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In Vitro Bioremediation: A Development Process of Cadmium and Mercury Removal by Environmental Biotechnologies of UV-Mutated *Escherichia coli* K12 and *Bacillus subtilis* 168

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Abstract:

E. coli K12 and *B. subtilis* 168 were investigated for their cadmium and mercury tolerance abilities. They were developed by UV mutagenesis technique to increase their tolerances either to cadmium or mercury, and their names then were designated depend on the name and concentration of metals. *E. coli* K12 $Cd3^R$ exhibited bioremediation amount of 6.5 mg Cd/g dry biomass cell. At the same time, its wild-type (*E. coli* K12 Cd3) was able to remove 5.2 mg Cd/g dry biomass cell in treatment of 17 mg Cd /L within 72 hours of incubation at 37 °C (pH=7) *in vitro* assays. The results show that *E.coli* K12 Hg 20 was able to remove 0.050 µg Hg/g dry biomass cell and more removal by its mutant E.*coli* K12 Hg 20^R to 0.060 µg Hg/ g dry biomass cell in the treatment of 0.15 µg Hg /L. On the other hand, *B. subtilis*168 Cd2 was able to remove the least amount of cadmium (5 mg Cd/ g dry biomass cell) and of mercury (0.045 µg Hg/ g dry biomass cell) under the same conditions were used for *E. coli* K12. Also, the complete removal of both metals was confirmed by scanning electron microscopy (SEM) showing that the effect of cadmium and mercury on the bacterial mass. Also, the SEM images showed that the removal amounts had relationships in changing the morphology of cells under *in vitro* assays.

Key words: *B. subtilis*, Bioremediation, Cadmium; *E. coli*, Mercury.

Introduction:

The contamination of environments by heavy metals has been increased over the times leading to water and soil pollution (1). Cadmium and mercury non-degradation metals, which are difficult to remove from solution. Physiological and chemicals techniques were used for cadmium and mercury removals by eliminating the contamination firstly and then treated it by adding chemical compounds. These processes are expensive and adding a new type of pollution coming from the added chemicals. An alternative method in low cost and hygiene method has been more used, such as using living microbes (2). Currently, bacteria are being used in biotechnology as sustainable alternative methods for biological remediation to remove metals, and this process is called bioremediation. The main mechanisms of bioremediation of cadmium are via biosorption or bioaccumulation mechanisms (3).

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These mechanisms are caused by biotic or abiotic activities of cells that lead to the absorption of cadmium ions in the cell surface or accumulation inside or outside the cell (4). Intracellular accumulation is a more complicated process, and the collection could be involved within specific enzymatic detoxification and organelles systems. However, there are differences in the bioremediation for mercury as the main mechanisms include bioaccumulation and volatilisation rather than biosorption or bioaccumulation mechanisms as for cadmium. Bacteria have a detoxification system of transport Hg (II) into cellular space to reduce it into Hg (0) by volatilisation (5). All these mechanisms, either for cadmium or mercury bioremediation, depend on the resistance ability of bacteria as higher resistance to metal leading to increase the process and the efficiency of removal (6). Thus, the resistance ability of bacteria is one of the most biotechnological approaches in the bioremediation process. One of the simple methods for improving resistance ability of bacteria is using ultraviolet radiation (UV). UV in wavelength above 260 nm can enter into DNA bases, producing pyrimidine

dimers that can cause an error in replication of DNA and growing within media that had specific metal ions which could improve the gens response of resistant to that metal ions during the duplication of the wild-type sequence (7). It is challenging to find bacteria which can remove cadmium or mercury efficiency. As, few researchers have studied the mechanisms of cadmium or mercury removal by either *E. coli* ATCC25922 (8) or *B. subtilis* BNi11 (9), so in this study, we evaluate to use *E. coli* K12 and *B.subtilis*168 as biosorbents for cadmium or mercury.

