Mercury bioremoval from water by probiotics in simulated microgravity

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Abstract

Heavy metals contamination is one of the major concerns of food industry professional's foodstuffs. So that, they are looking for a safe, cheap, easy and efficient method to eliminate this type of pollution. There are studies that reported the bioremoval of some heavy metals by probiotics from water and foods. Mercury (Hg) is one of the most dangerous heavy metals and its decline is a serious environmental management effort. Due to safety and several other advantages of probiotic bacteria including their ability to heavy metal binding and adsorbing, there are a lot of tendency to employing them for bioremoval of heavy metals. Earth gravity is an effective force under which life appeared and developed. There are several studies about the effect of microgravity on living organisms, including microorganisms. In the current research, capability of *Lactobacillus acidophilus* for mercury ions bioremoval from water was assessed in microgravity conditions. In addition, thermal and alkaline pretreatment effect on Hg absorption power of *L. acidophilus* was evaluated. Furthermore, effects of simulated gastrointestinal were assessed on strength of *L. acidophilus*-Hg binding. The results demonstrated that *L. acidophilus* can decrease Hg concentration excellently in both conditions of simulated microgravity and normal gravity. In addition, *L. acidophilus*-Hg complex remained well stable under simulated gastrointestinal conditions, although the bond strength was higher in microgravity. Alkaline and heat-pretreatment of *L. acidophilus* has not positive effects on the binding as well as stability, especially in microgravity, however, the stability was less in normal gravity.

Keywords: *"mercury", "Heavy metal", "microgravity", "Lactobacillus acidophilus".*

1. Introduction

Heavy metal contamination such as mercury (Hg^{2+}) poses an extreme threat to food security worldwide because of enrichment through food chain and, finally, to the human body. Some studies have reported the bioremoval of heavy metals (mercury (Hg), lead (Pb), cadmium (Cd), mercury (As), copper (Cu), zinc (Zn), chromium (Cr) and iron (Fe)) by probiotics [1].

The rapid industrial development around the world caused a critical environmental issue of mercury (Hg) in water and ecosystem. Mercury as the sixth toxic chemical in the hazardous compounds list is a heavy metal and due to extensive commercial use, the consumption in most of country is quite high. Because of its silvery white appearance also called "Quick Silver". It has very wide commercial applications in industries, mercurial catalysts, health care sector for extensive use Thermometers, Sphygmomanometers, dental amalgams and etc. Mercury is a heavy, odorless, lustrous liquid metal that go down in water. It is ductile mobile and converts in to malleable mass on being solidified at -39ºC, which may be cut with a knife [2]. Hg^{2+} have lipid solubility and can enter to human cells, easily. furthermore, Hg^{2+} may cause neurological human disorders [3]. The United States Environmental Protection Agency (USEPA) and World Health Organization (WHO) have enunciated the maximum acceptable concentrations of Hg(II) to be 1 µg/L and 1 µg/L in drinking water, respectively [4]. Because of Hg toxicity of mercury, its decontamination is very important and several effective manners introduced for its removal to comply with legal limits [5].

There are various strategies for heavy metal removal from water. For example, adsorption, membrane filtration, ion exchange, chemical precipitation, and nanotechnology treatments[6]. Several studies have focused on the bioremoval of heavy metals by probiotics as well as toxins and pesticides. Probiotics are the beneficial live microorganisms that lives in the body and could create health profits such as treatment of urinary infection, diarrhea, and lactose intolerance in the host [7]. Many reports show the bioremoval of heavy metals by probiotics [8]. Using probiotics to heavy metals bioremoval is a cheap, safety and often beneficial manner for human [9]. The lactic acid bacteria (LAB) can decline the toxic metals bio-accessibility by surface-binding between the metals and bacterial cells [10]. Most LABs are introduced as probiotics. One of these probiotic bacteria is *L. acidophilus* that prevalent in food products. Various Lactobacillus strains were evaluated for heavy metals bioremoval potential [7]. The main heavy metals bioremoval strategy of bacterial cells' is the ion exchange of peptidoglycan and or teichoic acid cell wall with the ligands [11]. The cell walls of LABs have a large content of teichoic acids and peptidoglycan so that they have high valence for heavy metals bioremoval [12].

