Bio-Remediation Of Cu, Ni And Cr From Rotogravure Wastewater Using Immobilized, Dead, And Live Biomass Of Indigenous Thermophilic Bacillus Species.

H Al-Daghistani

Abstract

Heavy metals resistant thermophilic bacilli were isolated from Ma’een hot springs in Jordan and used as immobilized, dead and live biomass for bioremediation of heavy metals present in the wastewater effluent of Jordan Rotogravure Establishment (JRE). Four species of bacillus were isolated and identified. They include: Bacillus sphaericus, Bacillus pumilus, Panibacillus alvae and Geobacillus sterothermophilus. They all exhibit resistance to at least 100 mg/L Cupper (Cu), nickel (Ni), and chromium (Cr). A significant difference in Cu (p= 0.001), Ni (p= 0.013) and Cr (p= 0.035) mean biosorption capacity was recorded between Bacillus species. Heavy metals were reduced more efficiently with immobilized cells (p˂ 0.05). B. sphaericus have the highest ability to adsorb Cu (87.5%) and Ni (72.8%) present in rotogravure effluents in comparison to B. pumilus, Panibacillus alvae and G. sterothermophilus that showed 81%, 65.4% and 79.6% for Cu and 62.4%, 59% and 65.6% for Ni, respectively. A maximum removing efficiency was recorded for Cr by B. pumilus (86.5%) in comparison to 83%, 78.3% and 82.7% estimated for B. sphaericus, Panibacillus alvae and G. sterothermophilus respectively. Although the overall sorption efficiency doesn’t vary significantly within the temperature range 37°C- 70°C, thermophilic bacilli exhibit a maximum metal binding capacity at temperatures 60°C and 70°C. The affinity of Bacillus for heavy metals adsorption was found to be: Cu> Cr> Ni for B. sphaericus and Cr> Cu> Ni for other Bacillus species.

INTRODUCTION

Many waste materials discharged to the environment. Most are degraded slowly by living organisms into smaller harmless molecules; however some not easily broken down and instead they accumulate to levels which could pose health hazards. The disposal of heavy metals is a consequence of industrial activities like, electroplating, painting, petrochemical, chemical manufacturing, and textile industries [1]. Toxic metals such as Ag, Cd, Cr, Cu, Hg, Ni and Zn, make their way into water bodies which can produce harmful effects on human health when they are taken up in amounts that cannot be processed by the organism [2]. As a result, many new technologies are required to mitigate heavy metal concentrations to environmentally accepted levels.

Rotogravure effluents contain certain heavy metals like chromium and copper which are generated during chrome plating and preparing of the printing rolls [3]. Removal of heavy metals from industrial effluents has conventionally been accomplished mainly by precipitation, ion exchange, and electrolytic technologies [4]. Physicochemical methods which have been used widely are expensive and ineffective, especially when the concentration of the polluted metals is below 50 mg/L [5]. Besides that, they have disadvantages like incomplete metal removal, energy requirements and generation of toxic sludge’s and other waste products that need to be disposal [6]. Various biomass materials and agricultural by-products have been utilized in the removal of toxic heavy metals from waste water [7, 8, 9]. However, bio-remediation of heavy metals by microorganisms may provide an attractive alternative to physico-chemical methods and has a great potential in industry [10, 11]. Microorganism's uptake metals either by bioaccumulation and/or biosorption [9, 12]. These processes use the functional groups present in bacterial, algal, and fungal cell wall to form complexes with metal ions and thereby aid in the removal of heavy metals. Metabolically active (living cells) and inactive (dead cells) were used in bioremoval process and behave in different ways. The former can only immobilize metals by biosorption, whereas the later may immobilize soluble metal species both by biosorption and by other mechanisms that are part of and/or are due to the
microbial metabolism [13]. Researchers have been conducted in the area of mesophilic and certain thermophilic bacteria in the context of reduction, leaching and other forms of mineral extraction [14, 15]. The most important microorganisms which have been successfully used in bioremediation were bacteria such as Pseudomonas, Bacillus and several other algae and fungal isolates [16, 17, 18]. Thermophilic bacilli are a group of bacteria that can grow at an optimum growth temperature of 60°C [19]. They have been used in bioremediation of heavy metals associated with different environmental conditions [20, 21].