Four objectives of this study were carried out, firstly determine the minimum inhibitory concentration (MIC) of *E. coli* K12 and *B.subtilis*168 in the presence of Cd and Hg, separately, secondly develop their MIC ability by UV mutagen, thirdly evaluate their abilities to remove their concentrations *in vitro* assay and finally investigate the effect of cadmium or mercury onto bacterial surface by SEM.

Materials and Methods:

Bacteria, cultural medium, and cadmium or mercury stock solutions

E. coli K12 and *B. subtilis*168 used in this study were acquired from Sigma-Aldrich Co and chemicals also were purchased from Sigma-Aldrich Co. The stock culture was grown in 50 mL of a nutrient medium using 250 mL Erlenmeyer flasks. Stock solutions of cadmium and mercury (1 M) were prepared in distilled water and sterilised by filtration using paper size pore 0.22 µm.

Determination of MIC

The MIC values of *E. coli* K12 and *B. subtilis*168 against cadmium and mercury ions were determined separately in E-Basel Salts (EBS) media (10). 1 mL of cell inoculum was inoculated into 50 mL of EBS broth medium supplemented with fructose (10 mM) as a source of carbon-containing cadmium or mercury and incubated at 37 °C for 48 hours. Starting concentration of cadmium or mercury was started from 5 µM until the growth of cell inhibited. The MIC was forward growth where there was no growth at a known concentration.

Effect of cadmium and mercury on microbial growth and substrate utilisation

After determining the MIC of each strain, the effect of either cadmium and mercury on the growth of *E. coli* K12 and *B. subtilis*168 were evaluated by growing the strains in the presence of cadmium and mercury at concentrations 0.1 X MIC, 0.5 X MIC, and 1 X MIC. 1 mL of cell inoculum (OD_{440} = 0.1) was inoculated into 50 mL EBS both medium supplemented with fructose (10 mM) as carbon source with the presence of cadmium or mercury in 250 mL Erlenmeyer flasks in triplicate and incubated shaken at 150 rpm at 37 °C. The cell growth was determined from the measuring of optical density (OD_{440}) by spectrophotometry at interval times. The fructose utilisation by bacterial cells was determined by collecting samples at interval times through cultural growth. Collected samples were centrifuged, and the supernets were kept in a freezer to determine the amount of fructose by using fructose kit assay (Sigma, kit number FA-20).

The growth was observed by measuring the optical density (OD_{440}) as 0.1 of *OD* assumed to equal to 23 mg/l of cells. Monod`s equation (1947) was used to determine the growth rate (μ):

$$\mu = \text{In } (2)/T_D$$

where μ= growth rate ($h.^{-1}$), In (2) = 0.693 and T_D= doubling time of cells (h.).

And the dry biomass formed was measured from the differences between the final concentration (X_f) and the initial concentration of biomass (X_0) to the differences between the final concentration (S_f) and the initial concentration of substrate (S_0) (11).

Mutagenesis

The mutagenesis was done for *E.coli* K12 and *B. subtilis*168 in aiming for getting mutants with higher resistance either to cadmium or mercury. Cells at concentrations 6 $x10^6$ were collected from early stationary phase prior mutation assay. Firstly, to optimise the best time of exposure that kill 90% of cells, cells (50 CFU/mL) were irradiated by ultraviolet light distance from light 50 cm for different times of vulnerability and colony-forming units were counted after growing in nutrient agar medium. Then the mutation assay was done at the best times for exposures. The irradiated cells finally were grown in EBS liquid media supplemented with 0.5 X MIC, 1 X MIC, 1.5 X MIC, and 2 X MIC concentrations of either cadmium or mercury of both wild-types in triplicate and incubated at 37 °C for 72 hours. The screening of mutant was done in EBS agar medium at the cadmium or mercury concentrations of cultures that the mutants were obtained from it. One mutated colony appeared on an EBS agar plate having the highest concentration of Cd or Hg was selected and purified for use in *in vitro* bioremediation of either of cadmium or mercury.

