THE TREATMENT OF HEAVY METAL POLLUTION BY RALSTONIA SP.

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Abstract

Since the human being started the industrial activities without focusing on their fate and wastes, the environments suffer from pollutions. A major one of them is the heavy metals pollution. The resources of heavy metal come from a variety of human industrial activities and wastes, including photographic industries, printing, pigment manufacture, pesticides, petroleum refining, paint manufacture, battery manufacturing, tanneries, textile, metal processing, electroplating and mining. Enzymes have been used as a biological treatment for heavy metals such as bioremediation, degradation and others. These enzymes have given a unique pattern to remove the heavy metal toxicity in different products. Microorganisms are the owner of enzymes and some of them are capable to grow up at heavy metal polluted environments. Furthermore, some microorganisms started to be active when finding metal ions in their growth conditions. For that, *Ralstonia spp.* is coming strongly due to its ability to solve and resist the heavy metals pollution in different ways.

Keywords: heavy metal, enzyme, microorganisms, Ralstonia spp.

I.The Effects of Metals in The Environment.

Nowadays, the pollution of Heavy metal is a critical problem in the environment. The resources of heavy metal into environments, including photographic industries, printing, pigment manufacture, pesticides, petroleum re fining, paint manufacture, battery manufacturing, tanneries, textile, metal processing, electroplating, mining, came from the industries products and waste discharges [1-3]. Then, these kinds of metal resources become a serious cause of pollution. Three types of heavy metals were considered. They are radionuclides (such as Am, Ra, Th, U, etc.), precious metals (such as Ru, Au, Ag, Pt, Pd, etc), and toxic metals (such as Sn, Co, As, Cd, Ni, Cu, Zn, Pb, Cr, Hg, etc.) [4]. Due to the disability of the heavy metals from organic wastes to degrade in the process of biodegradable and their ability to accumulate in living tissues, it might cause a wide types of disorders and diseases [5]. Heavy metals can cause reduction or damage to the blood composition, central and mental nervous functions , lower energy levels, liver, kidneys, lungs, and other organs [3].

In the near past centuries, the acceleration of industries and rapid development of globe, have increased metal utilizing and released into environment, altered the fresh water bodies. In aquatic system, the slow leaching from rock/ soil produce heavy metals to water naturally, which presented at low levels, that might lead to a serious effect on human health [2]. The developments in agriculture and industry indicate the fast increasing of the metal pollution in environment. Further, heavy metals, causing aquatic pollutions are usually appear as Ni, Zn, Cu, Cd, Pb, Cr, Hg, etc. in the system of water [6]. The wastewater arises from a paper making industry, curry, plating chemical plant, metallurgy, mill run, and mining. The compound of metal could be adsorbed on the suspended sediments and particles. Consequently, under suitable conditions such as Eh and pH values, the compound released again into water. Some of heavy metals such as Pb, Cu, Ni, Cd, Cr, Hg, etc. presented in water system could be posing high toxicities on the organisms of aquatic system [7].

The major sink of heavy metals is the soil that released to environment. Soil in industrial countries can be affected by organic refuses, mine waste, and acid deposition, as an example sewage sludge (released pollutants to soil) [8, 9]. Soil pollution level by heavy metals usually depends on soil retention capacity, particularly chemo-physical properties (organic matter, grain size, mineralogy)are influencing metal chemical properties and also particle surfaces of soil [8]. Further, soil components retained these metals in soil surface horizons or could be co-precipitated or precipitated such as oxides, carbonates, sulphides or hydroxides with Ca, Mn, Fe, etc. [10]. Trace metals mobility has reflected the passing ability between soil compartments where energetic boundless in the elements, soil solution is the ultimate compartment, which determines the bioavailability. Metals distribution among chemical forms or different compartments can use sequential extraction procedure for measures. The known contaminant methods that partitioned between different chemical forms allow a better perception into water quality and degradation of soil following the input of metallurgical plants and metals around mining. Therefore, heavy metals polluted soil that occur in both soil deeper layer and on the surface show a great concern to the control quality of environments. Pollutant content pattern by mixing processes is a synergistic result, including soil layers leaching irregularly (assisted by rainwater), sediments contaminated in deposition fluvial [11]. Heavy metals revealed a weakened impact from ore roasting and mining on soils via different processes such as soil compounds with complex formation, precipitation, and adsorption. Heavy metals with soils pollution are multidimensional. Large amounts of the soil upon entering, affect the biological primary characteristics by heavy metals: soil enzymes are capable of decreasing the activity and basic microbiology intensity processes, leading to reduction of species diversity, and the changes of microorganism total content. In addition to, pH of soils, structure, and the changes of humus contents by heavy metals. Ultimately, these processes conduct to a complete or partial loss soil fertility. Crops productivity have adversely affected by slight increase of contamination emission [12].

