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Bioremediation of Lead by Iraqi Isolate of Thermophilic Bacteria *Bacillus Stearothermophilus*

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Abstract

This research came to screen the bioremediation of lead in water by thermophilic Iraqi isolate of *Bacillus stearothermophilus* at different temperature. Lauria Bertani broth with or without pb was used as control for assessing bioremediation. The bacterium *B. stearothermophilus* was grown for five hours as a starter culture, then LB or LB+ pb were inoculated by starter culture incubated for 24-72 h at 55°C and the remaining of pb was detected to screen bioremediation ability of studied bacterium. Another experiment was conducted to screen pb reduction in water at 55°C. The Iraqi isolate of *B. stearothermophilus*could eliminate more than half of pb after 48 h, time elongation had no effect on bioremediation. In water the elimination of pb was dependent on bacterial activity, temperature and time, in which cell biomass caused a decrease in pb concentration from 0.775 to 0.122 at 55°C compared with cell debris the eliminate 0.354 ppm of pb. Less reduction of pb concentration in water was recognized at 25°C for both cell biomass and cell debris.

Keywords: Thermophile; Lead; Bioremediation; *Bacillus stearothermophilus*

Introduction

Many Industrial operations and agricultural applications would lead to release high quantities of different heavy metals to the natural ecosystems including soil, water and air [1,2]. The accumulation of these heavy metals in soils may cause reduction in soil fertility through their adverse impact on heterogeneous microbial communities inhabiting soils. Also, via food chains, heavy metals are known to cause toxic effects on both plant and human health [3,4]. Therefore, due to the fact that heavy metals persist in the environment and that they are biologically nondestructive; it has become argent to find an effective approach to eliminate and clean up heavy metals contaminated area [5]. Various options would take place to remove heavy metals, conventional strategies like filtration, flocculation and ion exchange resin have been found at great value but they are expensive, disruptive, less practical value in field experience [6]. On the other hand, bioremediations processes using live organisms or their subcellular fractions are very effective with low cost and time consuming process [7,8].

These organisms have the ability of exploiting chemical contaminants as an energy source in their metabolic processes. Bacterial population have promising as a bioremediation organisms due to their active and different metabolic pathways, their high division rate and

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can survive at many ecosystem [9,10]. Thermophilic bacteria such *B. stearothermophilus* has an advantage in bioremediation application in waste removal and treatment. This bacterium has high growth rate at 50- 65 C, it take place its full growth no more than 5 hour, produce tolerant spores that can resist many environmental harsh conditions. Thermophilic bacteria tolerate and resist many heavy metals include lead, cadmium, chromium and lead. *B. stearothermophilus* isolated from Iraqi soil has exhibiting resistant to multiple heavy metals including lead, chromium, cobalt and mercury [11]. Ecosystem in Iraq suffered from heavy metals effluents that discharged into eco- system without complete treatment. Thus, the main objective of this research was to assess the bioremediation capability of Iraqi isolate *B. stearothermophilus* to reduce lead concentration in pollutant water.

Materials and Methods

Growth conditions of *B. stearothermophilus*

Bacterial culture of *B. stearothermophilus* maintained on nutrient agar slant was used in the experiment. This bacterium was isolated from Iraqi soil and characterized byits lead resistance was evaluated and determined. Laurea-Pertani broth (tryptone 1%, yeast extract 0.5% and NaCl 0.5%) was inoculated by a loopful of bacteria and incubated for 5h or 18h as indicated at 55C±2 with a constant shaking at 120 rpm to reach 1×10^5CFUml^{-1} or 1×10^8CFUml^{-1} respectively, this culture was considered as a starter culture.

Lead metal biosorption

A batch equilibrium method was used to determine the sorption of lead by *B. stearothermophilus*. Three replica of each treatment was done in fixed volume (50 ml) of LB broth using 250 ml Erlenmeyer flasks. Treatments were: LB broth without inoculum and lead, LB broth augmented with 0.75 ppm lead without inoculum, bacterial inoculated of LB broth without lead and bacterial inoculated LB broth augmented with lead at 0.75 ppm. All flasks treated with bacteria were inoculated with 2ml of starting culture 1×10^5 CFUml^{-1}. Bacterial cultures were incubated at 55 ^{0}C ±2 for 24-72h on an orbital shaking incubator at 120 rpm/min. Specimen from culture was withdrawn each 24 h, bacterial biomass was pelleted by centrifugation at 5000 rpm for 15 min and the supernatants were analyzed for residual metal concentration by atomic absorption spectrophotometer (Germany). The standard's absorption of metal solutions was measured at wavelengths 283.3 nm. A standard curve was plotted from the absorption of standard metal solutions with concentration against absorption. The supernatant was analyzed for residual metal concentration in the bacterial culture and controls. Similarly, the residual metal was also determined by intersecting the absorption of supernatant in the standard curve.