***In vitro* bioremediation of cadmium and mercury**

For the preparation of absorbent, cells from early stationary phase of growth cultures grown in nutrient broth medium were harvested by centrifugation at 40000g for 15 minutes. Cells were washed twice with buffer solution (pH 7) and kept at -8°C prior using for the bioremediation of cadmium and mercury. For bioremediation experiments, cells in concentration 2.5 g/L were inoculated into 50 mL of cadmium at concentration was 17 mg/L and of mercury solution at concentration was 0.15 µg/L in 250 ml flasks in triplicate and incubated at 37 °C shaken at 150 rpm. Samples were taken from the batch flasks at interval times and centrifugated. The pellets were washed with a buffer solution to observe on the cell surface under SEM. The supernets were used to determine the concentrations of either cadmium or mercury by ICP-MS. The amounts of determined Cd or Hg (concentration in mg/L) were normalised into mg Cd or Hg depending on the molecular weight of each metal and the cell mass as follows:

$$\text{Removal amount} = \frac{(Mi - Mf)}{m}$$

where M_i and M_f are the initial and the final amounts of metal (mg); m is the dried biomass of cell (g).

SEM

SEM was used to observe the cadmium and mercury that binding on cell mass and how the cell-surface changed morphologically. Pellets of cells loaded with cadmium/mercury were treated with 5% glutaraldehyde (12). The fixed pellets were viewed under SEM (JEM-6610LV).

Determination of the concentrations of cadmium and mercury after bioremediation

The concentrations of cadmium and mercury in the batch experiment solutions that bioremediated by *E. coli* K12 and *B. subtilis*168 or their mutants were determined using ICP-MS. The samples of 20 mL were collected from each bioremediation flask, and 1 % of nitric acid was added to acidifications the samples. An internal standard solution, indium at 50 mg/l concentration and gold at 1 %(*v/v*) were added to the acidic samples for precise the measurement of Hg to avoid the volatilisation of it.

Results and Discussion:

The MIC of *E. coli* K12 was 3 mM Cd to cadmium and 30 µM Hg to mercury; while the MIC of *B. subtilis*168 was lower to 2 mM Cd to cadmium and but had the same MIC to mercury as *E. coli* K12 (30 µM Hg). The strains were named depends on metal`s name and MIC values as *E. coli* K12 Cd3 (where Cd presents the symbol of cadmium and 3 presents the value of MIC), *B. subtilis* 168 Cd2, *E. coli* K12 Hg30 and *B. subtilis*168 Hg30. These results show that the strains were resistant to cadmium and mercury and their resistance compared with other MICs reported by other studies (Tables 1 and 2).

Table 1. MIC values of Hg- resistant bacteria obtained from other studies.

Hg- resistant *Bacteria*	Strain	MIC(µMHg)	Reference
Cupriavidus necator	UFLA 01-659	5	(13)
Sulfolobus solftaricus	98/2	2.5	(14)
Brevibacillus thermoruber	FB2	50	(15)
Anoxybacillus contaminans	FB5	200	
Bacillus sp.	HT4	150	
Geobacillus caldoxylosilyticus	HT10	275	
Psychrobacter	ORHg 1	100	(16)
	ORHg 3	75	
Pseudomonas spp.	ORHg 8	50	
	ORHg 4	75	
	ORHg 5	75	
B. cereus	MM8	60	(17)
Lysinibacillus sp.	HG17	100	
Bacillus sp.	CM111	20	
Kocuria rosea	EP1	100	
Microbacterium oxydans	HG3	60	
Serratia marcescens	HG19	60	
Ochrobactrum sp.	HG16		
P. putida	SP1	280	(18)
Pseudomonas sp.	B50A	2.7	(19)
Pseudomonas sp.	B50B	2.7	