Several items such as heavy metal concentration, biomass concentration, temperature, and pH, have influence on heavy metal bioremoval. Furthermore,

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pretreatment of bacterial cells may evaluated metal absorption capacity [7]. It has been reported that *L. acidophilus* heavy metals bioremoval were elevated after NaOH and heat pretreatments [13].

Earth's gravity is one of the most influential forces on the organisms living on it. The creation and evolution of all organisms occurs in the presence of this force. Gut microbiome, like other organisms are in the influence of gravity. Few researches had been reported microorganism's physiology, morphology, and even pathogenicity alterations in microgravity condition. These changes could be explained by shift in surface layer thickness and agglomeration properties of microorganisms [14].

Any changes in gravity have resulted in microbiome composition disruption, as well as, growth rate, secondary metabolites production, pathogenicity, gene expression, biofilm formation, and antibiotic resistance of them [15]. But it has not been clearly determined that these gravity changes have a positive or negative effect on human health. Some investigations have proved that gravity removal (microgravity) have influence on microorganism's characteristics. Therefore, microgravity can lead to changes in body homeostasis and health [16]. Based on our knowledge, there are limited studies regarding the effect of information about the microgravity effect on the heavy metals bioremoval by *Lactobacillus* bacteria. So that, in this study, microgravity influence on Hg bioremoval from water by *L. acidophilus* condition as well as heat and alkaline pretreatments have been investigated. Also, the effect of simulated gastrointestinal tract (GIT) conditions on stability of *L. acidophilus*-heavy metal complexes was evaluated.

2. Materials and Method

L. acidophilus ATCC 4356 was taken from Tak Gene Zist, Tehran, Iran. It was commonly aerobic cultured in MRS broth at 37°C for 24 h seed culture was prepared with 5 cc of master culture to 50 cc MRS broth and incubated at 37°C for 48h [17]. At the first for alkaline pretreatment, *L. acidophilus* cell mixed with 0.1 N NaOH at 37°C for 1h, then specimens were centrifuged to remove supernatants. Then bacterial cells were washed triple time with sterile distilled water and centrifuged them. For heat pretreatment, *L. acidophilus* cells autoclaved 20 min at 121°C. Then bacterial cells were ready to removal of mercury solution [18]. At the first, 700 µg of mercury (10 mgL^{-1} in HCl 2%) was mixed with 9.3 ml of sterile deionized water and adjusting pH to 4 by HCl, then added prepared *L. acidophilus* to a solution (2.6×10^{12} $CFU.ml^{-1}$) [19]. Then, this specimen was incubated for 24h at 37°C under simulated microgravity on clionostat. One-axis clinostat was used (UN00SA, USA) for microgravity simulation, which rotates samples perpendicular to the direction of the gravity vector (Fig.1). Clinostat was placed in an 37°C incubator. The falcons were filled with samples without any bubbles that disrupt microgravity. Then samples were well-fixed around the center, and rotational speed was adjusted to 15 rpm [20, 21].

Fig. 1: Clinorotation of samples

In order to investigation the strength of *the L. acidophilus*-mercury in the body, a simulation of the digestive system was prepared. Simulated gastric juice was prepared by pepsin (3 $g.L^{-1}$)(Sigma-Aldrich, Darmstadt, Germany) in a sterile NaCl (0.5 % w/v) and adjusting the pH to 2 using HCl. Simulated small intestinal juice was prepared by pancreatin (1 $g.L^{-1}$) (Sigma-Aldrich, Darmstadt, Germany) and bile salt (1.5 $g.L^{-1}$) (Sigma-Aldrich, Darmstadt, Germany) in sterile NaCl (0.5 % w/v) and adjusting the pH to 8 with 0.1 mol/L NaOH. Both gastric and small intestinal juices were sterilized using 0.45-µm membranes filter (Nalge Co., Rochester, USA). After 24 h of bioremoval under simulated microgravity, 10 ml of each metal-bacteria solution was inserted to 40 ml of simulated gastric juice then vortexed (Vortex Genie 2, Scientific Industries, Bohemia, USA) for 10 s, and incubated at 37°C for 2h under microgravity conditions on clinostat. After sampling for mercury analysis, 10 ml of gastric solution, was added to 50 ml of simulated small intestinal juice and incubated at 37°C for 2h under microgravity conditions, and repeated sampling for mercury analysis [22].

