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Chapter

Treatment of Solid Waste Containing Metals by Biological Methods

Marlenne Gómez-Ramírez and Sergio A. Tenorio-Sánchez

Abstract

Methods for the treatment of hazardous wastes are based on two main approaches: either hydrometallurgy or pyrometallurgy. Biological methods are considered viable environmental-friendly technologies and have been developed in the last years and have been associated with lower cost and energy requirements, in comparison with nonbiological processes. In these methods, it is important to find suitable microorganisms to degrade organic substances under favorable conditions to complete the treatment. The advantages of biotechnological treatment of hazardous wastes are biodegradation or detoxification of a wide variety of hazardous substances using natural microorganisms, as well as the availability of a wide range of biotechnological methods for the total destruction of these wastes without the production of secondary hazardous derivatives. However, to intensify the biological treatment, it is a necessary requirement to add nutrients and acceptors of electrons, including the control of the optimal conditions. Thus, biotechnology provides a solution for the ecological degradation of harmful heavy metals and toxic chemicals. The main purpose of this chapter is to present and discuss the biological methods used in the treatment of solid waste containing metals and the advantages and disadvantages of each method.

Keywords: solid waste, metals, biological methods, bio-treatment

1. Introduction

1

The fast-developing of industries, such as mining, smelting operations, farming, energy stations, processing in refineries, coal burning in power plants, petroleum combustion, nuclear power stations and high tension lines, plastics, textiles, microelectronics, wood preservation, paper processing plants, and agricultural and anthropogenic activities, generally use metal-containing compounds which, due to the inappropriate waste disposal practices, have contributed to the contamination of soil and water with organic compounds and heavy metals with permanent toxic effects on ecosystems and humans [1, 2]. To counteract the effects of such contaminants, several methods and techniques have been implemented, each having its advantages and disadvantages. Currently, there are various types of waste contaminated with metals, and various treatments are applied depending on the type of waste to be treated, among them are as follows:

- **A.** *Domestic agricultural and industrial water*: the conventional techniques for removing dissolved heavy metals include chemical precipitation, carbon adsorption, ion exchange, evaporations and membrane processes, electrodialysis, and photocatalysis [3, 4].
- **B.** Contaminated soils: remediation techniques such as excavation, soil leaching/acid extraction, and soil washing are inadequate, costly, and often involved the storage of contaminated effluents in designated areas. The use of plants in metal extraction (phytoremediation) has appeared as a safe and cost-effective alternative in the removal of heavy metals excess from soil and water [1].
- **C.** Municipal solid waste (MSW): it is a complex material, which varies greatly in composition. In most of countries, solid wastes in land fill (open dump sites) are the most common means of disposal [5]. Lack of MSW management and disposal is leading to significant environmental problems. This includes soil, air water, and esthetic pollution. Such environmental problems are associated with human health disorder [6]. Composting is one of several methods for treating biosolids. Compost production is normally produced by two methods, an aerobic process and anaerobic pre-treatment of MSW followed by an aerobic curing step. There are many methods for removal of metal and toxic elements from soil and compost such as hydrothermal; subcritical water treatment; chemical leaching using inorganic mineral acids like sulfuric acid, hydrochloric acid, and nitric acid; or use of chelating reagents like nitrilotriacetic acid (NTA), ethylenediaminetetraacetic acid (EDTA), and diethylenetriaminepentaacetic acid (DTPA). Alkaline solutions like ammonium and sodium hydroxides are also sometimes used. However, these treatments have some common disadvantages such as high cost and generating potential toxic by-products [5, 7]. On the other hand, the compost and sewage sludge additions to agricultural and other soils, with background concentrations of heavy metals, raise the soil content and the availability of heavy metals for transfer into crop plants [8].
- D. Hazardous waste: it includes waste batteries, electronic waste, waste X-ray films, fly ash, petroleum spent catalyst, and metal finishing industrial waste. Several technologies are used for the treatment of this kind of industrial waste; these are pyrometallurgy, hydrometallurgy, and bio-hydrometallurgy. Pyrometallurgical recovery consists of the thermal treatment of ores and metal containing wastes to bring about physical and chemical transformations. The hydrometallurgical recovery uses mainly the leaching process, by using aqueous solutions containing a lixiviant brought into contact with a material containing a valuable metal. The leached metals are concentrated and purified by using precipitation, cementation, solvent extraction, and ion exchange [9].
- **E.** Landfill mining: in many regions of the world, landfills have long been seen as a final way to store waste at minimum cost. Landfill mining has been suggested as a strategy to address such problems and in principle means the excavation, processing, treatment, and/or recycling of deposited materials [10].