Enterobacter sp.	B50C	4.1	
Pseudomonas sp.	B50D	4.1	
P. putida	V1	11.5	
Enterobacter sp.	M25A	5.0	
Enterobacter sp.	M25B	5.5	
Serratia marcescens	M25C	5.5	
Pseudomonas sp.	M100B	5.5	
P. putida	C50B	5.0	
Providencia alcalifaciens	L1.0	9.2	
P. alcalifaciens	L1.7	9.2	
Serratia sp.	P 0.5	8.7	
Serratia sp.	P1 (A)	9.2	
B. cereus	P1 (B)	9.6	
B. cereus	CP1.0	5.0	
Enterobacter sp.	A25B	400	(20)
P. entomophila	A50A	250	
Pseudomonas sp.	B50A	920	
Pseudomonas sp.	B50B	450	
Enterobacter sp.	B50C	250	
Pseudomonas sp.	B50D	822	
P. entomophila	B100A	450	
P. putida	V1	920	

Table 2. MIC values of Cd-resistant bacterial species obtained from other studies

Cd-resistant *Bacteria*	Strain	MIC (mM Cd)	Reference
Cupriavidus necator	UFLA 01-659	5	(13)
Pseudomonas aeμginosa	--	0.007	(21)
B. circulans	EB1	2.0	(22)
P. fluorescens	--	10.0	(23)
K. variicola	--	4.4	(24)
B. cereus	S5	10	(25)
K. Yangling	I2	1.51	(26)
A.feacalis	BCd33	7.5	
P. aeruginosa	BCr3	1.5	
B. subtilis	BNi11	2.5	
Bacillus	BCd16	5.0	
Proteus mirabilis	BNi6	2.5	
B. cereus	BCr26	0.2	(9)
A. feacalis	BCr32	2.0	
B. cereus	BNi12	0.1	
B. safensis	BCr7	0.5	
B. cereus	BNi22	2.5	
B. pumulis	BCd2	5.0	

The growth curves of both strains shown in Fig.1 and the growth rate for *E. coli* K12 was 0.14 and for *B. subtilis*168 was 0.16 h^{-1}.

