



A COMPREHENSIVE REVIEW ON PARAMETERS INFLUENCING BIOLEACHING OF METALS FROM E-WASTE

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Abstract

'Electronic waste' (E-waste) is considered as a mixture of various metals. In particular, it consists of copper, aluminium, nickel, iron and steel, with various types of plastics and ceramics. Recycling of E-waste has gained importance in recent years, not only from the point of waste treatment, but also from the valuable metals that can be recovered, thus having substantial economic value generation from waste products. In the last decade, biotechnology has emerged as the most promising technology for recovering metals from primary and secondary sources and wastes.

The use of microorganisms appears to be economically the most appropriate method for recycling the valuable metals from secondary resources. Bioleaching process offers a number of advantages compared to the conventional methods. In spite of the above advantage bioleaching is at its nascent state even in well developed countries and in India, it is yet to take off.

Based on exhaustive literature survey carried out, it is found that generally there are eight parameters which influence the bioleaching process. The eight influencing parameters which have been selected in the present study, namely: pH, temperature, oxidation reduction potential (ORP), pulp density (PD), metal dosage concentration (MDC), particle size (PS), Solid / liquid ratio (S/L ratio), oxygen content (OC) or dissolved oxygen

(DO) and their influence on bioleaching of metals from E-waste has been comprehensively reviewed based on published work for the past 15 years. The influence of the above said parameters on the performance of several bacteria i.e. 13 families investigated so far, on the bacterial growth and leaching efficiency of heavy metals from PCBs, have been highlighted. **Index terms:** Bioleaching, biohydro metallurgy, E-waste, influencing parameters.

I. INTRODUCTION

Nowadays, waste is not considered as waste, but, as more valuables which have the potential to be reused or converted to other usable forms by recycling [1]. In recent years, electronic waste (E-waste) or Waste Electrical and Electronic Equipment (WEEE) has attained the status of one of the fastest growing waste stream, as the rate of recycling (or) recovery of valuable metals is far less when it is compared to the rate of waste generation. It has been reported that the average yearly rate of E-waste generation in India is about 1.7 million tonnes (M.T), whereas, their average recycling rate is only 23.5% [2].

The available methodologies as on date to treat E-wastes are: pyrometallurgy, hydrometallurgy, and biohydrometallurgy. The scientific society has always been in the pursuit to adopt the best suitable method for recycling and recovery of metals from e-waste. Pyrometallurgical method is an ancient method that simply fumes all the components of E-waste and it lacks in the

recovery efficiency. Further, it generates dioxins and furans that affect the quality of atmospheric air [1] and it is also energy intensive [3]. In hydrometallurgy method, fractions of E-waste are dissolved in an acidic or alkaline solution to recover the base and precious metals. The hydrometallurgical method has proven to be an efficient method than the pyrometallurgical method, but, the economical viability of processing and their environmental effects due to the toxic reagent continues to be serious and remain unresolved issues, even today [3]. On the other hand, biometallurgy method is an efficient and sustainable technology and a least number of dedicated research works have been carried out to recover the metals from primary sources (ores) and secondary sources (E-waste). It has been widely classified into two main areas such as: bioleaching and biosorption [4]. Bioleaching is a process described as being “the dissolution of metals from their minerals by certain naturally occurring microorganisms” [5]. Biosorption is a physico-chemical process that occurs naturally in certain biomass which allows it to passively concentrate and bind contaminants onto its cellular structure. In recent years, there is an increase in the number of efforts to adopt the bioleaching process for recycling and recovery metals which shows that the method matches the expectation / emphasis of environment – friendliness. Further, it has lower operational cost and energy requirements [3]. In recent studies, microorganisms such as: mesophilic chemolithotrophic (Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans) and cyanogenic bacteria, (Chromobacterium violaceum) when used for recovery of metals from printed circuit boards (PCBs) have shown promising recovery efficiency [6]. In addition, from the comprehensive survey of literature carried out, it has been noted that there is very scanty of literature available on the interrelation between influential parameters such as: pH, temperature, oxidation reduction potential (ORP), pulp density, metal dosage concentration, particle size, solid / liquid ratio and oxygen content on the bioleaching process. However, such studies are fundamental in understanding the bioleaching process and in designing the experimental programme and in optimizing the parameters of the bioleaching

process. The objective of this paper is to offer a comprehensive literature survey exclusively on the interrelationship between the influencing parameters such as: pH, temperature, oxidation reduction potential (ORP), pulp density (PD), metal dosage concentration (MDC), particle size (PS), solid/liquid ratio (S/L Ratio) and oxygen content (OC) for the bioleaching of metals from PCBs on the basis of different bacterial species, studied so far.