In this study, the amount of mercury concentration was measured by ICP-MS (Perkin Elmer ELAN 6100 DRC-e). Device conditions for testing were included, Nebulizer Gas Flow: 0.69 L/min, ICP Radio Frequency (RF) generator power: 1100W, Lenz Voltage: 6V, Analog Stage Voltage: -2300V, Pulse Stage Voltage: 1600V.

In this study all experiments were carried out in triplicate. Statistical analysis was performed by SPSS software, version 22 (IBM SPSS, Armonk, NY, USA) and graphs were drawn using GraphPad Prism, version 9, (GraphPad Software, USA). Results were used in one-way analysis of variance (ANOVA) to estimate p-values and confidence levels. A *p-values* less than 0.05 were considered statistically significant.

3. Results and Discussion

The bioremoval process may be affected by pretreatment of *L. acidophilus* cells. The reason for this phenomenon is due to surface changes of bacteria after pretreatment [13].

As shown in figures 2, 3, and 4, the mercury bioremoval by *L. acidophilus* in water after 24 h of exposure to untreated, heat and NaOH pretreated, were well done and second concentration had significant differences with primitive. There were significant differences in bioremoval of mercury in all conditions (*p-values* < *0.*05). The most bioremoval of Hg for simulated microgravity and normal gravity conditions was done with untreated bacteria cells. Based on Figure 2, the best bioremoval of Hg untreated bacteria was (95.71%) for simulated microgravity and (99.01%) in normal gravity conditions and there were no significant differences between simulated microgravity and normal gravity conditions. Lactic acid bacteria are well known as probiotic microorganisms for aflatoxins bioremediation. The main structural component of the LAB cell wall is Peptidoglycan, teichoic, lipoteichoic acid, proteinaceous S-layer, and some neutral polysaccharides are the major ingredient of lactobacillus bacteria cell wall and negatively charged functional groups (such as O–H, C–H, C¼O, and C–O–C) participate on heavy metals linkage [23].

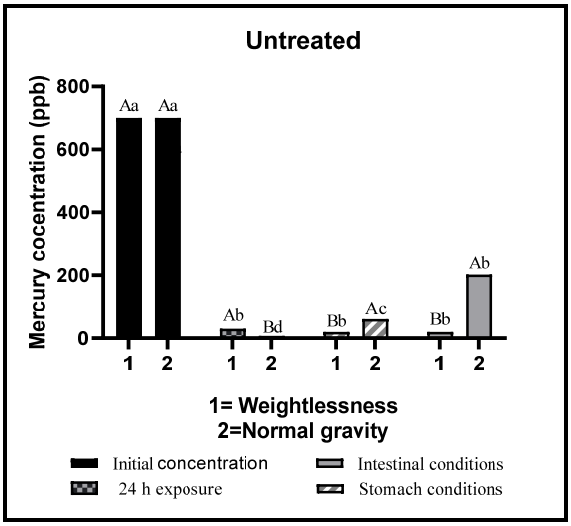

Fig. 2: Concentration of mercury after 24h exposure of Liquid phases with *L. acidophilus* in microgravity and normal gravity as well as simulated gastrointestinal conditions (C). Error amount 5% different small letters among the same samples, differ significantly (*P < 0.05*). Capital letters between microgravity and normal gravity conditions, differ significantly (*P < 0.05*).