The adsorption capacity of a microorganism was known to be affected by heavy metal concentrations, biomass type and weight, contact time, temperature, and pH. It was known that the highest adsorption occurs at pH ranges from 4-8 which is widely accepted as being optimum for heavy metal uptake of almost all types of biomass [14, 22, 23]. Although the effect of temperature on heavy metal biosorption by bacteria has not been clarified, especially when thermophilic bacteria were used as biosorbant, temperature exerts a significant effect on the adsorption efficiency. An increased in adsorption was recorded at higher temperatures [24, 25] which has been attributed to the decrease in the effective size of the adsorbing cations [26]. Microbial cells with adsorption abilities are immobilized either as entrapped biomass or as a biofilm to form a system for treating waste water known as a bioreactor [27, 28]. Inert (nonliving) microorganisms also have been used as biosorbants for heavy metals in wastewater treatment [29]. Although living biomass has an additional capacity for metal ions due to metabolic entrapment, non living biomass has a strong affinity for metal ions due to lack of protons produced during metabolism [30].

Biosorption is proven to be quiet effective for the removal of heavy metal ions from contaminated effluent in a low cost and environment friendly manner [31]. In Jordan high concentrations of heavy elements are found in the effluents of some industries including Rotogravure. These effluents end up in the surface and groundwater bodies and may be if connected to domestic wastewater treatment plants, kill the active bacteria and algae and hence stop the biological activities involved in wastewater treatment.

Many environmental technologists rely on application of microorganisms for the industrial waste treatment. The process is quite flexible and microbes can easily adapt the variations of conditions and metabolize the wastes present in the concerned discharge. This work aims to investigate the capability of available thermophilic gram positive Bacillus species in heavy metals sorption process, and to compare their efficiency if immobilized, dead, and live biomass were used.

MATERIALS AND METHODS

SAMPLING AND EFFLUENT CHARACTERISTICS

Wastewater effluent was collected from Jordan Rotogravure Establishment near Zarka city, distributed in 250 ml sterile conical flasks (100 ml in each) and stored at 4°C to stop any biological activity. The effluent quality exceeds all standards established by the sewer district and by the Jordanian standard (JS-893/2002) for reclaimed wastewater [32] (Table 1). The pH, temperature and chemical oxygen demand (COD) of the effluent were measured [33]. Samples were filtered and analyzed for total Cu, Ni and Cr using Atomic Absorption Spectrophotometer (AAS) (PerkinElmer, Germany) [34] (Table 2).

ISOLATION OF HEAVY METAL RESISTANT THERMOPHILIC BACTERIA FROM HOT SPRINGS

The bacteria used in our study was isolated from Ma’een hot springs water located north Jordan. These waters, springing out from below ground and flowing into the hypersaline Dead Sea. Water samples were collected from various points located 50 cm below the water surface using sterile thermal glass containers. The pH of the water was 6.9 and the temperature in this region varies from 50°C to 65°C. In order to isolate heavy metal-resistant bacteria, water samples were cultivated on Thermus agar media (ATCC medium 697) containing 0.5% NaCl, 0.5% peptone, 0.4% beef extract, 0.2% yeast extract and 2% agar, and incorporated individually with 100 mg/L of K$_2$Cr$_2$O$_7$, H$_2$O, NiCl$_2$, 8H$_2$O and CuSO$_4$, 7H$_2$O (analytical grade, Sigma), and incubated for 48 h at 60°C. Identical colonies from each plate were isolated and identified according to their morphological characteristics, microscopic appearances and biochemical tests using Analytical Profile Index 50 CHB (API 50 CHB, bioMerieux, France), which includes the following biochemical tests: Glycerol, Erytheritol, D-Arabinose, L-Arabinose, Ribose, D-Xylose, L-Xylose, Adonitol, β-Methyl-D-Xyloside, Galactose, Glucose, Fructose, Mannose E, Sorbose E, Rhamnose, Dulcitol, Inositol, Mannitol, Sorbitol, β-Methyl-D-Mannoside, β-Methyl-D-Glucoside, N-Acetyl-Glucosamine, Amygdalin, Arbutin, Esclulin, Salicin,

Four isolated Bacillus species were selected and identified. These include: Geobacillus sterothermophilus, Bacillus pumilus, Bacillus sphaericus and Paenibacillus alvei (Table 3). Heavy metal resistant bacteria were determined by plate diffusion method in which heavy metal salt solutions were prepared in different concentrations (40, 60, 80, 100, 120, 140, 160, 180 and 200 mg/L). Plates were spread with overnight cultures of each Bacillus species. To each plate, 100 µl of appropriate metal solutions were added in each wells of 10 mm in diameter and 4 mm in depth. After incubation at 37°C for 24h, the zone of inhibition was measured. A zone size less than 1.0 mm scored as resistance strain [35]. Bacterial species were maintained at 4ºC.

