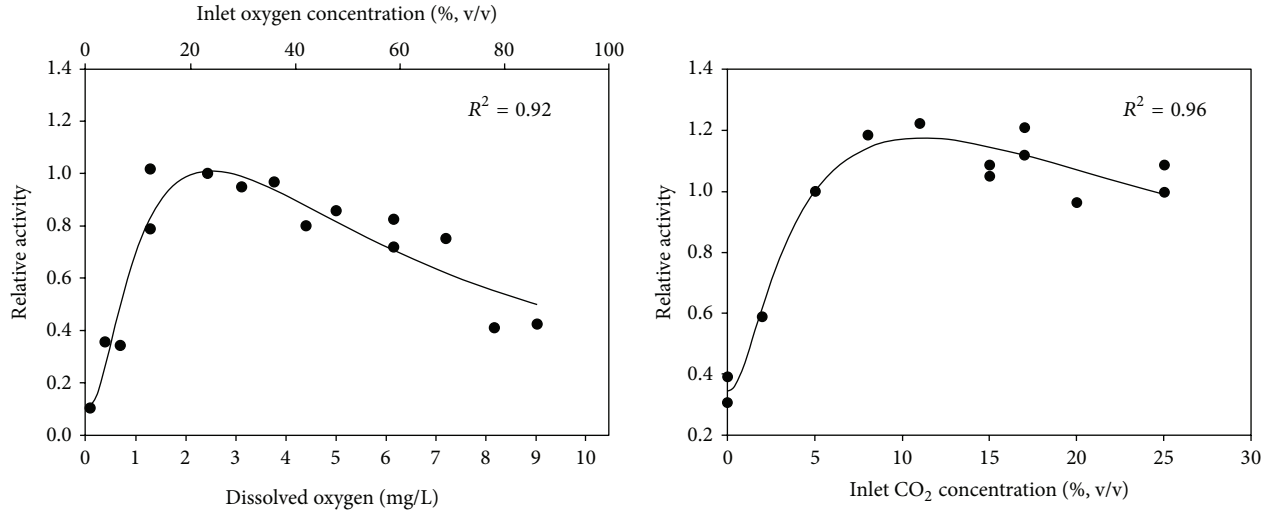
FIGURE 4: The effect of dissolved oxygen and gaseous CO_2 concentration concentrations on ferrous iron oxidation activity during autotrophic growth of Sulfolobus sp. U40813 [22].

were also investigated through a series of batch cultivations with increasing CO_2 concentration [23]. The relative activity data points were fitted using a four-parameter log normal fit function, where y and x are the relative activity and inlet CO_2 concentration, respectively:

$$y = 0.3450 + 0.8298l\left[-0.5\left(\frac{\ln{(x/11.02)}}{1.147}\right)^2\right]. \tag{21}$$

Such empirical models can be used for balanced gasification and agitation in the stirred tank reactors to achieve maximum cell growth and subsequent improved microbial activity necessary for controlled bioleaching and biooxidation in a reactor. The existing stirred-tank reactor design is relatively inefficient when it comes to aeration. Improved aeration designs will vastly improve the economics of use of stirred-tank reactors in biomining.

5. Temperature, pH, and Redox Potential

Physical conditions like temperature and pH are the inherent properties of microorganism and so they vary with microorganisms and mainly get decided by the dominant species in the consortium like *T. ferrooxidans* and *Leptospirillum ferrooxidans* [24]. The temperature has been shown to be clearly impacting the biomining rate on iron oxidation by mesophilic mixed iron oxidizing microflora [25]. By elevating from 20 to 30°C the rate was reported to increase and beyond this temperature the rate decreased due to denaturation of enzymes and proteins as well as the behaviour of ferric ion. Operating the reactor at higher temperature is advantageous in some cases such as during biooxidation of sulfidic-refractory gold concentrates; it allows growth of thermophilic microorganisms rendering oxidisation of partially oxidised sulfur compounds that are responsible for increasing cyanide consumption [26]. Also, bioleaching of chalcopyrite concentrates has been demonstrated using moderately thermophilic bacteria [27] and extremely thermophilic Archaea