Another experiment was conducted to evaluate the adsorption vs absorption behavior of *B. stearothermophilus* upon lead element. Starter culture counting 1×10^8 CFUml^{-1} was centrifuged at 5000rpm for 15min, bacterial pellet was washed twice time, suspended by DW, divided into two portions. One portion was boiling for 15 min and the other was used immediately. Only 2 ml of 1×10^8 CFUml^{-1} or the bacterial debris was mixed with 50 ml of DW augmented with 0.75ppm of lead. Live bacteria and debris was incubated for 48h at 55 ^{0}C or 25 ^{0}C respectively in a shaker incubator at 120 rpm min. Meanwhile, three replica of each treatment sample was withdrawn, centrifuged at 7000 rpm min for 10 min, supernatants were analyzed for residual lead concentration.

Laboratory scaled experiment for lead removal by *B. stearothermophilus*

Randomized water sample was collected from oil refinally/ Messan provenance at July 2018 and stored in sterilized bottle. Chemical analysis of water was done to estimate the concentration of lead. To analyze lead bio-sorbent into *B. stearothermophilus* bacterial cell procedure taken from *Selenska et al.* with some modification. Bacterial biomass was separated from 18 h growth culture by centrifugation at 5000 rpm for 10 min, bacterial biomass was washed three time with DW to remove the excess of adhesive medium which may gave false positive results. Biomass resulting from 2 ml growth was re-suspended by DW. Plane water was divided into 50 ml portion in conical flasks, trials were conducted at 55 ^{0}C and 25 ^{0}C, all flasks were autoclaved for 15 min at 122C, treatment group was mixed with bacterial biomass while, control group was immediately in use, both control and treatment groups were incubated for 48 hr in a shaker incubator at 120 rpm min^{-1}. Samples were withdrawn and supernatants were analyzed for residual lead comparing with untreated water samples.

Statistical analysis

Statically analysis was done for three replicate to each treatment using ANOVA analysis by gene stat program 12 editions.

Results and Discussions

Study result indicated the capability of Iraqi strain of thermophilic bacteria *B. stearothermophilus* to reduce the

concentration of lead from its corresponding growth medium (Table1), lead concentration was reduced to less of its half concentration from 0.804 ppm to 0.244 ppm after 48 h of treatment. Results indicated that lead reduction was time and concentration dependence in which no differences in lead concentration was recorded when native concentration was exist in LB broth, while differences was recognized with time at high concentration of lead. Bacterial growth caused elimination of lead concentration that depends on time, concentration reached to less than its half concentration after 48 h of treatment, otherwise no more reduction recorded after 72 h. Iraqi strain of *B. stearothermophilus* showed resistance to many heavy metals including lead as indicated by *al- Khafaji et al*. [11]. Such ability described for other strains of *B. stearothermophilus* isolated from other sources and places as its capability to eliminate and adsorbed cadmium, Crom and lead [12].

Treatment	Lead concentration					
	0 ppm			1ppm		
	24	48	72	24	48	72
LB broth	0.0917a	0.0867a	0.0867a	0.8883e	0.8047d	0.7793d
LB broth+ bacteria	0.0923a	0.0690a	0.0673a	0.6680c	0.2440b	0.2700b

Table1: The bioremediation of lead by *Bacillus stearothermophilus.*

Experiment recorded no differences in pb concentration at its low concentration 0.08839 ppm (control medium broth) while, more than half of pb concentration eliminated by *B. stearothermophilus* at 0.8241 ppm as shown in Table 2.

Treatment	Concentration ppm	
	0	1
LB broth	0.08839[a]	0.8241[c]
LB+ bacteria	0.0762[a]	0.3940[b]

Table 2: Removal of lead by *Bacillus stearothermophilus.*

Statistical analysis between lead concentration and the time required for biodegradation at low concentration of lead (control medium) revealed that no differences in pb reduction with time proceed while, at high pb concentration (0.8241) ppm, the reduction process was time dependent in which more lead elimination was seen after 48 hr (Table 3).

Concentration (ppm)	Time (hr)		
	24	48	72
0	0.0920[a]	0.0778[a]	0.0770[a]
1	0.7782[c]	0.5247[b]	0.5243[b]

Table 3: Removal of lead by *Bacillus stearothermophilus* at different period.