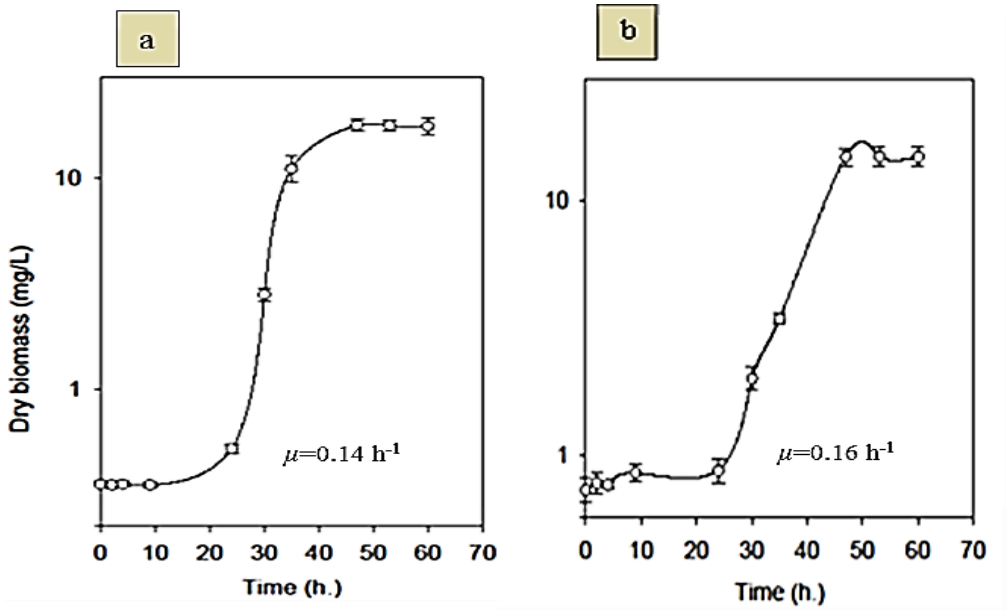

Figure 1. The growth curves of (a) *E. coli* K12 and (b) *B. subtilis*168. Cells were grown in EBS media and incubated for 72 hours at 37 °C and shaken at 150 rpm. At several time points after incubation of the cells, aliquots were taken for OD_{440nm} determination, 0.1 of *OD* was assumed to be equivalent to 23mg/L cell dry biomass. Error bars represent the standard error of the mean (*n* = 3).

Effect of cadmium and mercury on growth activity of bacterial cells

The effects were estimated by measuring of fructose utilisation by *E. coli* K12 and *B. subtilis*168. Based on the measurements, the results show that 100% (10 mM) of fructose was consumed by *E. coli* K12 and 93.7% (9.6 mM) was consumed by *B. subtilis*168 grown in EBS with 10 mM fructose for 72 hours at 37 °C and shaken at 100 rpm (Fig. 2).

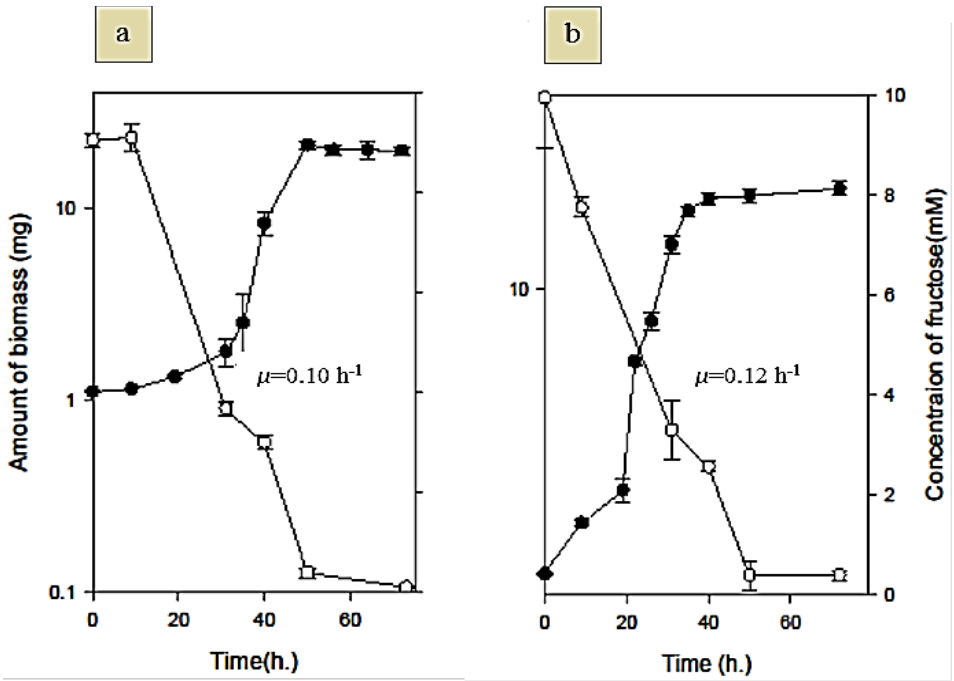

Figure 2. The amount of biomass (●) and fructose utilisation (○) of (a) *E. coli* K12 and (b) *B. subtilis*168. Cells were grown in EBS liquid medium containing 10 mM fructose and incubated for 72 hours at 37 °C and shaken at 150 rpm. At several time points after incubation of the cells, aliquots were taken for $OD_{440\ nm}$ and fructose concentration determination. Error bars represent the standard error of the mean (*n* = 3).