[28]. However, operating the reactor in thermophilic range increases both capital and operating costs of the process. Hence, more promising approach is to use a combination of mesophilic/thermophilic process, but such approach requires more developments before practice [3]. Besides temperature, since the bacteria involved in biomining are chemolithotropic and acidophilic in character, the optimisation of pH is needed for its growth. The major reason for the dominance of species such as "*Leptospirillum*" and *T. ferrooxidans* in industrial processes is almost certainly the Fe^{3+}/Fe^{2+} ratio, that is, redox potential (Eh) [14]. To estimate the rate of ferrous iron oxidation throughout the bioleaching process to obtain explicit data of $[H^+]$ and $[Fe^{3+}]$ from the values of Eh and pH, a preliminary calibration procedure should be developed. For this purpose, Nernst expressions which relate the solution redox potential with the ferric/ferrous ratio in solution were obtained [17], given as follows.

In basal medium,

$$Eh = 0.471 + 0.0579\log\left(\frac{[Fe^{3+}]}{[Fe^{2+}]}\right). \tag{22}$$

In nutrient-free solution,

$$Eh = 0.492 + 0.0644\log\left(\frac{[Fe^{3+}]}{[Fe^{2+}]}\right). \tag{23}$$

In continuously operating stirred tank reactors, a steady state is reached once the redox potential inside tank remains approximately constant and high. Under such conditions the ability of *T. ferrooxidans* to oxidize ferrous iron is severely inhibited by the presence of ferric iron [18] whereas the iron-oxidizing ability of *Leptospirillum* is much less affected because of its ability to oxidize pyritic ores better at high redox potentials. Sundkvist et al. [29] also developed a model on *Leptospirillum ferrooxidans*-dominated culture in chemostat and suggested that the apparent Fe^{3+} inhibition
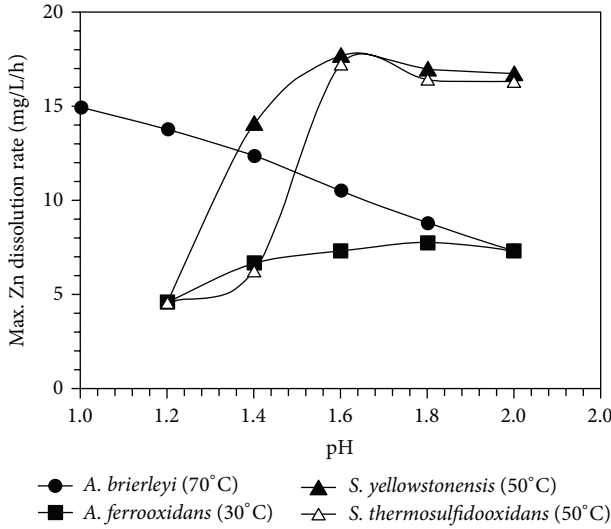
FIGURE 5: Effect of pH on the initial (maximum) dissolution rate of zinc from the ore by mesophilic moderately thermophilic and extremely thermophilic bacteria [31].

on specific Fe^{2+} utilization rate was a direct consequence of the declining biomass yield on Fe^{2+} due to growth uncoupled Fe^{2+} oxidation when the dilution rate was decreased.

However, there may be many other factors which may contribute to the dominance of particular species in consortia. The optimum pH for the growth of *T. ferrooxidans* is within the range pH 1.8–2.5, whereas *L. ferrooxidans* is more acid-resistant than *T. ferrooxidans* and can grow at a pH of 1-2 [16]. With regard to temperature, *T. ferrooxidans* is more tolerant of low temperature and less tolerant of high temperature than is *L. ferrooxidans*. Some strains of *T. ferrooxidans* are able to oxidize pyrite at temperatures as low as 10°C [30] but 30–35°C is considered to be optimal. *Leptospirillum*-like bacteria have been reported to have an upper limit of temperature for growth around 45°C with a lower limit of about 20°C [31]. Deveci et al. [32] showed that the bioleaching activity of mesophile *A. ferrooxidans*, as indicated by the dissolution rate of the zinc, Figure 5, was adversely affected with decreasing pH to ≤1.4. But, above this pH, there seemed no significant difference in the dissolution rate and extent (91–93%) of zinc. In contrast to *A. ferrooxidans*, the extraction rate of zinc by the extremely thermophilic *A. brierleyi* (at 70°C) was observed to increase with increasing acidity. In case of thermophilic *S. yellowstonensis* and *S. thermosulfidooxidans* the increase in the acidity to pH 1.2–1.4 led to a decrease in the oxidising activity of both strains, indicating the inhibitory effect of increased acidity on the strains [32].