II. Parameters Influencing Bioleaching

In this section the relation between the following parameters and the bacterial growth and metal recovery efficiency are summarized. The pH, temperature, oxidation reduction potential (ORP), pulp density (PD), metal dosage concentration (MDC), particle size (PS), solid/liquid ratio (S/L Ratio) and oxygen content (OC) are chosen as the unique influential parameters which highly influence the bioleaching process. Brief outlines of their influences are given below.

A. pH

pH is the prime influential parameter that governs the bacterial growth. Further, it is the environmental factor for microbial production and it also influences the leaching of heavy metals from PCBs into the solution. The details of the optimum pH conditions for the bacterial growth and bioleaching of PCBs have been summarized in Table 1, based on previous reported research findings. It can be seen that in general, the pH range varies with the family of microorganisms, and for the majority of the microorganisms, excepting, three of them, the pH lies in the very high acidic range of 1.0 – 4.0. Only for the family of microorganisms Neisseriaceae and Pseudomonadaceae, the optimum pH range lies in the very high alkaline range of 6.0 – 11.0. Further, the only family which can survive and be effective both in the high acidic to alkaline range in the bioleaching process are Bacillaceae. Thus, it can be safely stated that a very high acidic pH has to be maintained for bioleaching of PCBs, which calls for careful preparation and maintenance of pH range and continuous monitoring during the entire period of bioprocess.

B. Temperature

Temperature is another important influencing factor, which directly influences the rate of

leaching kinetics and growth of bacterial population in chemical and bioleaching reactions. The classification of microorganisms based on temperature for their sustainability has been given in Table 2. The optimum temperature for the bacterial growth and bioleaching of PCBs has been summarized from published literature in Table 3. Further, the above temperature ranges are categorized into the five temperature groupings given in Table 2, for further

discussion. It is seen that the optimum temperature varies widely from 25°C to 75°C for the various family of microorganisms investigated so far. For the majority of the families of microorganisms such as: Acidithiobacillaceae, Nitrospiraceae, Neisseriaceae, Pseudomonadaceae, Desulfovibrionaceae and Enterobacteriaceae lies in the mesophilic temperature range of 20°C to 45°C.

Table 1: Details of microorganisms and their optimum pH range for bioleaching of PCBs

Family of the microorganisms	Name of the microorganism	Optimum pH range	References
Acidithiobacillaceae	Acidithiobacillus ferrooxidans	1.3-4.5	[5] [9] [3] [14] [13] [15] [11] [16] [8] [10] [12] [1] [7]
	Acidithiobacillus thiooxidans		
	Thiobacillus ferrooxidans		
	Thiobacillus thiooxidans		
Nitrospiraceae	Leptospirillum ferrooxidans	1.0-2.0	[15]
Clostridiales Family XVII. Incertae Sedis	Sulfobacillus	1.9-2.4	[6] [19] [20] [21]
	thermosulfidooxidans		
Alicyclobacillaceae	Sulfobacillus acidophilus	1.6-2.3	
Bacillaceae	Bacillus stearothermophilus	2.0-11.0	Nil
Sulfolobaceae	Metallothermobaculum sedula (Archaeae)	1.0-2.5	Nil
Acidithiobacillaceae	Acidithiobacillus sp.	1.3-4.5	[13]
Thermoplasmataceae	Thermoplasma acidophilum (Archeae)	0.5-4.0	[6] [20] [21]
Neisseriaceae	Chromobacterium violaceum	7.0-11.0	[23] [24] [25]
Pseudomonadaceae	Pseudomonas aeruginosa	6.0-10.0	[24]
	Pseudomonas fluorescens		
	Pseudomonas stutzeri		
Gallionellaceae	Gallionella	1.0-2.5	[17]
Desulfovibrionaceae	Desulfovibrio desulfuricans	2.0-3.5	[26]
Enterobacteriaceae	Klebsiella pneumoniae		[27]

Note: PCBs considered on the above studies are preprocessed and pretreated, and do not belong to the categories of ‘as-is-where-is’.

The above temperature is typical of south India, especially in the Union Territory of Puducherry, where there is an ongoing research work in this emerging area. The recorded minimum and maximum average temperature for Puducherry respectively is: 21.4°C and 36.8°C (Indian Meteorological Department, IMD). For the group of families of microorganisms such as: Clostridiales Family XVII Incertae Sedis, Sulfolobaceae, Acidithiobacillaceae, Thermoplasmataceae and Gallionellaceae their optimum temperature range lies in the

thermophilic temperature range of 45°C to 70°C. Further, the family of Alicyclobacillaceae and Bacillaceae has an unique property that it can survive even in a lower temperature of 28°C to the higher temperature range of 75°C, i.e. covering both mesophilic and thermophilic range. From the above analysis it can be stated that microorganisms are sensitive to temperature, as well as they require continuous monitoring and maintenance for their effective and successful growth in the bioleaching process.