However, the growth rates of both strains were slightly affected by 1 and 1.5 mM Cd compared to the control cultures grown without cadmium (Fig. 3) leading to decrease in growth rate that was resulting in increasing the biomasses. However, at increasing Hg concentrations, the growth rates were considerably reduced of growing strains at 10 µM Hg showed the growth rate of *E. coli* K12 and *B. subtilis*168 were 0.10 and 0.12 $h.^{-1}$, respectively (Fig. 4).

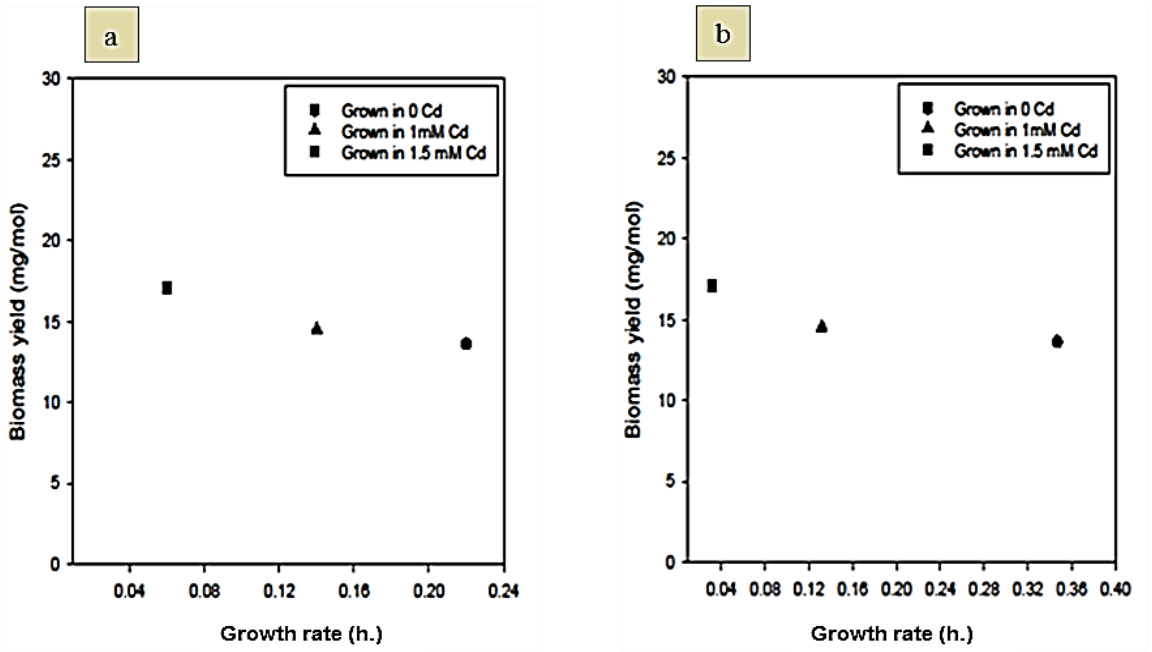

Figure 3. The amount of biomasses and the growth rates of (a) *E. coli* K12 and (b) *B. subtilis*168. Strains were exposed to (●) 0 (▲) 1 and (■) 1.5 mM Cd. Cells were grown in EBS liquid medium containing 10 mM fructose and incubated at 37 °C for 72 hours and shaken at 150 rpm.