In an organisms life processes, metals have an integral and essential role such as zinc, nickel, sodium, manganese, magnesium, potassium, iron, copper, chromium, and cobalt. They functioned as micronutrients such as osmotic pressure regulation,

acting as various enzymes components, stabilizing electrostatic interactions molecules, and redox processes [13].Other metals, like, mercury, lead, gold, cadmium, and silver, without any biological function, are potential toxic and nonessential organisms. Further, nonessential metals could change essential metals by interaction with ligand conducting to toxicity or native binding sites [13, 14]. For example, Ag^{2+}, Cd^{2+}, and Hg^{2+} could bind to protein SH groups and make inhibition for enzymes activity [14]. Both nonessential and essential metal could (i) damage high concentration of DNA structure (ii) disrupt cell functions (iii) alter the specificity of enzymes and (iv) damage the membranes of cell [13]. Most of metals ions should entire the cells to be toxic. Many of metals divalent citations (e.g. Zn^{2+}, Cu^{2+}, Ni^{2+}, Co^{2+}, Fe^{2+}, and Mn^{2+}) show a resemble in the structure. The oxy-anions structure e.g. phosphate resembles that for sulfate, as well as arsenate and chromate. The uptake system of organism regulates tightly to make differentiation between metal ions structures.

II. Metal Resistant Enzymes

The enzymes used for the purposes of environments have increased recently due to peculiar properties and specificity of these classes of protein. Enzymes are specific catalysts, efficient, and versatile acting in living organism's chemical reactions. They have abilities to preform many of specific reactions via non reachable elevated rate by physical catalysis or traditional chemicals. Whitely et al found that enzymes playing a major role in the remediation of biology, enzymes processes are used to remove, transform, and target specific heavy metal (pollutants) in wastes [15]. The roles could range as natural processes e.g. intrinsic bioremediation that transform the toxic heavy metals to less toxic in a contaminated environment [16], to contaminate environment manipulation via adding bio-accumulated or biochemical additives or specific chemicals (all processes involving removal and presenting in living cells), enzymatic bioprecipitation of metal ions, accumulation, and degradation of organic pollutants [15, 17].

Also, enzymes can be quantified and detected by environmental status before, during, and after remediation-process. Furthermore, enzymes could be acting as a specific molecule sensors or its products could evaluate their concentration and amounts, and assess the contamination site restoration effectiveness. Moreover, they can be acting as biosensors and could provide simples, high sensitive assay to detect the initial indication for pollution of environment or obtain restoration [18-26]. Enzymes have another function which is the ability to act as bioindicators of water/sediment/soil health and quality. Furthermore, they can give more information than pollution impact in environment as that, they may do remediation treatment such as actual restoration [27-42].

Also, numerous of published reports have been supported [27, 29, 30, 33, 36-38, 43, 44] as enzymes which could be useful for knowing whether it is an estimation, how much and when in general an antropogenic-factors or a pollution with terrestric environments and/or aquatic healthy impaction. Indeed, enzymes could have a rapid change to ecosystem variations that can be measured easily leading to provide fast tools to trace the modification of environments.

Enzymes have three functions, first of them is behaving as a main effector for occurring the transformations and other is acting as verification of transformation occurred and the third one is knowing

how much and whether the substance-pollutants have been removed, the environmental pollution has been remediated sufficiently in term-of quality and health, and has initial regained properties.

For our knowledge, reviews or papers have found integration on enzymes roles as polluted-environment bioindicators, pollutant-assaying agents, and pollutant-degrading catalysts available in literature as M.A. Rao et al [45].

To improve the efficiency, effectiveness and applicability in pollutant transformation via enzyme-catalyzed processes, it is important to make activity on two enzymatic-properties: activity and stability. Fast and high transformation of target-substrate will be guaranteed by higher activity. Stability enhancement will allow a more operational life prolongation. As previous reports, the great concern of enzyme catalyzed process is the target enzyme stability [46-48]. Enzymes should have storage stability (maintaining the unaltered of catalytic activity until using in the process) and operational stability (maintaining the activity during the entire conditions or transformation for use) together. The stability of enzymatic-proteins have been increased by several methods [46-49]. They could act to enzymatic molecule directly, by molecular features changes, or could alter the environmental-conditions favourably where the enzymatic-reaction occurs. The enzymes involved catalytic-properties in both cases, positive or negative effects, may derive [46-49].