Table 2: Classification of microorganisms based on temperature range for their sustainability

Classification of microorganisms	Temperature (°C)	Reference
Psychrophiles (PSP)	-15 °C to 10 °C	[28]
Psychrotrophs (PST)	0(zero) °C to 20 °C	
Mesophile (M)	20 °C to 45 °C	
Thermophile (T)	45 °C to 70 °C	
Hyperthermophile (H)	75 °C to 110 °C	

Table 3: Details of microorganisms and their optimum temperature range for bioleaching of PCBs

Family of the microorganisms	Microorganisms	Optimum Temperature Range (°C) and Grouping	Reference
Acidithiobacillaceae	Acidithiobacillus ferrooxidans Acidithiobacillus thiooxidans Thiobacillus ferrooxidans Thiobacillus thiooxidans	45-50 (M)	[5] [9] [3] [14] [13] [15] [11] [16] [8] [10] [12] [1] [7]
Nitrospiraceae	Leptospirillum ferrooxidans	20-30 (M)	[15]
Clostridiales Family XVII. Incertae Sedis	Sulfobacillus thermosulfidooxidans	50-55 (T)	[6] [19] [20] [21]
Alicyclobacillaceae	Sulfobacillus acidophilus	28-62 (M/T)	
Bacillaceae	Bacillus stearothermophilus	30-75 (M/T)	Nil
Sulfolobaceae	Metalloshera sedula (Archeae)	50-75 (T)	Nil
Acidithiobacillaceae	Acidithiobacillus sp.	45-50 (T)	[13]
Thermoplasmataceae	Thermoplasma acidophilum (Archeae)	55-60 (T)	[6] [20] [21]
Neisseriaceae	Chromobacterium violaceum	30-40 (M)	[23] [24] [25]
Pseudomonadaceae	Pseudomonas aeruginosa	30-45 (M)	[24]
	Pseudomonas fluorescens	25-30 (M)	
	Pseudomonas stutzeri	25-30 (M)	
Gallionellaceae	Gallionella	40-50 (T)	[17]
Desulfovibrionaceae	Desulfotribrio desulfuricans	25-40 (M)	[26]
Enterobacteriaceae	Klebsiella pneumoniae		[27]

Note: (M), (T) etc. refer to the temperature range and grouping given in Table 2.

C. Oxidation Reduction Potential (ORP) oxidation or reduction reaction which takes place on precious metals in a solution. The oxidation reduction potential (ORP) is an indicative parameter for the growth of microorganisms that can be identified by microorganisms (i.e. it indirectly conveys the

information that, if the bioleaching system have a good growth of microorganisms then it is evident that the system can have higher oxidation reduction potential) which can convert the metals into ionic form by means of chemical or biochemical reactions. It is possible due to the release or accept of electrons which are the fundamental leaching mechanisms that can be done by the selective microorganisms.

D. Pulp Density (PD)

Pulp density (PD) is defined as the amount of addition of various metals and plastic consisting of crushed fine powder of printed circuit boards (PCBs) to the selective species inoculums. It directly affects the species growth during the bleaching process, the influence may occur due to the presence of various metals, plastics and resins which are considered highly toxic to the metabolic activity of microorganisms. The optimum pulp density for the bioleaching process as collated from various published research work is given in Table 4 for the various

microorganisms. It is seen that the optimum dosage for Acidithiobacillaceae has been fairly established, whereas, for other family of microorganisms, studies are rather rare.

E. Metal Dosage Concentration (MDC)

MDC is defined as the addition of amount of metals to the selective species inoculums. Further, it highly influences the bioleaching system such as growth of microorganisms and metal recovery efficiency. From the various studies carried out it is seen that the addition of higher amount of metal containing pulp will not give higher metal recovery efficiency. Instead, it inhibits the total metabolic growth of microorganism. Hence, the study on optimum metal dosage addition has a high interest to explore further research in this area. The optimum MDC for the bioleaching process as reported in published literature is also given in Table 4 for the various microorganisms. It can be seen that studies on MDC for a chosen bacteria is rather rare.