Given the increase in the generation of waste contaminated with metals, strategies have been sought in which microorganisms are small factories for the transformation and/or decontamination of the waste through different mechanisms of each microorganism, thereby reducing the metal load in the residue, changing the oxidation state of the metal by making it less toxic, or recovering it either soluble

or insoluble for reuse. Some metal tolerant microorganisms have the potential to be used in biotechnological processes for the recovery of valuable metals [11]. The bacteria have developed various resistance mechanisms to tolerate the harmful effects of toxic metals and have been abundant on the planet earth, and microbes have been exposed to them since basically the beginning of life, nearly 4 billion years ago [12]. Among them are mainly those that involve (1) cellular components that capture ions, neutralizing their toxicity, (2) enzymes that modify the redox state of metals or metalloids, turning them into less toxic forms, and (3) expulsion of metals or metalloids from cytoplasm through membrane-located transporter proteins [13].

The sections covered in the chapter are as follows:

- 1. Introduction
- 2. Biological systems for the treatment and recovery of metals.
- 3. Microorganisms with potential for the treatment and recovery of metals.
- 4. Treatment of spent catalysts of the petrochemical industry by microbial route.
- 5. Conclusion

2. Biological systems for the treatment and recovery of metals

Biotreatment is a biological process in which live, dead microorganisms or metabolites produced by them are used such as enzymes, biopolymers, siderophores, organic acids, inorganic acids, and biosurfactants. To eliminate pollution caused by metals or other contaminants, defense mechanisms are activated to detoxify their environment causing a transformation of the contaminant into less toxic compounds or the internalization of the contaminant inside the microbial cells [14, 15].

To understand and know the type of mechanism that each microorganism will use depend on how they interact with the environment and the contaminants; some factors are (1) microbial specie, (2) microbe-metal interactions, (3) growth medium composition, (4) pH, (5) temperature, (6) contact time, (7) oxygen, (8) osmotic pressure, (9) culture age, (10) microbial tolerance, (11) population density, (12) chemical and metal composition of solid wastes, (13) pulp density of waste, (14) size of particle of solid waste, (15) oxidation state of metals, and (16) presence of other toxic compounds [16–21]. The isolation of microorganisms from contaminated environments has led to finding microorganisms adapted to them, which have also developed certain metabolic strategies to detoxify their environment and, which are used for the treatment of different types of waste. The solid wastes generated from agricultural, electronic scraps, medical activity, metal finishing industry, industrial effluents, auto catalysts, manufacturing and recycling of batteries, fly ash, mining tailing, spent catalyst by petrochemical and petroleum refining industry mostly contain Ag, As, Ba, Be, Cd, Co, Cu, Fe, Li, Mo, Mg, Zn, Cr, Hg, Ni, V, Pb, Se, Zn, Ti, and so on and precious metals such as Au, In, Ag, Pd, Pt, and so on [9, 16–18, 20, 22–27]. Due to high metal content, waste containing metals are considered as artificial source of minerals and valuable metals that can be recovered [9, 18, 22, 23]. During treatment of solid waste, generally a low pulp density is used with ranges between 0.01 and 10% (w/v) and rarely higher than 16-80% (w/v) [19, 21, 22, 24, 26, 27]. While in biohydrometallurgy of low-grade ores, pulp density is generally 10% or higher

because the ores are mainly reduced sulfides ores, which do not contain alkaline matter or toxic compounds that could inhibit the microbial growth or production of metabolite of interest to metal removal [16, 17].