**TEMPERATURE TOLERANCE**

Temperature tolerance profile of the isolates was checked on Luria-Bertani medium (Difco, UK) by incubating the inoculating tubes for 24 h at different temperatures and the growth was measured by determining OD at 620 nm [36].

**PREPARATION OF MICROBIAL CULTURES**

One ml of each Bacillus species was mixed with 1L of nutrient broth and incubated for 48 h at 60°C on the rotary shaker (100 rpm). The cells were centrifuged for 15 min at 10,000 rpm, then washed with sterile 0.5% (w/v) NaCl solution followed by sterile distilled water. The cells were dried at 105°C for 2 h. To assess complete death of the dried cells, samples of the dried cells were inoculated to Petri-dishes containing Blood agar medium, absence of any growth indicating positive results (complete death of the bacteria). The dried cells were then ground in a porcelain mortar to obtain a fine powder (0.2 mm), and stored at 5°C, until use. The dried cells of Bacillus species (200 mg/ml) were prepared by suspending in distilled water and homogenized in a mixer to destroy aggregated cells. The wet cells were also suspended in 100 ml of 0.5% (w/v) NaCl solution to obtain a suspension of 200 mg/L equivalent to the dead cell concentration [15].

**IMMOBILIZATION OF BACTERIAL CELLS**

Immobilization of the cells was carried according to the method of Champagne and Gardner, 2001 in which an equal volume of overnight microbial broth culture was mixed with 4% sterile sodium alginate. This mixture was dropped gently in 0.1 M calcium chloride solution using a sterile syringe to get even sized beads. Alginate drops solidified upon contact with CaCl₂ forming beads and thus entrapping biosorbent particles. The beads were allowed to harden for 30 min and were then washed with sterile physiological saline solution to remove excess calcium ions.

**ADSORPTION OF HEAVY METALS**

The adsorption test was conducted using 100 ml of effluent incubated with 1% sucrose and 1% of overnight grown culture in conical flasks [37]. The pH was adjusted to 7 [22]. The four Bacillus species were used as immobilized, dead, and live biomass. Flasks were kept on rotary shaker at 100 rpm (Fisher Scientific, UK) at 37°C, 50°C, 60°C and 70°C for 48 h. Samples were collected, centrifuged at 10.000 rpm for 15 min. The supernatants were analyzed for the remaining heavy metals in the solution using atomic absorption spectrophotometer. Determination of Cu, Ni, and Cr was done by using its specific lamp for each metal and at specific wavelength. The concentration of metal adsorbed to bacteria in each sample was calculated by subtracting the heavy metals concentration remaining in the supernatant from the original concentration of 10 ppm, 1.66 ppm, and 5ppm for Cu, Ni, and Cr respectively. The efficiencies of biosorption were calculated according to the following equation:

\[
R\% = \frac{C_0 - C_e}{C_0} \times 100
\]

Where \(C_0\) and \(C_e\) represent initial and final Cr, Ni, and Cu concentration [38].

**STATISTICAL ANALYSIS**

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software version 13. Results were presented as mean values with deviations (±SDs). Significance of differences was performed using one-way ANOVA correlation and descriptive statistics. A p-value of < 0.05 was considered significant.

**RESULTS**

Wastewater effluent obtained from JRE contains a high
concentration of Cu, Ni, and Cr (10, 1.66 and 5 ppm respectively) which exceed the normal limit. According to Jordan standard, the allowed dischargeable limit to streams, wadis and water storage areas for Cu, Ni, and Cr was 0.2 mg/L, 0.2 mg/L, and 0.02 mg/L, respectively. In order to reduce heavy metals concentration in the effluents, thermophilic Bacillus species were selectively used.

**THERMOPHILIC HEAVY METAL RESISTANT BACILLUS**

Analysis of water samples obtained from hot springs in Ma‘een revealed the presence of different species of thermophilic Bacilli which showed optimum growth rate at temperature of 60°C as detected by temperature tolerance test. Four Bacillus species were isolated and identified by morphology and biochemical tests. These include; B. sphaericus, B. pumilus, G. sterothermophilus and Paenibacillus alvei which are resistant to Cu, Ni, and Cr. In plate diffusion method, heavy metal-resistant bacteria show no inhibition of growth for higher concentration of heavy metals, whereas heavy metal-sensitive bacteria show inhibition of growth for higher concentration of heavy metals [39]. According to this concept, Bacillus species were identified as resistant to Cu, Ni, and Cr respectively (Figure 1). Thermophilic Bacilli exhibited an increasing order of tolerance for the metals (mg/L) Cu (160), Ni (120), and Cr (140).