Table 4: Details of microorganisms and their optimum value for Pulp Density (PD) and Metal Dosage Concentration (MDC) for bioleaching process

Family of Microorganisms	Microorganisms	Optimum PD (mg/l)	Reference
Acidithiobacillaceae	Acidithiobacillus ferrooxidans	15, 10	[1] [3]
	Acidithiobacillus thiooxidans		[7]
	Thiobacillus ferrooxidans		
	Thiobacillus thiooxidans		
Nitrospiraceae	Leptospirillum ferrooxidans	10	[7]
Gallionellaceae	Gallionella	10	[8]
Clostridiales Family XVII. Incertae Sedis	Sulfobacillus thermosulfidooxidans	10	[6]
Optimum Metal Dosage Concentration (MDC) (mg/l)			
Acidithiobacillaceae	Acidophilic bacteria	4	[14]

F. Particle Size (PS)

Particle size is a prime physical parameter which also directly influences the bioleaching process. Particle size is in the sense that the reduced size of printed circuit board is up to micrometers and millimetres. It was noted from published work that an increase in size of PCBs affects the microbial growth and metal recovery efficiency. Several studies have found the

optimum particle size of PCBs for various selective microorganisms. The optimum particle size for the bioleaching process as reported in published literatures summarized in Table 5 for the various microorganisms. It can be seen that the optimum value of PS of Acidithiobacillaceae ranges from 0.5 mm to a few terms of micrometer.

G. Solid/liquid ratio (S/L Ratio)

Solid/liquid ratio is a prime influential parameter that directly influence the microbial metabolic growth and metal recovery efficiency. It is the combination of two physical systems such as solid and liquid that governs the total bleaching process, PCBs are the solid part and growth media and energy sources for the microorganisms are the liquid part of the systems. Increase in any one of the above physical system causes an inhibitory effect to the bioleaching process. If the solid content is increased the dissolution of metals in the liquid will also get increased due to higher amount of solid containing higher metal concentration. Obviously, metal dissolution will influence the complete bioleaching. The optimum S/L ratio for the bioleaching process has been summarized

and given in Table 5 for the various microorganisms.

H. Oxygen Content (OC)

Oxygen content (OC) (or) dissolved oxygen (DO) is prime influential parameter. The oxygen content has to be maintained in an optimum level always, and the decrease in OC will drastically affect the microbial metabolic activity and indirectly influence the metal recovery efficiency. The reduction in oxygen content denotes the active microbial metabolisms and it will be compensated for the maintenance of optimum oxygen level by introducing the oxygen through the diffused air aerators. The optimum oxygen content for the bioleaching process as summarized from various literatures is given in Table 5 for the various microorganisms.

Table 5. Details of microorganisms and their optimum value for Particle Size (PS), Solid/Liquid Ratio (S/L Ratio) and Oxygen Content (OC) for bioleaching process

Family of Microorganisms	Microorganisms	Optimum PS	Reference
Acidithiobacillaceae	Acidithiobacillus ferrooxidans	0.4-0.8 mm	[3]
	Acidithiobacillus thiooxidans	0.5-1.0 mm	[11]
Acidithiobacillaceae	Acidophilic bacteria	270-48 μ m, 60-80 mesh	[13] [14]
Optimum Solid/Liquid Ratio (S/L Ratio) (mg/l)			
Acidithiobacillaceae	Acidithiobacillus ferrooxidans	01 /100	[12]
Optimum Oxygen Content (mg/l)			
Acidithiobacillaceae	Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans	Minimum Level 0.2	[9]
Clostridiales Family XVII. Incertae Sed	Sulfobacillus thermosulfidooxidans	7.3-8.0	[6]

III. INFLUENCE OF VARIOUS PARAMETERS ON THE BIOLEACHING OF METALS – CRITICAL ASSESSMENT

In this section, published literature for the past 15 years have been reviewed, with respect to the bioleaching of selection metals, namely, copper and zinc (two of the most predominant metals found in PCBs), by various bacterial species.

A. Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans for the removal of copper and zinc

A. Influence of pH on removal of copper

The increase in optimum pH value (2.0) adversely affects the growth of Acidithiobacillus ferrooxidans [7] which the following experimental procedure shows it evidently that when the experiment was conducted only in acidic medium of pH 2.0 (initially adjusted) without microorganisms and ferrous ions shows lower leaching rate of copper of 21% at 120 hrs. But, when the experiment was conducted with microorganisms and ferrous ions in three

different pH such as 1.5, 1.7 and 2.0 with their corresponding copper leaching efficiency of 99.06%, 93.25% and 99% for the leaching period of 48 hrs, 48 hrs and 66 hrs respectively.