Adapted microorganisms are used to carry out these biotreatments, or they are adapted to the characteristics that the pollutant presents (high concentration and variety of metals); however, in works related to the recovery of contaminated sites, the use of metabolic activity of microorganisms such as bacteria and fungi,

Biological process	Process description
Bioadsorption	The union between the microbial biomass and the metal can occur at an extracellular level and is carried out by coordinated complexes. The microorganisms used as biosorbents retain heavy metals on the surface of the cell when they come into contact with the metals through ionic interactions between them and the cell wall. The microbial cell can be alive or dead, and energy expenditure by the microorganism is not required [35]
Bioabsorption	The intracellular accumulation of a metal occurs in two stages: process of adsorption of the metal and subsequently the transport of the metal into the cell by an active transport system. The bioaccumulation processes require the metabolic activity of the cells; they also involve a transmembrane transport system, which is responsible for letting the captured metal into the cell wall or membrane inside the cell, once it was incorporated, the metal can remain sequestered by specific genetically encoded proteins, so it is required to expose the cell to metal ions to induce their expression [36]
Bioleaching/mobilization	It uses the ability of a variety of microorganisms (bacteria and fungi) to mobilize and leach metals from a solid matrix based on three principles: (1) the production of organic and/or inorganic acids; (2) through oxide-reduction reactions; and (3) secretion of complexing agents (siderophores, lipopeptide biosurfactants). The microorganisms used are autotrophic, heterotrophic bacteria as well as fungi. These microorganisms are capable of producing organic and inorganic acids, and the processes can be carried out directly, using the microorganism and its by-products, or indirectly in which only the acid or metabolite produced by the microorganism is used [21, 37–44]
Membrane transporters expelling harmful species from the cell cytoplasm	(1) Cation diffusion facilitators (CDF) are proteins that are distributed in the three domains (Bacteria, Archaea, and Eukarya). They generally not only transport zinc but can also expel other cations such as Cd, Co, Ni, and Fe. (2) P-type ATPases constitute a superfamily of metal transporters that are energized by the hydrolysis of ATP. They are widely distributed in the three domains of life, and their substrates are ions such as H ⁺ , Na ⁺ , K ⁺ , Mg ²⁺ , Ca ²⁺ , Cu ⁺ , Ag ⁺ , Zn ²⁺ , and Cd ²⁺ . This type of ATPases is located in the inner membrane and can transport ions into the cellular interior, commonly physiological ions such as Mg ²⁺ , or function as expulsion systems, removing toxic metals to the periplasmic space. (3) RND proteins, these are involved in resistance, nodulation, and cell division processes in different bacterial species, have only been identified in bacteria. RND proteins that participate in the expulsion of metals are commonly associated with a pair of auxiliary polypeptides: a small outer membrane protein and a periplasmic protein that binds (or fuses) to the inner and outer membranes [11, 45, 46]. Anion ejection systems In this group, there is a system of expulsion of toxic inorganic ions that use transporters to expel arsenic and chromium oxyanions. This system can work in a dual way: driven by the hydrolysis of ATP or by a chemiosmotic process [11].

Table 1.Biological mechanisms involved in the removal of metals.

isolated from environments exposed to metals has been proposed [28]. This condition allows to obtain microorganisms adapted and/or resistant to metals of interest, whose metabolic activity could favor the mobilization and/or immobilization of metals from a contaminating matrix; this is through the accumulation or adsorption of metals by biomass or the production of metabolites such as organic and mineral acids, chelating agents (siderophores and biosurfactants), and enzymes [15]. **Table 1** shows some of the mechanisms used by microorganisms for the removal of metals. In these processes, the *cell wall* plays the main role since it is the first one that comes into contact with the metal ions, and these are deposited on the surface or between the cell wall structures, which, depending on the type of cell, will contain functional groups such as carboxyls, phosphonates, amines, and hydroxyl groups, among others [29]. These metal ions will be attracted by the negatively charged sites of cellular components. The cell wall in Gram-positive bacteria has a thickness of 20-80 nm and is made up of peptidoglycan or murein and is located on the plasma membrane, in which there are a lot of teichoic acids, which are polymers of glycerol or ribitol linked to a phosphate group, which are attached to the peptidoglycan layer by covalent bonds with the acid of acetyl muramic acid (6-hydroxy-N-acetyl-muramic) [30, 31] also called lipoteichoic acids because they leave the cytoplasmic membrane and have a negative charge. Teichoic acids extend over the entire surface of the peptidoglycan, and given their negative charge, they give the cell wall of the bacterium its net negative charge, being the teichoic and lipoteichoic acids the ones that participate in the entrapment of metal ions on the surface of the cell wall. The Gram-negative cell is a bit more complex, in which the peptidoglycan layer (cell wall) has a thickness of 2–7 nm surrounded by an outer membrane of 7–8 nm, and the peptidoglycan is between the plasma membrane and the outer membrane, which is composed of phospholipids, lipopolysaccharides (LPS), enzymes, and other molecules such as lipoproteins. The polysaccharide chains constitute the O-antigens of the Gram-negative bacteria; the lipopolysaccharides (LPSs) are formed by lipid chains and carbohydrates; these LPSs are joined to the outer membrane by ionic and hydrophobic interactions; the groups of LPSs and phospholipids have a net negative charge, so that they are attributed to the cell surface charge of Gram-negative cells; and these are the primary sites of interaction with metal ions [30, 31]. Due to these characteristics, bacteria can be used as biosorbents; in addition to that, their small size and rapid growth ability under pre-established conditions (temperature, pH, nutrients, aeration, etc.) allow the recovery of various metals or specific metals depending on the conditions used and the type of microorganism. Some species like Bacillus [16, 22, 32], Pseudomonas [33], Streptomyces [34], and Microbacterium [24, 27] have already been tested for the recovery of some metals such as Cr (VI), Cu, Cd, Fe (III), Pb, Hg, Ni, Zn, Pd, Pt, Th, U, and V.