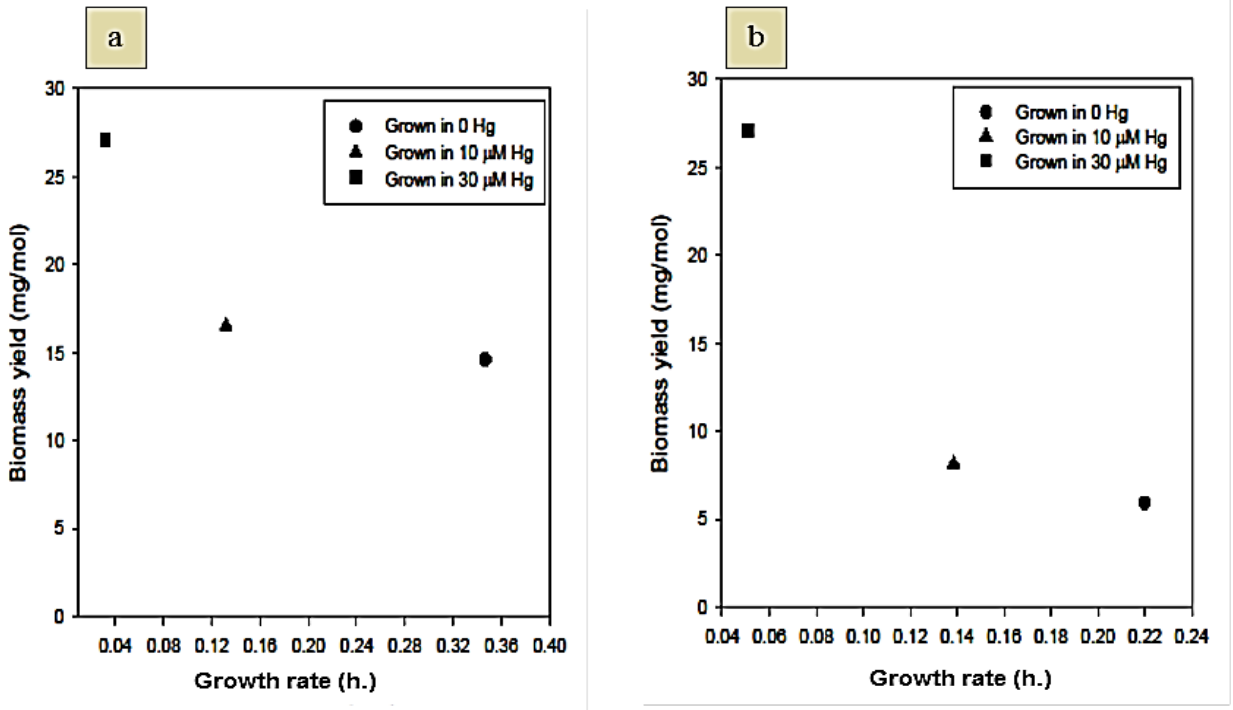

Figure 4. The amount of biomasses and the growth rates of (a) *E. coli* K12 and (b) *B. subtilis*168. The strains were exposed to (●) 0 (▲) 10 and (■) 30 µM Hg. Cells were grown in EBS broth medium containing 10 mM fructose and incubated at 37 °C for 72 hours and shaken at 100 rpm.

Mutagenesis

The killer curves of *E. coli* K12 and *B. subtilis*168, as presented in Fig. 5 a,b show that 20 minutes kill 90% of cells in exposure to UV light, and this time was the best time to generate a mutant.