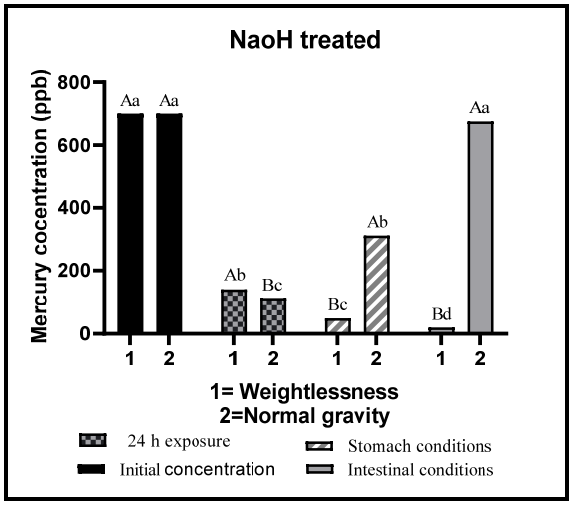

Fig. 3: Concentration of mercury after 24h exposure of Liquid phases with *L. acidophilus* in microgravity and normal gravity as well as simulated gastrointestinal conditions (NaOH- treated). Error amount 5% different small letters among the same samples, differ significantly (*P< 0.05*). Capital letters between microgravity and normal gravity conditions, differ significantly (*P < 0.05*).

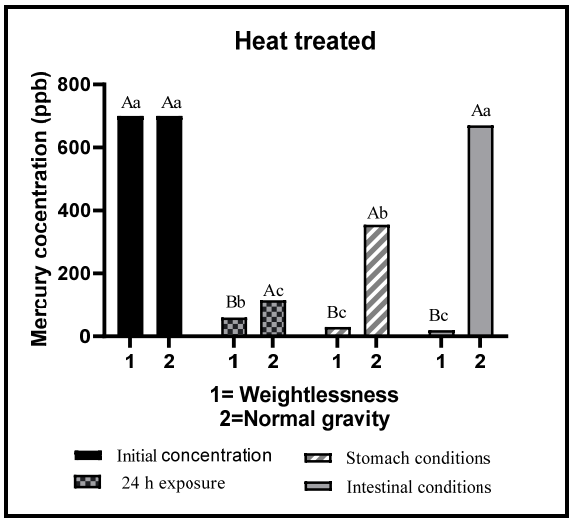

Fig. 4: Concentration of mercury after 24h exposure of Liquid phases with *L. acidophilus* in microgravity and normal gravity as well as simulated gastrointestinal conditions (Heat- treated). Error amount 5% different small letters among the same samples, differ significantly (*P < 0.05*). Capital letters between microgravity and normal gravity conditions, differ significantly (*P < 0.05*).

The *L. acidophilus*–mercury complex stability was evaluated through simulated gastrointestinal conditions treatment. In such a way that after primary 24 h exposure, the specimen was entered and incubated into simulated gastric juice for 2 hours. Then, 2 h exposure to the simulated small intestinal condition were done. As shown in Figs.2, 3 and 4, the stability of *L. acidophilus*–mercury complex was upper in simulated microgravity compare to normal gravity and

significant differences (*p-values* < *0.05*) were observed in all conditions (untreated, heat and alkaline pretreatment). Furthermore, the stability was upper in untreated bacteria compare to heat and NaOH pretreated. In other words, release of *L. acidophilus*-Hg complexes did not happen completely in simulated GIT conditions especially in simulated microgravity. Even, there was no significant difference between the initial concentration and after exposure to simulated intestinal conditions (except untreated bacteria). In simulated microgravity conditions, the trend of increasing adsorption simulated GIT conditions of all treatments was rising, so that in gastrointestinal conditions, Hg concentration reached its lowest level, and the highest stability belong to *L. acidophilus*-Hg complexes in intestinal conditions was observed for all treatments.

literature review reveal that microbial strains, cell wall structure and surface charge, environmental conditions such as pH and temperature are the items affecting the metal removal by microorganisms [23, 24].