Also enzymes can monitor the water/soil pollutants and their restoration by different roles such as enzymatic biosensors and enzymatic (bio) indicators [45]. They play an important role in assisting the quantification and detection of pollutants amount and indication of the quality and health status of polluted resorted target-environment.

III. Metal Resistant Microorganisms

Ion imbalance is the heavy metal concentration major problem. Microorganisms have developed mechanism to fix ion imbalance problem by using two kinds of metal ions uptake systems. One is bacterial cytoplasmic across membrane by chemiosmotic gradient which is driven, unspecific, and fast. This mechanism is constitutively expressed by many of the substrates [14]. Uptake system second type is slower, high specificity, cell produce energy source via ATP hydrolysis in needing times, special metabolic situation or starvation [50]. Even microorganisms are nonessential metals with highly concentrations, have uptake system specificity and could transport across cells by unspecific system constitutively expressed.

Because, metal ions modification or degradation are impossible as organic compound, metal resistance system has six options for mechanisms- (i) reducing the cellular target sensitivity to metal ions (ii) detoxification the metal enzymes cation to lower toxic form (iii) using protein binding for sequestration of metal intracellular (iv) sequestration of the extracellular (v) activation of the organism cell to transport the metals away and (vi) using permeability barriers to exclude metals [13, 50, 51]. The mechanism of detoxification could be conducted against a single metal or assemble of chemicals related to metals. Further, the mechanisms of detoxification are variable. They depend on the kind of microorganism [50, 51]. Specific genes in majority of microorganisms are used for recognizing heavy metal toxic ion resistant and most of these genes found on chromosomes or on plasmids [14, 52]. Chromosomes are more different than plasmids based on the system

of metal resistance. The essential system for metal resistance is based on chromosomes. Plasmid systems are less complex than chromosome systems. Also, plasmid- encoded systems are the mechanism of toxic-ion efflux and reported as inducible [53-55].

Plasmid of bacteria has genes that can be resistant to metalloids and man toxic metals such as Zn^{2+}, T^{1+}, TeO_2^{-3}, Sb^{3+}, Ni^{2+}, Hg^{2+},Cu^{2+}, CrO_2^{-4}, Co^{2+}, Cd^{2+}, AsO_3^{-4},AsO^{-2}, and Ag^+. Also, on bacterial chromosomes there are related system such as efflux of arsenic in *E. coli*, efflux of Cd^{2+} in *Bacillus*, and resistant of Hg^{2+} in *Bacillus* [56]. Bacterial metal resistance in general could include that (1) the mechanism of resistance has efflux from enzymatic detoxification and the cells, (2) plasmids have resistance systems in all bacterial groups that have been tested and (3) high specific plasmid determined resistances [14, 56, 57]. Moreover, interactions of other less specific, such as sorption, could help to response for the over all. Chromosomal and plasmid systems can determined efflux pumps (are either chemiosmotic or ATPases) that may contribute to show a similarity in a variety of bacteria. The resistance of Cd^{2+} may include (1) Gram- positive bacteria efflux ATPases, (2) Gram- negative bacteria cation H^+ antiport, and (3) cyanobacteria intracellular metallothionein [56, 58]. Gram- negative bacteria arsenic resistant have an arsenate reductase and arsenite efflux ATPase (arsenate [As (V)] reduce to arsenite [As (III)]) which involve the mechanism of biochemical underlying [59, 60]. Hg^{2+} resistance has similar systems that occur on plasmids of bacteria from Gram-negative and Gram-positive with genes component that involved in the transportation from Hg^{2+} to the detoxifying enzymes (reductase of mercuric) which reduce Hg^{2+} to Hg^0 element [56, 61].

C-Hg bond broke by organomercurial lyase enzyme [62]. In polluted habitats, Hg^{2+}-resistant bacteria with high frequencies selecting contaminating mercury [56, 63]. Chromate resistance by plasmid determined decreases to Cr (III) and unconnected with chromate [Cr (VI)] [64-66], resistances depending on the reductase of CrO_2^{-4} uptake [66]. In Gram-positive bacteria as *Bacillus* spp. widely found Cd^{2+} efflux ATPase [57]. *Alcaligenes eutrophus* large plasmids have a numerous resistant determinants of toxic metals such as two divalent cations, *cnr* (Ni^{2+} and Co^{2+} resistance), *czc* (Co^{2+}, Zn^{2+} and Cd^{2+}), one for Cr^{6+} ,and three Hg^{2+} [56, 67, 68]. The functions of czc like divalent-H^+ antiporter of chemiosmotic [50]. In *Enterococcus hirae*, there are two genes, *copB* and *copA* that efflux P-type ATPase and determine uptake respectively, can determine copper resistance [69]. The descriptions of *Pseudomonas* including plasmid-determined Cu^{2+} resistances [70, 71]with *Escherichia coli* [72], and *Xanthomonas* [73, 74]. Cu^{2+} resistance and transport the chromosomal affected genes via determining functions e.g. intracellular Cu^{2+} binding, efflux and uptake [75].