The performance of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* was studied [8] with respect to the addition of lower and higher particle size of PCBs with the corresponding change in pH. It is evident that at the higher particle size, the performance of *Acidithiobacillus ferrooxidans* is significantly much better with respect to the longer leaching duration. At the same time, the performance of the *Acidithiobacillus thiooxidans* was comparatively lower to obtain an optimum pH for the better recovery. At lower particle size the performance of the both *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* shows similar effects on change in pH with respect to their durations. A recovery performance of *Acidithiobacillus ferrooxidans* in nine days of leaching duration has been reported for the recovery of copper range from about 74.9 to 99.9% for 0.5 -1.0 mm of sieve fraction from printed wiring boards (PWBs).

The addition of particle sizes of 0.42 – 0.84 mm and greater than 0.84 mm shows a decrease in pH from 2.0 to 3.5 over a period of time. The above significant inter-relation was claimed due to the reasons such as oxidation of iron sources and alkaline property of PCBs. Further, a notable claim was made that there is no influence of culture medium pH on copper leaching efficiency [9].

The various dosage additions of inoculums such as 10%, 20%, 50% and 100% in bioleaching solution with respect to the duration, changes the pH values [10]. At the initial stage of process the value of the pH rose from 1.8-2.0 to about 2.2 – 2.4. Further, the addition of bacterial dosage of 50% and 100% shows noted level of growth and reasonable increase in pH which leads the bioleaching process in a notable way.

The influence of pH on single cultured and mixed cultured systems shows that the single culture of *Acidithiobacillus ferrooxidans* requires an optimum range of pH values such as 1.74, 2.56, and 3.51 where it can survive and leach the metals effectively [11]. For the single culture of *Acidithiobacillus thiooxidans*, requires an optimum range of pH values such as 1.42,

2.02 and 2.53. But for the mixed culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*, the pH values 1.38, 1.82 and 2.21 of the bioleaching system reduces the metal leaching efficiency of bacteria because of the lower pH when compared to the optimum range. It obviously, reveals the fact that single culture of suitable bacteria leads the bioleaching system in a better way.

The interrelated mechanism of parameters such as pH with their respective leaching duration and bacterial dosage reveals that each parameter is dependent parameters where their influence is high in bioleaching [12].

The interrelation between the energy source additions with the change in pH value gives an idea that the increase in dosage of elemental sulphur from 1.5 to 2.5% increases the acidic level of the bioleaching solution from 1.7 to 1.4 and 1.3 [6]. The path of H⁺ consumption for the recovery of metals through bioleaching includes direct and indirect consumption. In both the cases it is found that the H⁺ consumption also contributes to the mechanisms of oxidation process of ferrous by bacteria [1].

The alkaline nature of e-waste has a greater impact on maintaining the pH for the *Acidithiobacillus ferrooxidans* growth. The survivability of *Acidithiobacillus ferrooxidans* requires an acidic environment ranges from 1.3 - 2.5 of pH and it was maintained by adding the sulphuric acid externally which leads to a 90% copper recovery from waste electric cables. [13].

B. Influence of pH on removal of zinc

The change of pH i.e. from 1.8 – 2.0 to about 2.2 – 2.4 has been reported that the addition of various bacteria dosage such as 50% and 100% at the initial stage was a predominant reason [10], where the 10%, 20% of lower bacterial dosage requires longer adjustment in pH nearly 16 and 12 days. Whereas, in the 50% and 100% of bacterial dosage, it has been found that reasonable increase in pH which leads to the optimum recovery of zinc 57%.

The acidic pH range of 2-3 of bioleaching solution triggers the faster recovery of zinc such as 48% from e-waste rather than the higher removal efficiency has been reported, where 93%, 48.5% and 53% corresponding to the metals cadmium, nickel and copper for the same pH range. At the same time, chromium shows

significant removal efficiency even at the pH range of 6-8 [14].

C. Influence of temperature on removal of copper and zinc

The performance of the microorganisms *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* over temperature reveals that the optimum temperature for the growth of microorganisms were 32°C. The study was conducted in various temperatures such as 20°C - 50°C [15].

The increase in temperature leads to sustainable growth and recovery efficiency to some extent [13]. But it was surprising phenomenon happened due to the higher chemical activity independently in the presence of bacteria. Further, the study was conducted at 50°C to check the growth of microorganisms but there is no growth was found.

F. Influence of ORP on the removal of copper and zinc

At the initial stage of bioleaching process, it has been found that ORP was in lower level due to the growing stage of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. The increase in ORP was indentified with increase in leaching time. The both pH and ORP shows a similar performance trend for the single and mixed culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. The above said phenomenon contributes to the copper recovery of 99%, 74.9% and 99.9% for 0.5 to 1.0 mm particle size of PCBs for single culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* and mixed culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* [8].