3. Microorganisms with potential for the treatment and recovery of metals

Currently, there are commercial systems of biorecovery of metals that use different biosorbent matrices, among which are AlgaSORB™, AMT-BIOCLAIM™, BIO-FIX®, B.V. SORBEX, BIO-FIX®, MetaGeneR, and RAHCO Bio-Beads; the first uses a biosorbent material based on algae *Chlorella vulgaris* with a thickness of 1–3 mm consisting of an immobilized biofilm on a silica-gel matrix and is the most popular of these sorbents; this biological ion-exchange resin was able to bind both metallic cations and metallic oxoanions and could be competitive to commercial ion-exchange resins. The second consists of a biosorbent material based on

immobilized *Bacillus subtilis* cells on extruded beds of polyethyleneimine (PEI) and glutaraldehyde, removes metals ions from wastewater, and recovered precious metals [47, 48]. Several works have reported improvement of the adsorption capacity of biosorbents after immobilization of microorganisms on matrices, and some of them are *Aspergillus niger*, *Rhizopus nigricans*, *Trichoderma viride*, *Pseudomonas fluorescens*, *Microbacterium oxydans*, *Cupriavidus* sp., *Sphingobacterium*, *Bacillus* strain CR-7, *Bacillus subtilis*, *Candida albicans*, *Saccharomyces cerevisiae*, *Saccharomyces uvarum*, and *Saccharomyces lipolytica* [47, 48].

The siderophores are molecules produced by some microorganisms and have been used to reduce the level of metal contamination in the environment specifically from soil and water. The siderophores are low molecular weight (<10 kDa) iron chelating compounds synthesized by many bacteria of which can be mentioned *Pseudomonas, Azotobacter, Bacillus, Enterobacter, Serratia, Azospirillum,* and *Rhizobium* [43, 44, 49] and are extremely effective in solubilizing and increasing the mobility of a wide range of metals such as Cd, Cu, Ni, Pb, Zn, and the actinides Th(IV), U(IV), and Pu(IV) [44]. This ability of siderophores mainly depends on their ligand functionalities, by which means siderophores may have a strong affinity or selectivity for a particular metal other than Fe with regard to the stability constants of this metal-siderophore complex [43].

However, bioleaching processes are the most reported for the treatment of metal contaminated waste or for the recovery of metals. The genus of *Acidithiobacillus* is the most reported autotrophic sulfur-oxidizing bacterium for metal solubilization, particularly because it has been able to tolerate high concentrations of heavy metals. Obtaining the energy required for its metabolism is received through aerobic oxidation and the reduction of sulfur compounds, including sulfides, elemental sulfur, thiosulfates, and Fe²⁺, producing H₂SO₄ [38, 42, 50, 51]. During bioleaching, several mechanisms are involved, including (1) acidolysis, (2) complexolysis, (3) redoxolysis, and (4) bioaccumulation. However, operating costs are higher for fungal leaching (by heterotrophs) than bacterial leaching (by autotrophs) due to the need for an organic carbon source for their growth and organic acid excretion [5].