Clear understanding of living cells with metal toxicity mechanisms may mitigate such toxicity by development of novel technology. Studying a useful models of microorganisms by oxidative stress of various aspects at cellular level, molecular, and biochemical. The factors of natural stress and the damage caused via oxidative stress to lipid, protein, nucleic acids, and other components of cell, are same as all organisms' types. This right due to cell organization at all levels, the oxidative stress against cellular defense are similar e.g. to decrease ROS concentrations by performing the role and nature of antioxidative and antioxidants enzymes and protein,

damaged macromolecules repair, irreparable protein elimination [76]. Numerous of microorganisms have also developed complex mechanisms to act against metals toxic effects [56]. Thus fungi, algae, yeast, and bacteria have been isolated from and located in heavy metal with contaminated site, and they are putative strongly to environmental contamination bioremediators [77-79]. In metal-polluted locations with toxicity, numerous of microbes flourish and grown, and mechanisms-variety, both incidental and active, resistance contribution [80-84]. Microbial resistant spreads widely in metal toxic environments, with different ranging between 100% of heavy-metal environments until a few percentage of pristine-environments [85].

IV. Ralstonia sp. Metal Resistant.

In fact, 40% of isolated bacteria on nonselective media as heavy metal resistant from β-Proteobacteria *Ralstonia spp.* [86]. *Ralstonia sp.* are defined as a heavy-metal resistant bacterial strain that consider in extremophiles as metallophiles.

Ralstonia sp. capable to grow in media containing up-to 5mM Cd^{+2}, 20mM Co^{2+}, or 20mM Zn^{2+} [87]. The ability of genetics to survive heavy-metal concentration on plasmid (pMOL30), and designed czc as cobalt-zinc-cadmium resistant [88]. The description of czc system is the detoxification of cells via cation efflux. Heavy metals have 3 cations that used for transport system to take-up into the cell, which is fast and unspecific [89], and extruded out of the cell by product of the czc-resistance determinant [90].

The real efflux-protein is composed by 3 subunits, CzcA, CzcB, CzcC [90]. The complex of Czc efflux driven by chemiosmotic gradient, and not ABC-ATPase [50]. CzcA is RND-protein [91], and considered in the Czc complex as the only single protein, which has further than single transmembrane α-helix and cation proton-antiporter function [92]. The CzcA is located in the periplasm and has 2 large hydrophilic domain as CzcB, and CzcC subunits [93]. Although CzcA alone resulted to a weak metal resistant, it needs CzcB and CzcC for full function and the $CzcCB_2A$ complex may be capable to transport the toxic across the cell wall membrane of complete Gram-negative from cytoplasm through the cell wall to the outside of the cell. The $CzcCB_2A$ efflux complex amount is tightly regulated in the cell. Six regulated proteins, an unknown sigma factor (RpoX), CzcI, CzcN, CzcS, CzcR, and CzcD, are involved in 3 structure-genes *czcCBA* regulation, which have been transcribed as a pentacistronic-*czcNICBA* mRNA, a tetracistronic- *czcNICBA* mRNA, or a tricistronic-*czcCBA* [94, 95].

In addition, there are some other systems for heavy metal resistant presented in *Ralstonia sp.* In *cnrCBA* gene structure of cobalt-nickel-resistance system *cnr* [96] have *czcCBA* a relationship. Furthermore, the determinant of *cnr* has been located in *Ralstonia sp.* on second megaplasmid, pMOL28 [97]. The form of $cnrCB_2A$ is like a transmembrane efflux-pump for Ni^{+2} and Co^{+2}. However, *cnr* regulation is different from *czc* regulation. The location of ECF-sigma factor gene (*cnrH*) is on *cnrC* upstream directly and transcribed in *cnrCBA* the same direction.

V. Conclusion

This review presents the problem of heavy metal in environments, resources, and treatments by enzymes, which came from microorganisms. Also, it explains the microorganisms that can be able to survive, resist and remove the heavy metal toxicity. Then gives in details a clear and strong candidate to solve the

pollution of heavy metals. Finally, it has been concluded that *Ralstonia sp.* is considered to be a candidate of heavy metal treatment and resistance.

VI. Acknowledgment

VII. References

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