**BIOSORPTION OF HEAVY METALS**

Immobilized, dead and live biomasses of thermophilic Bacillus species were found to be effective in biosorption of Cu, Ni and Cr. The comparison of the sorption performance using different species of thermophilic Bacillus was achieved under the same conditions (pH, agitation speed, concentration of heavy metals and incubation time). The highest reduction efficiency was recorded using immobilized biomass of all Bacillus species. A significant differences in the mean sorption efficiency percentages of Cu, Ni, and Cr among G. sterothermophilus, B. pumilus, B. sphaericus and Paenibacillus alvei being used as immobilized, dead, and live biomass were recorded (p= 0.004, 0.05 and 0.023 respectively) (Figures 2, 3, and 4). Cu and Cr are removed more efficiently than Ni by the four species (Table 4).

B. sphaericus have the maximum ability to adsorb Cu, and Ni present in rotogravure effluent with means removing efficiency of 87.5%, 72.8% as immobilized and 82.2%, 59.45% as dead biomass, respectively. On the other hand, B. pumilus showed a maximum result for Cr biosorption as immobilized, dead and live biomass (86.5%, 81.7%, and 73.5% respectively) as compared to other Bacillus species. The affinity of heavy metals adsorption was found to be as follows; Cu> Cr> Ni for B. sphaericus and Cr> Cu> Ni for B. pumilus, G. sterothermophilus and Paenibacillus alvei.

**EFFECT OF TEMPERATURES ON HEAVY METALS BIOSORPTION**

Heavy metals biosorption by Bacillus species at temperature 37°C, 50°C, 60°C and 70°C are shown in Table 5. Experimental data indicate that there are no significant differences in the extent of heavy metal adsorption with temperatures range 37°C-70°C for all Bacillus species. Under our experimental conditions, including pH 7, agitation 100 rpm, time 48 h, and 60°C-70°C, thermophilic Bacillus species adsorbed Cu, Ni, and Cr.

**Figure 2**

Figure 1. Growth of Bacillus species at different concentrations of heavy metals

**Figure 3**

Figure 2. Mean biosorption efficiency percentages of Cu (10 ppm) using Immobilized, dead and live Bacillus species.
Figure 4
Figure 3. Mean biosorption efficiency percentages of Ni (1.66 ppm) using Immobilized, dead and live Bacillus species.

Figure 5
Figure 4. Mean biosorption efficiency percentages of Cr (5 ppm) using Immobilized, dead, and live Bacillus species.

Figure 6
Table 1. Jordanian Standard (JS: 893/2002) for wastewater to be discharge to streams, storage, ground water recharge, and reuse for agricultural irrigation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Discharge to streams, walks and water storage areas</th>
<th>Ground water recharge</th>
<th>Reuse for agricultural Irrigation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenol</td>
<td>&lt; 0.002</td>
<td>&lt; 0.002</td>
<td>&lt; 0.002</td>
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<tr>
<td>TDS mg/L</td>
<td>1500.0</td>
<td>150.0</td>
<td>1500.0</td>
</tr>
<tr>
<td>Total POM</td>
<td>350.0</td>
<td>350.0</td>
<td>400.0</td>
</tr>
<tr>
<td>NO₃</td>
<td>300.0</td>
<td>300.0</td>
<td>500.0</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>400.0</td>
<td>400.0</td>
<td>400.0</td>
</tr>
<tr>
<td>Na</td>
<td>200.0</td>
<td>200.0</td>
<td>530.0</td>
</tr>
<tr>
<td>Ca</td>
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<td>5.0</td>
</tr>
<tr>
<td>Al</td>
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<td>2.0</td>
<td>5.0</td>
</tr>
<tr>
<td>As</td>
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<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Be</td>
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<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Cu</td>
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<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>F</td>
<td>1.5</td>
<td>1.5</td>
<td>1.5</td>
</tr>
<tr>
<td>Fe</td>
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<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Li</td>
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<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>Mn</td>
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<tr>
<td>Mo</td>
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<tr>
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<tr>
<td>Pb</td>
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</tr>
<tr>
<td>Se</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Cd</td>
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<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>Zn</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Cr</td>
<td>0.02</td>
<td>0.02</td>
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</tr>
<tr>
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<td>0.002</td>
</tr>
<tr>
<td>V</td>
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<td>0.1</td>
<td>0.1</td>
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<tr>
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<td>0.05</td>
</tr>
<tr>
<td>B</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*0.975 mg/L used for citrus crop