A separate culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* shows that a significant increase in ORP in the bioleaching solution, but adding PCBs to the solution immediately decreased the ORP. Further it has been noted that the formation of sulphuric acid and oxidation of Fe^{2+} to Fe^{3+} indicates the growth of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* addition of high amount of PCBs. The performance of mixed culture shows a good response to the higher ORP, when compared to a single culture [11].

The parameters such as chemical ORP and biological ORP was studied comparatively with the addition of oxygen, Fe^{2+} and 10% and 20% of bacterial dosage. A separate culture of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* oxidizes the Fe^{2+} to Fe^{3+} initially, where the chemical process dominates the biological one in which, the rate of leaching of copper and zinc was slow which indirectly means the growing phase of bacteria. Further, the gradual increase in leaching duration strongly helps to increase the biological ORP from 390 mV to 610 mV which indirectly represents the microbial growth in bioleaching solution where the biological process dominates the chemical one in a longer duration [10], [12], [13], [16].

G. Influence of pulp density (PD) on the removal of various metals

The increase in an addition of amount of PD directly affects the growth of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* [7] and also it affects the production of organic acids from the above bacteria [3]. The addition of PD (PCBs) to the bioleaching process accelerates the pH from acidic zone to alkaline zone due to the alkaline nature of PCBs [9]. The limited availability of air distribution and oxygen mass transfer rate affects the growth of bacteria [17].

The addition of PD (PCBs) obviously forms the Fe^{3+} precipitate contributes to the lower metal recovery from the bioleaching solution. For the bioleaching of metals, *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* requires a complete acidic environment but the addition of PD (PCBs) may lead to alkaline environment which is primarily considered as an unfavourable condition for the oxidation of metals, as well as bacterial growth [11]. The formation of sulphuric acid is possible when *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* was used as a bioleaching agent. The formation of sulphuric acid is a fundamental mechanism that contributes to the leaching of metals such as copper, zinc, and lead etc [8].

H. Influence of metal dosage concentration (MDC) on the removal of various metals

Generally, metals acts as an inhibiting compound in the bioleaching process, but, the

intention of the study is to recover the same metals from the PCBs by using microorganisms. PCBs contain lots of metals combined. At the same time, the recovery of selective metals from the PCBs with selective single microorganism poses great challenge for the bioleaching of metals from E-waste [3].

I. Influence of particle size (PS) on the removal of various metals

The particle size such as > 0.84 mm, 0.42-0.84 mm and 0.25-0.42 mm were taken and added to the bioleaching solution. Further, the results possess the effect such as increase in value of pH from 2.0 to 3.5 for >0.84 mm PS at 24 hrs and it was 2.0 to 2.5 for 0.25 – 0.42 mm at a period of 24 – 48 hrs. The reason claimed for the increase in pH is the alkaline nature of PCBs which accelerates the pH from acidic zone to alkaline zone. The bigger particle size will not suddenly induce the alkalinity due to the low contact surface area. At the same time, it was found that 78-97% of copper leached from the size 0.42 – 0.84 mm the reason claimed for the maximum leaching efficiency was the surface contact area between bacteria and PCBs [9].

The performance study on particle size with dependent parameters such as bacterial growth and metal leaching efficiency was studied. A two size of particles such as 0.5-1.0 mm and 1.0-3.0 mm of 7.8 g/l of PD have added in the bioleaching solution which leads to the leaching of metals from PCBs. The leaching efficiency of copper such 89-94 % and 99-99.9% for the PS of 0.5-1.0 mm and 1.0 – 3.0 mm respectively was noted [8].

J. Influence of solid/liquid ratio (S/L ratio) on the removal of various metals

The increase in value of S/L ratio decreases the bacterial growth as well as metal leaching efficiency. The reason for the above said reduction is mainly due to toxic nature of the metals which induces the high inhibition to the bacterial growth and it was found by conducting the experiments that the 65% & 56% of cobalt recovery was achieved for 5g/L and 10g/L of S/L ratio respectively [18].

K. Influence of oxygen content (OC) on the removal of various metals

From the study, it was found that the optimum OC range was suggested that 0.29-0.7 mg/L for *Acidithiobacillus ferrooxidans*. The oxygen

content is a necessary parameter which influences the oxidation of Fe^{2+} to Fe^{3+} and for the metabolic activity of bacteria [13].