According to the results, bioremoval of heavy metals using *L. acidophilus* was somewhat reversible under simulated GIT conditions in normal gravity conditions. It might be due to the simultaneous chemical and physical adsorption in the heavy metal adsorption process. Zoghi et al., reported reversibility of complex indicating the importance of non-covalent electrostatic bonds (hydrogen and Van der Waals bonds) [13].

The GIT treatment results of microgravity conditions showed considerable performance of *L. acidophilus* to mercury bioremoval. So that in the simulated intestinal conditions had the highest absorption of Hg compared to the simulated stomach conditions. This differences may be due to the effect of pH on the mercury removal. According to studies, the lowest heavy metal removal occurs at pHs below 2-3 and the highest Heavy metal removal occurs at pHs above 3 (especially at pH 4-6) [25].

The Earth gravity is permanent force and played a vital role in the evolutionary expansion of organisms. All living creatures on earth has adapted to this physical force by developing structures and functions at the levels of molecular, cells, tissues, and organisms. Only experiments in microgravity provides information about the effect of gravity [26]. Some alteration has occurred in microgravity can lead to changes in the cells. Some of the considerable effects of microgravity environment for cells include reduction or elimination of shear stress and low turbulence environment. Being in such an environment makes changes in microorganisms, such as growth rate disruption, secondary metabolism production alteration, changes in pathogenicity, changes in resistance to environmental stresses, including antibiotics, changes in genetic, and changes in morphology and physiology. In the human body, environments with low shear stress and low turbulence due to microgravity were found in in utero and in the protected environment between the brush border microvilli of epithelial cells [26, 27].

According to the experiments performed, different microbial behavior in microgravity depends on the mobility characteristic of strain. It seems that motile bacteria have better access to food and oxygen by their flagella and therefore, both in poor and enriched culture environments have the same action. But in relation to non-motile bacteria, due to their static nature, in poor environments because of poor access to food and oxygen, their number has been lower in natural gravity conditions. While in microgravity condition, due to the removal of the sedimentation force resulting from gravity, the distribution of available food, air bubbles and waste materials have changed, and hence we will see an increase in the number and some physiological characteristic of motile bacteria compared to normal gravity [28].

The results demonstrated that *L. acidophilus* can make complex with mercury and decrease its concentration excellently in both conditions of simulated microgravity and normal gravity. In addition, *L. acidophilus*-Hg complex remained well stable under simulated gastrointestinal conditions, although the bond strength was higher in microgravity. Alkaline and heat-pretreatment of *L. acidophilus* has not positive effects on the binding as well as stability, especially in microgravity, however, the stability was less in normal gravity. Accordingly, the finding of the current study demonstrates the probiotic efficiency to bioremoval of mercury contamination, especially using microgravity.

4. Concluding Remarks

Living organisms are suffering from polluted water very much and it is very detrimental to the environment. In this category, heavy metal contamination is the major problems for public health Acute and choric illnesses are caused by heavy metal pollution in drinking water that exceed the permissible limits. These can range from nonfatal, such as muscle and physical weakness, to fatal, such as brain, nervous system, and even cancer. Water quality testing is necessary for the protection of human health and the environment [29].

Removal of heavy metals contaminations by safe strategy is very important. Probiotic bacteria application can be a safe solution to omit these pollutions. Polymeric substances in bacterial outer membrane are useable to efficiently complex with cationic heavy metals and bioremoval them from water [30]. Any factor that increases the thickness of the polymeric outer material of the bacteria will improve the heavy metal bioremoval valance. Due to the bacterial exopolymeric thickness in microgravity compared to natural gravity [31], in the current study mercury bioremoval by *L. acidophilus* was evaluated. The results demonstrated that 24 hours bioremoval of mercury was high in microgravity as well as normal gravity and no significant differences was seen. Furthermore, bioremoval of Hg by untreated bacterial cells were more effective in both conditions of microgravity and normal

gravity than the pretreated bacterial cells. Anyway, *L. acidophilus* is capable probiotic bacteria to decline Hg from water. In the category of strength in GIT conditions, the data show that stability of complex was highest in microgravity compare to normal gravity. The results of this research can be used in the foodstuff industry.

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