Figure 7
Table 2. Characteristics of Rotogravure industrial effluent

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Temperature</th>
<th>pH</th>
<th>COD (mg/L)</th>
<th>Copper (ppm)</th>
<th>Nickel (ppm)</th>
<th>Chromium (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values</td>
<td>40°C</td>
<td>7.0</td>
<td>46(0)</td>
<td>10.0±1.24</td>
<td>1.6±0.42</td>
<td>5.0±0.70</td>
</tr>
</tbody>
</table>

Values represent means of three sample ± standard deviation

Figure 8
Table 3. Morphological and biochemical characteristics of G. sterothermophilus, B. pumilus, B. sphaericus and Paenibacillus alvei
Bio-Remediation Of Cu, Ni And Cr From Rotogravure Wastewater Using Immobilized, Dead, And Live Biomass Of Indigenous Thermophilic Bacillus Species.

DISCUSSION

Chromium, Nickel and Copper have been recognized as toxic heavy metals and it is essential to remove them from wastewater both to decrease the amount of wastewater produced and to improve the quality of the wastewater before it is released into the environment [40]. Wastewater obtained from JRE contains Cu, Ni and Cr which were generated during operation. The removal efficiency of heavy metals by thermophiles, and the quality of recycled waste water were tested during six months of experimental work.

Prior to our research, wastewater was subjected to coagulation, flocculation and flotation to reduce the concentration of heavy metals and neutralize the pH. These processes were not well enough therefore waste liquid cannot be discharged unless they meet the environmental protection target [33]. The accumulated treated wastewater in the overhead tank contained heavy metals which is not suitable to be reused in side or out side the factory unless additional treatment steps were applied.

Biosorption is defined as a metabolism independent adsorption based on the partition process on a microbial biomass [41], or it is a passive non metabolically-mediated process of metal binding by biosorbent [42]. Limited studies have been published addressing metal adsorption using thermophilic microorganisms. Adsorption reactions and stoichiometries revealed that the metal adsorption behaviour of thermophiles parallels the adsorption behaviour of bacteria grown at lower temperatures [43].

As with biotechnological applications, thermophilic biological treatment systems have many advantages compared to conventional technique, including faster biodegradation rates, greater overall process efficiencies, and a significantly improved ability to destroy pathogens [44]. However, if thermophiles can survive in the presence of heavy metals contaminants, their usefulness can be extended to bio-remediation. The limiting factor preventing the widespread use of thermophilic technology in environmental engineering is the cost of raising system temperatures which can be avoided in two situations: first, when wastewater are produced hot (e.g. pulp, rotogravure and paper industries), and second which is more common, when wastewaters are highly concentrated such that the heat release during contaminant biodegradation is sufficient for autothermal operation.

Thermophilic bacilli are a common genus with high potential of metal sequestration that used in commercial biosorbent preparation [45, 46]. In this paper, the capability of thermophilic bacillus isolated from Ma’a’en hot springs in the biosorption of heavy was examined taking an advantage of the persistent high temperatures in the JRE to enhance the removal of heavy metals. A significant difference was

**Table 4. Mean sorption efficiency percentages of Cu, Ni and Cr achieved by immobilized, dead and live biomass of B. sphaericus, B. pumilus, Panibacillus alvae and G. sterothermophilus.**

**Table 5. Copper, Nickel and Chromium remediation by immobilized, dead and live biomass of thermophilic Bacillus species at different temperatures.**
Bio-Remediation Of Cu, Ni And Cr From Rotogravure Wastewater Using Immobilized, Dead, And Live Biomass Of Indigenous Thermophilic Bacillus Species.

appeared between B. sphaericus, B. pumilus, Panibacillus alvae and G. sterothermophilus in the means biosorption efficiency percentages of Cu (p=0.0001), Ni (p=0.013), and Cr (p=0.035). Immobilized and dead biomass of Bacillus species showed a significant higher reduction efficiency of Cu (p=0.004), Ni (p=0.05) and Cr (p=0.023) in comparison to live bacterial cells. Similar study revealed that immobilized fungal cells exhibited the maximum biosorption ability for heavy metals [47]. Such cells have a benefit in cases of heavy metal toxicity and other extreme properties of waste effluents that might limit the use of living cell system. Immobilized cells appear to be of greater potential in controlling particle size, with better capability of regeneration, high biomass loading, minimal clogging and reduced depletion of nutrient source. These cells were found to be most effective in designing small and large-scale bioreactors for heavy metal biosorption [48].

