Bacterial exploitation for heavy metal bioremediation and sequestration from wastewaters

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This PhD project has the purpose to study bacterial challenges to heavy metals (HMs) through the implementation of microbial resistance mechanisms for the decontamination of industrial and the subsequent recovery of HMs. It consists in an eco-friendly biotechnology which will permit to convert a waste product into a valuable one, since the metal-organic complexes can serve as commercial salts or catalysts. The final aim is to design a process that has a lower water footprint and fits in the concept of circular economy.

Biorisanamento e sequestro di metalli pesanti da acque reflue tramite l’utilizzo di batteri

Il presente progetto di dottorato si propone di studiare la capacità batterica di resistenti ai metalli pesanti tramite l'implementazione di meccanismi di resistenza microbica per la decontaminazione di acque reflue industriali e il successivo recupero di metalli pesanti. Si tratta di una biotecnologia eco-friendly che permetterà di convertire un prodotto di scarto in un prodotto di valore, poiché i complessi metallo-organici possono essere utilizzati come sali commerciali o catalizzatori. L'obiettivo finale è elaborare un processo che abbia una minore impronta idrica e si inserisca in un concetto di economia circolare.

# **1. State-of-the-Art**

Over the last few decades, several industrial processes such as industrial welding, dyes and pigments manufacturing, electroplating processes, leather tanning, agricultural activities, and wood preservation, have been considered the major causes of HMs water pollution (Singh *et al.*, 2023). Such contaminants deteriorate water quality, with a negative impact on human health and ecosystems, due to their highly toxic, non-biodegradable and persistent nature (Nyika *et al*., 2022). The HMs that cause harmful impacts include, among others, chromium (Cr), copper (Cu) and nickel (Ni). These metals are responsible for reducing profitability and causing damage also to the agricultural systems (Yaashikaa *et al*., 2022). The United Nations world water development report 2021 claimed that around the world, 80% of all the industrial and municipal wastewater is directly released into the water ecosystems without any pre-treatment, leading to severe adverse effects on the environment (Jain *et al*., 2022).

Among possible treatment strategies, the use of microorganisms represents a suitable solution (Jain *et al*., 2022). Their resistance mechanisms can be divided into: i) generic stress-related, and ii) HM-dependent ones. To the first one belongs the production of exopolymeric substances (EPS), outer membrane binding, precipitation as salts and sequestration by stress related peptides like glutathione. The second group includes periplasmatic accumulation and HM subsequent transformation to insoluble compounds by metallothionein-mediated transport, enzymatic activities of efflux pumps, and specific oxido-reduction mechanisms to transform metals into harmless substances. Moreover, HMs can undergo dissimilative processes when they are used as electron acceptors/donors for microbial metabolic purposes to produce energy (Nyika *et al*., 2022). However, there is still a gap of knowledge regarding the specific interactions metal-microorganism, in particular regarding interactions in multimetal contaminated environments. Furthermore, there are several challenges for large-scale industrial bioremediation application which feasibility needs to be explored. In addition, the optimal environmental conditions to enhance bacterial growth, the operating conditions and mechanisms of removal need to be critically evaluated (Jain *et al*., 2022).

Mechanisms of wastewater decontamination exploit HM bindings with EPS bacterial surface (Singh *et al.*, 2023). EPS functional groups such as hydroxide (-OH), carboxylate (-COO), amino (-NH), and carbonyl (C = O), help to bind to HM cations, removing them from the contaminated solutions (Priyadarshanee *et al.,* 2021). A technique used to evidence the presence of EPS-producing cells is flow cytometry (FC), based on the specific interaction of EPS carbohydrates with lectins labelled with fluorophores (Hendrickson *et al.,* 2019).