L. *Sulfobacillus thermosulfidooxidans*, *Thermobacillus acidophilum*, *Sulfobacillus acidophiles* for the removal of copper and zinc

L. pH and Temperature

The interrelated phenomenon between the pretreated electronic scrap (ES) such as washed ES, unwashed ES and washed ES with sulfur as energy source and pH of the bioleaching process. It was found that the pH of the unwashed ES rose up to 3.4 in 3 days and it was maintained to 2.0 after addition of sulphuric acid. For washed ES, the pH was not as high as in case of the unwashed ES. It was observed that the third condition, washed ES with sulfur, has not shown any increase in pH, due to the oxidation of sulfur. A mixed consortium of moderately thermophilic strains of acidophilic chemolithotrophic and heterotrophic (code A1 TSB) isolated from local environments established maximum metal leaching efficiency for copper (79%) and zinc (83%), apart from other metals investigated [19].

The Lack of growth of bacteria was identified at the initial stage i.e. up to 42 days with a pH of 2.0 but there was an increase in growth of bacteria mainly due to hydrolysis of metal ions by microbial action from 43rd day to 196th day at a pH of 1.6. At the final stage, i.e. from 196th day to 238th day, the pH slowly decreased from 1.6 to 1.5 and remained the same up till the end of the bioleaching study. The 86% of copper and 80% of zinc in PCBs after pre-treatment has been leached out when a mixed culture of the above consortium of bacteria, was used [20].

M. Influence of ORP

At the initial stage of bioleaching process, there was a lag in bacterial growth was found. From the 21st day, there was an increase in redox potential from 390 mV to 620 mV until 30th day, whereas, the pH reduced from 2.2 at the initial stage to 1.8 at 30th day. After the 30th day, consistent increase in metal extraction was observed, as well as increase in ORP from 620 to 647 mV by the 120th day. From the above study, it is observed that, the initial stage of bioleaching contributes to the growth of microorganisms, the second stage, as a transition period from the first to the third where the mixed culture starts to oxidize the iron compounds and grows; and the

third stage as a stage of metal extraction, where real bioleaching takes place [21].

N. Influence of pulp density, particle size and dissolved oxygen content on the removal of various metals

The bioleaching study was conducted with the various pulp densities (PD) such as 10-12% and 10-14% with the addition of biogenic sulphur as energy source. The metal leaching efficiency of 94% was achieved for the PD of 10-14% and it was 79% for the PD of 10-12%. The contribution of energy source towards the leaching of metals have been noted that the dosage of biogenic sulphur such as 2.5% help significantly but increase in dosage to 3.5 or 4.5 shows no increase in metal leaching efficiency [6].

The study on particle size was analysed to determine the relation between PS, bacterial growth and metal leaching efficiency. From the literature it was noted that removal efficiency has increased with decrease in particle size. It has been found from the experimentation that less than 75 μm of PS was considered as an optimum PS for the microbial growth and leaching efficiency. At the same time, a controversial aspect was found by author that the particle size of 250 μm to 150 μm , increased the metal removal efficiency instead of adopting lesser PS such as 270 μm to 48 μm . The reason for the above said phenomenon is the surface area of particles that gets increased when the PS reduced, where the bacteria can leach more efficiently due to the large surface area of particles. At the same time, there is no noticeable observation was found on the metal leaching efficiency and bacterial growth, if the PS were further more decreased below 150 μm [21].

The study on dissolved oxygen in the bioleaching shows that the DO content gets decreased with respect to the time. This was claimed due to the reason of oxygen deprivation caused by bacteria which further affects bacterial growth and oxidation of elemental sulphur. The addition of 10% of pulp density sustains the DO of 7.3-8.0 mg/L in the bioleaching [6].

O. Acidophilic bacteria, *Chromobacterium violaceum*, *Acidithiobacillus* sp., *Bacillus subtilis*, *Bacillus cereus* for the removal of various metals

O. Influence of metals dosage concentration, particle size, dissolved oxygen content on the removal of various metals

The experiment on MDC has studied to analyse the interrelated phenomenon with bacterial growth and metal leaching efficiency. The metal dosage concentration such 4g/L, 8g/L and 12g/L were used and their respective metal leaching efficiency of 97.5%, 93.1% and 94.2% were achieved for the period of 8 days. The study reveals that the higher concentration of metal dosage have not shown the favourable higher metal leaching efficiency, the reason claimed for the above said aspect is the higher MDC limited the air distribution, oxygen mass transfer and acts as an inhibitor for the growth of bacteria and indirectly influences the metal leaching efficiency of bacteria [22].