Immobilized cells of B. sphaericus has the best ability to reduced Cu, Ni present in rotogravure effluents with maximum removing efficiency means of 87.5%, and 72.8% respectively in comparison to immobilized B. pumilus, Panibacillus alvae, and G. sterothermophilus that removed Cu with an efficiency of 81.2%, 65.4% and 79% and Ni with an efficiency of 62.4%, 59.1%, and 65.6%, respectively. It was reported that B. sphaericus are enveloped by a highly ordered crystalline proteinaceous surface layer (S-layer) [49, 50] which is generally composed of identical protein or glycoprotein subunits and possesses pores of identical size and morphology covering the cell surface that facilitated ions trapping and accumulate selectively some of heavy metals (like Cu) in dense deposits at the cell surface [51]. Because of the ability of the S-layer to self-assemble and replace the “older” S-layer sheets on the cell surface, one can speculate about the mechanism of its protective function against some toxic heavy metals. The metal binding sites in the cell wall of Bacillus species have been described [52]. Anionic groups such as carboxylate and phosphate groups of peptidoglycan and teichoic acids are considered the major metal binding sites [53].

Comparison on using dead and live biomass for the adsorption of heavy metals showed that the dead cells exhibited higher adsorption potential for Cu, Ni, and Cr for the four Bacillus species being used. Although living biomass has an additional capacity for heavy metals biosorption due to metabolic entrapment, non living cells dose not only depend on requirements for growth, metabolic energy, or transport yet also has a strong affinity for metal ions due to the lack of proton produced during metabolism [30]. In addition, the maintenance of healthy microbial population is difficult due to metal toxicity and other unsuitable environmental factors resulting in cell death. It often requires the addition of nutrients and this will increase the biological and chemical oxygen demands of the treated water. Other disadvantages of using live suspended microbial biomass are the small particles size and low mechanical strength [13, 54]. Metal uptake by dead biomass is mainly in passive mode through adsorption and ion exchange [2, 55] which is independent on energy. It is mainly occur through chemical functional groups on the materials, comprising the cell and particularly cell wall whereas active mode is metabolism dependent and related to metal transport and deposition [6].

On the other hand, immobilized B. pumilus showed the highest mean biosorption efficiency (86.5%) for Cr in wastewater effluent present initially at a concentration of 5 ppm. This result agrees with that of Pattanapiipitpaisal et al. [56] who reported the removal of Cr (VI) (90%) by B. pumilus after 48 h. The biosorped Cr was deposited as an exocellular precipitate with the formation of cell-bound CrPO which was demonstrated by energy dispersive X-ray microanalysis (EDAX).

Bioaccumulation of heavy metals by Bacillus species appeared to be temperature independent. The range of temperatures used in our biosorption batch experiment was (37°C-70°C) and was comparable to the range of isolation temperatures of Bacillus species. This suggests that the selection for these isolates have been influenced on the heavy metals biosorption temperatures. No significant differences in the extent of heavy metal adsorption with temperatures range being used. However, maximum removal of Cr, Cu and Ni was observed at 60°C and 70°C for all of the isolates. Johnson et al. [27] finds that increasing the temperature will increase the rate of diffusion through the internal pores of the adsorbate particles and leads to a detectable decrease in the viscosity of the liquids. Their effects on the equilibrium capacity of the adsorbate depending on whether the process is exothermic or endothermic [57]. Most importantly, it appears that as the temperature increase, a decrease in the solubility of the dissolved oxygen was noticed and this would adversely affect oxygen transfer, but the concomitant changes in viscosity and diffusivity correspondingly improve the
overall state of aeration. As a result, the overall oxygen transfer rate is not affected greatly, and can slightly increase with temperature. Goyal et al. [26] find similar increase in Cr (VI) biosorption by S. cerevisiae at 60°C. Increased temperatures to certain extend leads to an increase in the energy and metabolic activity which could promotes the active uptake or attachment of metals to cell surface [58].

Thermophilic bacillus showed significant efficiencies in removing Cu, Ni, and Cr from wastewater to levels as low as required by environmental legislation. However further studies are still needed in order to optimize the process and assess its applicability.

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Author Information

Hala I. Al-Daghistani
Department of Medical Allied Sciences, Al- Salt College for humanitarian Sciences, Al-Balqa Applied University