EPS is one of the significant components of biofilms which are clusters of microbial cells attached to a substratum and embedded within a matrix of EPS. The formation of bacterial biofilms can be employed in water treatments since the toxic metal ions can be entrapped within the biofilm. This technique has lately gained attention owing to its high microbial biomass density and immobilization capability, as well as more adhesive properties. The biological activation of adsorbing biomaterials (*i.e*. agro-wastes, biochar, activated carbon, lignite) with specific metal-removing bacterial strains can lead to the formation of microporous microcarriers activated with bacterial biofilms. These systems are characterized by selective HM extraction while providing cells with high resistance to environmental stress (Priyadarshanee *et al.,* 2021). The material used for adsorption should be easily available, non-toxic, and cost-effective (Manikandan *et al.,* 2023). In addition to the above-mentioned technology, bacteria may form biofilm on the anode of microbial electrochemical systems (MES), considered innovative tools of wastewater treatment and bioremediation of HMs (Sivasankar *et al.,* 2019).

The focus of the present research will be to characterize HM resistant bacterial strains to provide insights for their possible exploitation in metal removal and recovery from industrial wastewaters. The present research will contribute to lower the industrial water footprint since the treated waters will re-enter the production cycle.

# **2. PhD Thesis Objectives and Milestones**

The objective of the present PhD study is to test isolated bacterial strains for their feasibility to remove HMs from industrial wastewaters. According to the Gantt diagram given in Table 1 the activities of this PhD project will be subdivided as follows:

A1) ***In vitro essay*** will be set to test isolated bacterial strains in biofilm and planktonic experiments in the presence of HMs such as Ni(II), Cu (II) and Cr(VI) that will be determined by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS) and spectrophotometric methods. Subsequently, trials on real HM contaminated electroplating wastewater will be conducted to assess adsorption and desorption kinetics and metals recovery from bacterial biomass.

A2) ***Characterization of mechanisms in HM/microbe interaction*** which will be assessed through i)EPS compositional characterization by nuclear magnetic resonance (NMR); ii) interaction between EPS components and specific lectins by FC; iii) definition of HMs detoxification pathways.

A3) ***Assessment of HM biofiltration unit*** through microporous microcarriers activated by bacterial biofilms monitored through lectins labelled with fluorophores.

A4) ***State of the art, dissemination, and thesis preparation*** by scientific literature revision, manuscript preparation and oral/poster communications to national/international conferences.

***Table 1***Gantt diagram for this PhD thesis project.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Activity/Months | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** | **11** | **12** | **13** | **14** | **15** | **16** | **17** | **18** | **19** | **20** | **21** | **22** | **23** | **24** |
| A1) | ***In vitro essay*** |  |  |  |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 1) Biofilm and planktonic experiments on Ni(II), Cu (II) and Cr(VI) |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
|  | 2) Adsorption/desorption kinetics |   |   |   |  |  |  |  |   |   |  |  |  |  |   |   |  |  |  |   |   |  |  |  |  |
|  | 3) Metal recovery from biomass |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |   |  |   |   |   |  |  |  |  |
|  | 4) Trials on real electroplating wastewaters |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Characterization of mechanisms in HM/microbe interaction***  |   |  |   |   |   |   |  |   |   |   |   |   |  |   |  |  |  |  |   |   |   |   |   |  |
|  | 1) ESP (NMR and flow cytometry) |   |  |   |   |   |  |  |   |   |   |   |  |  |   |  |  |  |  |   |   |   |   |  |  |
|  | 2) HMs detoxification pathways  |   |  |   |  |  |   |  |   |   |  |  |   |  |   |  |  |  |  |   |   |  |  |   |  |
| A3) | ***Assessment of HM biofiltration unit*** |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
|  | 1)Active biofilm on microporous microcarrier |   |  |   |  |  |   |   |   |   |  |  |   |   |   |  |  |  |  |   |   |  |  |   |   |
| 2) International internship |   |  |   |  |  |  |  |   |   |  |  |  |  |   |  |  |  |  |   |   |  |  |  |  |
| A4) | ***State of art, dissemination, and thesis preparation*** |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |   |  |   |   |   |   |   |   |   |

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