The relation between particle size and bacterial growth and metal leaching efficiency was studied with different size of particles such as 40-60 mesh, 60-80 mesh and < 80 mesh and their corresponding copper leaching efficiency such as 98.5%, 98.7% and 93.1% were attained for a period of 8 days. Significant phenomenon were claimed such as smaller particle size contributes to high metal leaching efficiency and at the same time, it was found that the very smaller particles may tend to attribute to higher attrition between two particles which causes cell damage to bacteria [22].

The performance of dissolved oxygen on bacterial growth and metal leaching efficiency was studied. The oxygen content is a primary physical parameter which contributes to the metabolic growth of bacteria as well as oxidation of energy sources. In this study, initially the oxygen content such as 6.8 mg/L was determined and used for the respiration. Further, it was tend to decrease up to 0.06 mg/L. The optimum pH for the gold and copper recovery was 11.0 and 10.0 noted when the oxygen content was 6.8 mg/L. The higher pH and oxygen content tends to higher gold recovery [4].

IV. CONCLUDING REMARKS

Based on the above comprehensive literature review, following are the critical observations / concluding remarks:

- 1) The overall performance of the bioleaching process, is an interdependent process with

- the following influential parameters such as pH, temperature, ORP, Pulp Density, Metal Dosage Concentration, Particle Size, Solid/Liquid Ratio, Oxygen Content, etc.
- 2) It is found that most of bioleaching process has been carried out using a variety of microorganisms for the recovery of metals such as: copper, zinc, nickel and Aluminum, gold (in that order of preference). Both isolated and mixed cultures have been used, but the use of mixed culture, is rather comparatively rare.
 - 3) pH is found to be predominant influential parameter in the bioleaching process. It directly influences the microbial growth, i.e. if the pH of a conducting liquid medium decreased from the optimum pH, it affects the total bioleaching process. At the same time, if the pH of a conducting liquid medium increased, instead of not maintaining the optimum pH, it affects the bacterial metabolisms such as: microbial growth, which is a direct influential parameter for recovery of metals from PCBs.
 - 4) The life system of microorganisms mainly concerned with the parameter temperature for its metabolic activities such as: intake of food, sustainability and growth. The range of temperature in which various families of microorganisms could survive that has been summarized, based on the published literature so far, will help to plan experimental programme in bioleaching. The other unique property of microorganisms is their high sensitivity to the temperature, thus necessitating continuous monitoring and maintenance to attain higher bioleaching efficiency.
 - 5) ORP is an indicative parameter, and it does not affect the bioleaching process directly, as is the case with pH & temperature. Increase in pH and temperature affects the oxidation-reduction potential of microorganisms.
 - 6) Particle Density (PD) is found to be predominant influential physical parameter in the bioleaching process. It directly influences the microbial growth, i.e. if the PD of PCBs increased over the optimum PD, it affects the total bioleaching process by means of bacterial metabolisms such as: microbial growth, which is a direct influential parameter for recovery of metals from PCBs and it affects the production of organic acids from microbes indirectly.
 - 7) The life system of microorganisms directly gets affected by the parameter - metal dosage concentration. If the MDC is increased in higher amount it will drastically affects the sustainability and growth of microorganism. Finding the optimum MDC for the every selected microorganism will help to plan experimental programme in bioleaching. The other unique property of microorganisms is their high sensitivity to the MDC, thus necessitating continuous monitoring and maintenance to attain higher bioleaching efficiency.
 - 8) Particle Size is one of the prime influential physical parameter where the smaller PS will help in higher metal leaching efficiency due to the larger surface area of particle (PCBs). Further, if the PS is too low then it is difficult to attain the higher metal leaching efficiency due to the attrition of two particles leading to no biological growth in the medium.
 - 9) Solid/Liquid Ratio is another important influential physical parameter that directly affects the bacterial growth and metal leaching efficiency. If the proportion of PCBs is not in the range to the liquid volume which trigger dissolution of metal in the liquid medium. Further it turns to be a potential toxicity to bacteria. Determining the optimum S/L ratio leads to better metal leaching efficiency and bacterial growth.
 - 10) The oxygen content of the liquid medium has to be properly maintained for the development of bacterial cell growth and oxidation of energy sources such as: elemental sulphur and ferric ions. The oxidation of energy sources is considered as a very important aspect for the development of bacterial cell and metal leaching efficiency.
 - 11) Application of bioleaching for removal of metals from PCBs is still in its nascent state, even in developed countries. In the case of India, systematic and comprehensive studies are required considering the economy of the country and the safety of millions of people who are indirectly involved in E-waste processing, in an unscientific manner,

thereby posing a grave threat not only to their health & but also to the environment.

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