In a bioleaching process, neutralisation is required at different stages. As the microorganisms used in bioleaching processes are chemolithotrophic and acidophilic having optimum activity at low pH, therefore, the addition of neutralising agents is required to maintain the desired pH depending on the reactor configuration. Neutralisation of the acid produced during bioleaching of sulphide minerals is generally practised using limestone. Gahan et al. [33] concluded in their

study that considerable savings in operational costs could be obtained by the replacement of lime or limestone with steel slag, without negative impact on bioleaching efficiency.

6. Solid Suspension and Mixing

The reactors which are mostly used in commercial mining plants operating continuously and in biohydrometallurgical laboratories are the stirred tank reactor (STR) and the airlift reactor (ALR) or pneumatic reactor (PR) of the Pachuca type. Aeration in these reactors depends on suspension agitation, and "flooding" may cause some limitations, while very strong agitation may cause cell damage to the extreme [8]. Normally, the stirred suspension flows through a series of pH and temperature-controlled aeration tanks in which the mineral decomposition takes place. Thus maintaining the uniformity of solid-liquid ratio in reactor system, solid suspension is important for efficient biomining. The solid-liquid ratio becomes a very critical parameter in the bioreactor applications as the solids packed density that can be maintained in suspension is limited to 20% in the operation of stirred tank reactors, beyond which the physical and microbial problems occur. The shear force induced by the impellers causes physical damage to the microbial cells and the liquid as well becomes too thick for efficient gas transfer [34]. In biomining, higher mixing characteristics and lower oxygen transfer rates are needed compared to common industrial fermentations. Hence, impellers that generate axial flow patterns are preferable [13]. Among the axial flow type impellers, the hydrofoil design offering several advantages such as good mixing characteristics, low shear, and gas transfer capabilities are most compatible with the biomining processes demands [8, 35]. A disk turbine at the bottom and a propeller at the upper part of the shaft have been proposed to meet higher oxygen demand situations [36].

Most of the existing mixing models concern single impeller nonaerated systems in turbulent flow conditions and consider generally one geometrical factor, which is the impeller to reactor diameter (d/D ratio). Only the influence of the off-bottom clearance and the spacing between impellers on the power consumption was studied. In the present instance it can be assumed that the mixing time in an aerated and agitated vessel is governed by a multitude of geometrical ($h, D', d, d', d'', z, B, l, w, n_b, n_{imp}, n_{baf}$) and technological ($N, G, \rho, \eta$) factors. The analysis leads to the following dimensionless grouping and variables [37]:

$$NT_m = K \left(\frac{h}{D'} \right)^a \left(\frac{d'}{d} \right)^b \left(\frac{d''}{d} \right)^c \left(\frac{z}{d} \right)^e \left(\frac{d}{D'} \right)^f$$
$$\times n_b^h X n_{im}^i n_{baf}^j \left(\frac{B}{d} \right)^k \left(\frac{l}{d} \right)^m \left(\frac{w}{d} \right)^n Re^P Fr^q F_g^r, \quad (24)$$

with NT_m being the dimensionless mixing number; Re = Nd^2/η the impeller Reynolds number; Fr = N^2d/g the impeller Froude number; Fg = $G/Nd^3 n_{imp}$ the modified aeration number related to a single-impeller system; and (h/D'), (d'/d), (d''/d), (z/d), (d/D'), (B/d), (l/d), (w/d) dimensionless geometric groups. The use of a specific reactor

requires maintaining some of the dimensionless geometric groups constant. Therefore, the general expression becomes

$$\mathrm{NT}_m = K_1 \left(\frac{d'}{d}\right)^b \left(\frac{d''}{d}\right)^c \left(\frac{z}{d}\right)^e \mathrm{Re}^p \mathrm{Fr}^q F_g^r. \tag{25}$$

After evaluating the coefficients b, c, e, p, q, and r, by trial and error the final form of the equation can be determined for the specific system.