In the bioleaching, once the acid is produced in a medium, it favors a decrease in pH and creates a highly protonated medium (H⁺) in which a series of electrochemical reactions are carried out, where solid compounds are transformed into soluble and extractable forms and subsequently can be recovered [5, 41]. There are iron-oxidizing species within Acidithiobacillus genus, as Acidithiobacillus fer*rooxidans*, which obtain their energy by oxidation of Fe^{2+} to Fe^{3+} [38]. In addition to Acidithiobacillus genus, some fungi, as Aspergillus niger [39, 41] and Penicillium simplicissimum [40], have been studied for the production and secretion of organic acids such as oxalic, malic, gluconic, and citric acid. The production of these acids involves a large number of enzymatic reactions, for example, gluconic acid is produced extracellularly in two steps, and the glucose in the medium is oxidized in a glycolysis process mediated by glucose oxidase. The secretion of these acids by the cell also lowers the pH in the medium, protonating it, causing the solubilization of metals from a solid matrix to the liquid medium. Given the solubility characteristics of metals in these acidic media, leaching processes have been successfully applied since the 1980s in large-scale treatments for the recovery of metals such as Ni, Co, Zn, Mo, V, Cd, Al, Cu, V, Fe, and Mn, from solid waste using microorganisms listed in **Table 2** [5, 25, 26, 38, 39, 40, 41, 51].

Indirect bioleaching is mainly used in industrial applications since it is believed to be the most appropriate for increasing the efficiency of leaching processes, avoiding toxicity problems toward microbial cells by being in the presence of the solid waste. However, some authors mention that the presence of cells and metabolite produced increases the percentages of metal recovery compared to

Bacteria and archaea	Fungi
Acidithiobacillus albertensis	Aspergillus flavus
Acidithiobacillus caldus	Aspergillus foetidus
Acidithiobacillus ferrooxidans	Aspergillus niger
Acidithiobacillus thiooxidans	Penicillium chrysogenum
Ferrimicrobium acidiphilum	Penicillium simplicissimum
Ferroplasma acidiphilum	Penicillium tricolor
Leptospirillum sp.	
Leptospirillum ferriphilum	
Leptospirillum ferrooxidans	
Sulfolobus metallicus	
Sulfolobus yangmingensis	
Sulfobacillus acidophilus	
Sulfobacillus thermosulfidooxidans	

 Table 2.

 Microorganisms used in bioleaching treatments.

indirect bioleaching processes [52]. Currently, the bioleaching process is the only one reported for the recovery of metals from depleted catalysts, where treatment efficacy has been proven up to 90% using Acidithiobacillus and Aspergillus. The first bioleach process development that was commercialized for agitated tank bioleaching of sulfide concentrates was at the Fairview Gold Mine in South Africa in 1986 with a current capacity of 65–80 t/d. Another plants of refractory gold tank bioleaching operations are located in Brazil (1991); West Australia (1993); Obuasi, Ghana (1994); Tasmania, Australia (2000); Shandong, PR China (2001); Krasnoyarsk, Russia (2001); Kazakhstan (2005); Victoria, Australia (2005); Ghana (2006); PR China (2006); and Uzbekistan (2008), with different capacities of operation [51]. At present, there are patented processes that are used in the world, among which stand out BIOX[®], BioCOP™, BROGIM[®], GEOCOAT[®], and BacTech[®] [51]. GeoBiotics originally developed the GEOCOAT[®] system for the treatment of refractory gold deposits and has since expanded the technology for the treatment of copper, nickel and cobalt. The process incorporates elements of two successful and commercially proven technologies: heap leaching and biooxidation, depending on temperature of operation, the heap is inoculated with mesophilic or thermophilic microorganism [53]. The GEOCOAT® technologies, together with a wide variety of additional expertise and patents, constitutes the GeoBiotics technology suite, including high-temperature bioleaching, toxins removal, HotHeap™, BIOPRO™, and other complementary processes focused around pretreatment, aeration, stacking, and instrumentation [54].

4. Treatment of spent catalysts of the petrochemical industry by microbial route

In the case of the treatment of spent catalysts, the coke that is impregnated in the pellets modifies the particle size, in addition to restricting and decreasing the contact surface between microorganisms and metals, so a pretreatment is necessary to eliminate oil residues and subsequently enter the waste into the bioleaching process; therefore, the continuous treatment is often transformed into a batch treatment (batch processes) causing the process to slow down and increase the cost of the treatment [55, 56].