Recent work improved that the reduction of lead from water was dependent on temperature and treatment type. It was found that live intact bacterial biomass of *B. stearothermophilus* could eliminate high concentration of lead in comparison with bacterial debris, about 0.633 ppm and 0.421 respectively (Table 4), this may due to that whole bacteria may possess more than one strategy for lead removal such as active accumulation which need ATP to transport pb to interior of bacterial cell and the biosorption that involve the association of element covalently or electrostatically without need ATP (passive accumulation).

Treatments	0 ppm		1 ppm	
	55^{0}C	25^{0}C	55^{0}C	25^{0}C
DW	0.0097a	0.0093a	0.7550e	0.7463e
DW+ Bacteria	0.0053a	0.0083a	0.1220b	0.2930c
DW+ bacterial debris	0.0077a	0.0090a	0.354d	0.3433d

Table 4: Residual concentration of lead in water treated with *Bacillus stearothermophilus.*

Also, more biodegradation of pb was take place at 55 ^{0}C in comparison with 25 ^{0}C for bacterial biomass, while no recognized statistical difference between 55 ^{0}C and 25 ^{0}C for pb reduction by bacterial debris (Table 5). This may due to the thermophilic nature of *B. stearothermophilus* which could live, divide and resist high temperature only.

Concentration (ppm)	Temperature ^{0}C	
	55	25
0	0.0076[a]	0.0089[a]
1	0.4103[b]	0.4609[c]

Table 5: Residual lead concentration at different temperature.

Heavy metals (lead at recent work) can be accumulated, absorbed and adsorbed by either intact cell, bacterial cell wall (cell debris) and bacterial by product (polysaccharide, lipid, protein, DNA and any molecule has negative charge in live or dead cell. Live bacterial cell find a way to protect itself from the toxic accumulation of heavy metals inside bacteria by covering peripheral surface with a shield of Exopolysaccharide matrix leading to sequestration of metal and obstructs their penetration

inside the cell. Many other biological interactions take place between heavy metals and bacterial cell converting lead to either less toxic form or less available form.

Water sample collected from oil refinally was analyzed chemically and used as a substrate for bioremediation by *B. stearothermophilus* Iraqi isolate and Table 6 showed the chemical parameter compared with the permissionleast limit of WHO and IQS.

Unit	Parameter	Well water	IQS	WHO
	pH	7.31	6.5-8.5	6.5-8.5
ms/ cm	EC	4.01		
ppm	TDS	4500	1000	1000
	CL	193.51		
	Ca	92.7	150	100
	Mg	66.39	100	125
	Na	417.3	200	200
	K	4.34	12	12
	NH4	1.02	350	250
	PO4	0.02	400	250
	NO3	0.91	50	50
	Mn	0.402	0.1	0.4
	Zn	0.314	3	3
	Fe	0.210	0.3	0.3
	Cu	0.125	1	2.1
	Cr	0.04	0.05	0.05
	Pb	0.115	0.01	0.01
	Co	0.024	0.02	0.02
	Cd	0.009	0.003	0.003
	Ni	0.04	0.02	0.07
	SO4	195.17	300	200

Table 6: The chemical parameter of industrial water sample.

Biodegradation of pb from water sample was done using thermophilic isolate *B. stearothermophilus* following previous presented data, water samples 50 ml portion planted with 10^8 CFU ml^{-1} of thermophilic bacteria and grown at either 55 ^{0}C or 25 ^{0}C for 48h and residual concentration of pb was monitoring (Table 7).

Treatment	Pb concentration (ppm)	Residual pb (ppm)
Water + bacteria (55^{o}C)	0.1150	0.0054
Water + bacteria (25^{o}C)		0.0067

Table 7: Biodegradation of lead from oil refinally water/ Messan by *Bacillus stearothermophilus.*

The conclusions of resent work were gave an insight to apply the thermophilic *B. stearothermophilus* in bioremediation of lead from pollutant water. The whole bacteria or its cellular component debris could use efficiently in pb removal from water based on many strategies of bioremediations. Also, it gave flexibility in treatment such as using different temperature, low requirement for growth, easyfor maintenance and application, had no bacterial hazardous for human and animal and short time for bioremediations about 48 hr. Also, local isolate of thermophilic bacteria could remove about 0.633 ppm at 55 ^{0}C and about 0.4533 ppm at 25 ^{0}C at each run contained 10^8 CFU ml^{-1}. It concluded from recent work that thermophilic bacteria might had quorum sensing for high concentration of lead in water, it could response only to high concentration of pb in treatment comparable to low concentration of pb less than 0.1 ppm.

The recommendation of this application was to use *B. stearothermophilus* in removal of pb from pollutant water of river or industrial heavy water or high lead content of diesel oil.

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