A more complete model was given by Norwood and Metzner [38] which takes account of the influence of the Re and Fr numbers and shows clearly the existence of three zones corresponding to the laminar, transitory, and turbulent flow regime. In this model, the mixing time number is independent of the Re number. Scale up of mixing uses a correlation of Reynolds number with Power number, N_p, given as

$$N_p = \frac{P}{\rho N^3 d^5}. \tag{26}$$

Effective mixing is mandatory for all material transport processes involved in microbial and biooxidation kinetics of biomining and it attains its maximum effectiveness when all these processes are optimised in the bioreactor. However, mechanically agitated reactors have limitations with respect to their sizes due to increase in cost and mechanical complexity related to agitation above the volume of 500 m³ [13]. Pneumatically agitated reactors could be a better option when high reaction volumes are needed, as their construction and operation costs are lower due to the absence of an agitator.

7. Continuous Process Dilution Rate

In order to maintain solids packed density below 20% during the operation of stirred tank reactors, which deviates due to the feeding of mineral slurry it is necessary to account the effect of dilution rate. Carrying the mass balance across the reactor, the final biomass accumulation rate can be described as

$$\frac{dC}{dt} = \mu C - \left(\frac{F}{V}\right) C. \tag{27}$$

Dilution rate in agreement with the equation of product (P) balance in a CSTR vessel in steady state is given by Aiba et al. [39]:

$$\frac{dP_n}{dt} = D\left(P_{n-1} - P_n\right) + \left(\frac{dP_n}{dt}\right)_{\text{production}}, \tag{28}$$

where the desired dilution rate $D = F/V$ (time⁻¹) is a rate at which the mineral slurry was fed continuously to the bioreactor and the effluent is removed by means of variable-speed pumps. At steady state, $dP_n/dt = 0$ and assuming that $P_{n-1} = 0$ in a single reactor vessel, thus

$$\left(\frac{dP}{dt}\right)_{\text{total}} = 0 = \left(\frac{dP_n}{dt}\right)_{\text{production}} - DP,$$

$$\text{that is } \left(\frac{dP_n}{dt}\right)_{\text{production}} = DP. \tag{29}$$

Steady state can also be expressed in terms of growth rate of microorganisms which equals dilution rate, D, or the reciprocal of the residence time, $1/\tau$. Using this method, the calculation of dilution rate of the general model of industrial scale biomining processes can be done (generally between 0.05 and 0.6 h⁻¹). A continuous bioreactor for biomining operating at steady state ideally follows the dilution rate equal to the growth rate of the microorganisms. The rates of microbial oxidation obtained from (15) remain coupled for dilution rates yielding moderate concentrations of the individual cations. However, with increasing cation concentrations the reaction rate reduces. This was shown by Ojumu et al. [40], where microbial oxidation occurred better at lower ferric-to-ferrous ratios. These rates levelled off or began to decrease with increasing dilution rates in those runs with high cation concentrations. As $\mu_{\max}$ was lower at higher concentrations of dissolved ions, washout would be expected to occur already at lower dilution rates.