The most reported biological processes for the treatment of catalysts involve bioleaching processes using *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferro-oxidans* as producers of inorganic acids in direct bioleaching systems [38, 50]. Using inorganic acids and a concentration of 10% v/v of spent catalyst, recovery of Ni and V can go up to 98–99% and other metals, such as Mo, Co, Al, and Fe, in a smaller proportions (60–80%) [56]. Using a mixed culture of Fe/S oxidizers with *A. ferrooxidans*, *A. thiooxidans*, and *L. ferrooxidans*, a recovery was achieved of 83–90% of Ni and V from the spent catalyst at 10% (w/v) of pulp density [42].

Aspergillus niger is a heterotrophic fungus reported in bioleaching processes mediated by organics acids as oxalic acid, reaching recovery percentages of 62.8% of Ni when was used spent catalyst at a concentration of 1% (w/v) [39]. In another study, that used the same microorganism, recovery was 45.8% of Ni in a concentration of 3% (w/v) of spent catalyst [41]. Although these microorganisms are the most reported in the literature for the treatment of spent catalysts, other microorganisms of the genus Bacillus are being evaluated for these bioleaching processes, and Bacillus megaterium was studied for leaching Re and Pt using the cyanide produced by this microorganism [57]. The ability of *Bacillus megaterium* MNSH1-9K-1 and Bacillus subtilis PRGSd-9K-4 for the removal of Ni and V was evaluated by using a mineral medium plus a spent catalyst at 16% (w/v) of pulp density, finding removals of Ni and V of 149.5 and 920.5 mg/kg, respectively [22]. Species of genus Bacillus have been reported to remove metals Ni, V, Al, Fe, As, and Mg at different extents, and cell morphology changes have been detected at the end of biological treatment as a higher quantity of spores for Bacillus thuringiensis MNSH2-AH-3, 2 μm cells in pairs for *Bacillus megaterium* MNSS-AH-4, and long chain-vegetative cells having inclusions into the cell surface in *Bacillus* sp. PRGSd-MS-2 [16]. The ability of Microbacterium oxydans MNSH2-PHGII-1 and Microbacterium liquefaciens MNSH2-PHGII-2 has recently been reported for the removal of Ni and V metals by using a rich medium added of 16% of spent catalyst (w/v) finding removal percentages for Ni 45.4 and 51% and for V 30.4 and 41.4% for each microorganism, respectively [24]. *Microbacterium liquefaciens* is able to remove Ni and V from spent catalyst at 80% (w/v) pulp density in a glass-column system under the following laboratory conditions: 80% (w/v) pulp density, inoculum at 20% (3×10^8 CFU/ml), air at 80 ml/min, incubated at 30°C during 14 days. Under this condition, it was able to remove 1007.4 mg/kg of Ni, while V was removed at an extent of 5360.5 mg/kg [27]. Suspensions containing bioemulsifier produced by *Microbacterium* sp. strains were shown to be able to remove cadmium and zinc from contaminated industrial residue and its ability varied according to carbon source [58]. About the biosorption capacity of cadmium by the biopolymers Microbactan and MC3B-22, both synthetized by marine bacteria *Microbacterium* sp. MC3B-10 and *Bacillus* sp., respectively, the maximum sorption capacity of Cd²⁺ was 97 mg/g for Microbactan and 141 mg/g for B. firmus EPS, both at pH 7 and 28°C. In addition, microbactan and B. firmus exopolymeric substances (EPSs) were nontoxic to Artemia salina nauplii, which is an aquatic model organism widely used in aquaculture activities [59].

5. Conclusion

Microorganisms can adapt to different environments. Biological treatments consider characteristics of interest of the microorganisms used, such as resistance to metals, at acidic and alkaline pH, at low and high temperatures, taking advantage

Treatment of Solid Waste Containing Metals by Biological Methods DOI: http://dx.doi.org/10.5772/intechopen.92211

of organic and inorganic sources as a carbon source and energy, and even the ability to secrete substances such as polymers, enzymes and/or proteins, acids or siderophores, and so on, which allow their use directly or indirectly using microbial by-products. However, more studies are required to optimize the processes and conditions for each microorganism and the type of residue to be treated.

Acknowledgements

The authors would like to acknowledge the National Polytechnic Institute, Mexico.

Conflict of interest

The authors declare no conflict of interest.

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