8. Microbial Community Structure

Although microbial community structure is not an engineering parameter, it is an integrated part of biomining processes which catalyses the biochemical reaction involved and should be considered properly in process designing approach. For the robust modelling of the system, knowledge regarding microorganisms present should be acquired as each species is kinetically different from the other. Therefore, the simplistic assumption of lack of diversity in the characteristics of the species within the microbial groups may lead to over- or underestimation of the model output when compared to observed data. There have been considerable studies in the last years to understand the biochemistry of iron and sulphur compounds oxidation, bacteria-mineral interactions, and several adaptive responses allowing the microorganisms to survive in a bioleaching environment which has been reviewed by Valenzuela et al. [41]. There are different groups of microorganisms capable of growth in situations which simulate biomining commercial operations [31]. The biology of leaching bacteria becomes more and more complex due to increasing data on 16S rRNA gene sequences as many new species of leaching bacteria have been described and known species have been reclassified [42]. Moreover, it was believed that only mesophilic bacteria play a role in biomining, but the discovery of new genera of moderate and extremely thermophilic bacteria belonging to *Sulfolobus, Acidianus, Metallosphaera*, and *Sulfurisphaera* has made the bioleaching process more economically attractive to the industries where coiling used to be a necessary step to operate the reactors at mesophilic temperature [41, 43]. Change in microbial community dynamics during moderately thermophilic bioleaching of chalcopyrite concentrate has been recently studied by Zeng et al. [44] and it was observed that *A. caldus* was dominant at the early stage while *L. ferriphilum* predominated at the later stage in a stirred tank reactor.

The bioleaching performance of chalcopyrite for various hydraulic residence times (HRTs) was estimated by Xia et al. [45] and they observed that *Leptospirillum ferriphilum* and *Acidithiobacillus ferrooxidans* compete for oxidising ferrous iron, leading to large compositional differences in the microbial community structure under different HRTs. It was observed that *Leptospirillum ferriphilum* and *Acidithiobacillus thiooxidans* dominated under the longer HRT (120 h), while *Acidithiobacillus ferrooxidans* was dominant when the HRT was decreased. It has been suggested that for the proper establishment of active biomining consortia, a substantial period of adaptation is necessary as consortia (mesophiles or moderate thermophiles) cannot be assembled "off the shelf" [46].

9. Conclusions and Perspectives

The future of biomining is much demanding and the incorporation of engineering principles in the field will make the process industrially more acceptable. Stirred-tank bioreactors have clearly opened new opportunities for processing in mining industries as it allows better control than conventional technologies leading to superior leaching efficiency and metal recovery. Aeration, temperature, pH, and so forth can be continually adjusted to achieve maximum microbial activity in stirred-tank reactors. Present studies demonstrate that in designing a biomining process, the bioleaching and biooxidation performance, microbial growth kinetics, and other characteristic properties (gas-liquid mass transfer, solid suspension and mixing, and the characteristic dilution rate of a continuous process) should be reasonably well understood. To evaluate the feasibility and effectiveness of the controlled process in wide-scale metallurgy, it is essential to make predictions of the biomining performance. The performance assessment and prediction based on both equilibrium and dynamic studies will help in the extended process control increasing the commercial viability.

There are several specific needs for more advancement in the commercialisation of biohydrometallurgy. A prolific area for research is a comprehensive study of microbial community structure with rapid, accurate, and simple techniques for monitoring the microbial activity in bioleach/mineral-biooxidation systems which will enable the operators to gain more control on these processes. More advanced research in applied microbiology will throw light in this regard. In most instances, these advancements will help the metallurgical engineers understand the role and specific operational needs for microorganisms and to improve the designing approaches maximizing the system's efficiency. Another area for development is in reactor engineering such as materials of construction. New materials for high temperature, highly corrosive conditions that are of relatively low cost, would also advance the technology. Such reactors would amplify opportunities to bioleach whole ores. Robust modelling of the relevant parameters and processes including the microbial community dynamics and structure would make biomining more established and economically feasible option over other hydrometallurgical processes worldwide.

Symbols

α_M:	Metal recovery
α:	Specific surface area of pyrite ($m^2 \, mol^{-1}$)
$r_{Fe^{2+}}^{chem}$:	Chemical ferrous iron production rate ($mol \, Fe^{2+} \, l^{-1} \, h^{-1}$)
$r_{Fe^{2+}}^{bac}$:	Bacterial ferrous iron production rate ($mol \, Fe^{2+} \, l^{-1} \, h^{-1}$)
$r_{Fe^{2+}}$:	Ferrous iron production rate ($mol \, Fe^{2+} \, l^{-1} \, h^{-1}$)
A:	Kinetic constant in pyrite oxidation (dimensionless)
c_R:	Oxygen concentration in static medium layer ($mol \, l^{-1}$)
c_P:	Oxygen concentration in mineral matrix, ($mol \, l^{-1}$)
D_0:	Diffusion coefficient ($m^2 \, s^{-1}$)
D_{eff}:	Diffusion coefficient in surface matrix of membrane ($m^2 \, s^{-1}$)
$\zeta_{Fe^{2+}}$:	Area specific ferrous iron production rate ($mol \, Fe^{2+} \, m^{-2} \, h^{-1}$)
$\zeta_{Fe^{2+}}^{max}$:	Maximum area specific ferrous iron utilization rate ($mol \, Fe^{2+} \, m^{-2} \, h^{-1}$)
f_e^0:	Electrons from the donor transferred to the terminal electron acceptor
f_s^0:	Electrons from the donor that is used for net cell synthesis
K:	Kinetic constant (dimensionless)
k:	Rate constant
t:	Time of bioleaching (day)
μ_{max}:	Specific growth rate of the bacterial cell (h^{-1})
F:	Total feed rate ($kg \, h^{-1}$)
$[Fe^{2+}]$:	Ferrous iron concentration ($mg \, l^{-1}$)
$[Fe^{3+}]$:	Ferric iron concentration ($mg \, l^{-1}$)
Fr:	Mixing time number
$[H^+]$:	Proton concentration ($mg \, l^{-1}$)
H:	Henry's law constant ($mol \, dm^{-3} \, atm^{-1}$)
K_s:	Michaelis-Menten constant ($mg \, l^{-1}$)
K_I:	Ferric iron inhibition constant (no units)
$k_L a$:	Overall gas mass transfer coefficient
P_g:	Substrate partial pressure in gas phase (atm)
P_l:	Substrate partial pressure at equilibrium with the substrate concentration in the bulk liquid phase (atm)
$N_{transported}$:	Gas mass transfer rate per unit volume of reactor ($mol \cdot s^{-1} \cdot l^{-1}$)
$q_{Fe^{2+}}$:	Bacterial specific ferrous iron oxidation rate ($mol \, Fe^{2+} (mol \, C)^{-1} \, h^{-1}$)
$q_{Fe^{2+}}^{max}$:	Maximum bacterial specific ferrous iron oxidation rate ($mol \, Fe^{2+} (mol \, C)^{-1} \, h^{-1}$)
q:	Specific uptake rate ($mol \, cell^{-1} \, h^{-1}$)
q_{max}:	Maximal cell-specific oxygen uptake rate ($mol \, cell^{-1} \, h^{-1}$)

V: Volume of processing system (l)

V_L: Liquid volume (l)

V_{oxi}:   Specific ferrous iron oxidation rate (mg (hr cell)^{-1})

V_{chem}:   Maximum specific ferrous iron oxidation rate non affected by ferric (mg (hr cell)^{-1})

V_{max}:   Maximum specific ferrous iron oxidation rate (mg (hr cell)^{-1})

X: Cell density (cells l^{-1})

Y: Bacterial growth yield on ferrous iron oxidation ($\text{cell mg}^{-1}\text{ Fe}$)

z: Bottom clearance (m)

D':   Tank diameter (m)

d: Impeller diameter (m)

d':   Impeller spacing (m)

d'':   Top clearance (m)

B: Baffle width (m)

l: Impeller blade length (m)

w: Impeller blade width (m)

n_b: Number of blades

n_{imp}:   Number of impellers

n_{baf}:   Number of baffles

N: Impeller rotation speed (rpm)

G: Volumetric gas flow rate ($\text{m}^3\text{ h}^{-1}$)

ρ: Liquid density (kg m^{-3})

η: Liquid kinematic viscosity (Pa·s)

$a, b, c, e, f, h, i, j, k, m, n, p, q, r$:   Numerical coefficients in (24) and (25)

P: Power required to drive the agitator (W).

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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