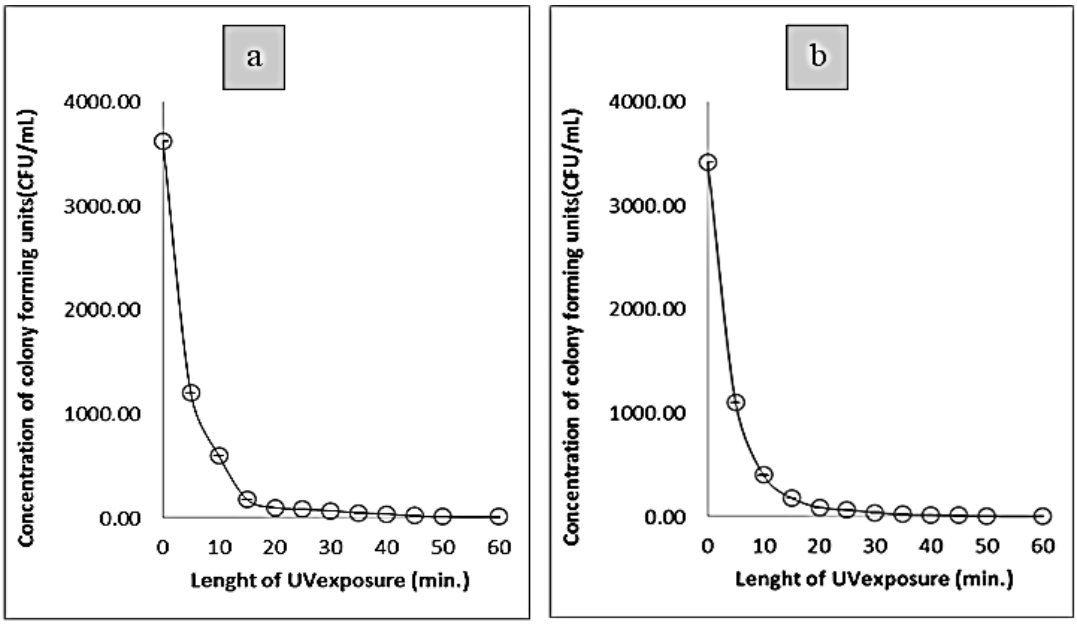

Figure 5. The killer curves of (a) *E. coli* K12 and (b) *B. subtilis*168. Strains in concentration (50 CFU/plate) were exposed to UV light. Total viable count (CFU/mL) was accounted, and error bars represent the standard error of the mean (*n* = 3).

Mutant characteristics

One mutant *E. coli* K12 $Cd3^R$ was obtained from the UV mutagen of *E. coli* K12 Cd3 in that media had cadmium (9 mM) and no mutant of *B. subtilis* 168 Cd2 was obtained. Also, one mutant *E.coli* K12 $Hg20^R$ was obtained in grown in the media that had mercury from *E.coli* K12 Hg20 and no mutant obtained from *B. subtilis* 168 Hg20. The MIC of developed mutant, *E. coli* K12 $Cd3^R$ was 9 mM Cd much higher than the MIC of its wild-type, *E. coli* K12 Cd3 (3 mM Cd). And the MIC of developed mutant, *E.coli* K12 $Hg20^R$ was 30 µM Hg bit higher than the MIC of its wild-type, *E.coli* K12 Hg20 (20 µM Hg).

In vitro bioremediation of cadmium and mercury

E. coli K12 Cd3, mutant *E. coli* K12 $Cd3^R$ and *B. subtilis* 168 Cd2 were used as biosorbents for cadmium removals in batch flasks containing 2 mM[Cd]. While, *E.coli* K12 Hg20, mutant *E.coli* K12 $Hg20^R$ and *B. subtilis* 168 Hg20 was used as biosorbents for mercury removals in batch flasks containing 20 µM Hg. The removal amounts were compared between wild-types and mutants to evaluate the improvement of their MIC in the upgrades of the bioremediation process. The precipitation of cadmium was observed in the EBS broth medium after 72 hours in flasks incubated at 37 °C, as shown in Fig. 6. The results, as shown in Fig.7b showed the mutant *E. coli* K12 $Cd3^R$ removed 6.5 mg Cd/ g dry biomass cell from solution. In comparison, its wild-type *E. coli* K12 Cd3 was able to remove less amount to 5.2 mg Cd/ g dry biomass cell within 72 hours of incubation at 37 °C (pH 7) in vitro assays (Fig. 7a). But *B. subtilis* 168 Cd2 was able to remove a similar amount of *E. coli* K12 Cd3 to 5 mg Cd/ g dry biomass cell (Fig 7c). The removal by E.*coli* K12 Hg20 and *B. subtilis*168 Hg20 were to 0.050, and 0.045 µg Hg/g biomass cell, respectively(Fig 8a and c) and more removal by mutant *E.coli* K12 $Hg20^R$ to 0.060 µg Hg/ g dry biomass cell (Fig. 8c).

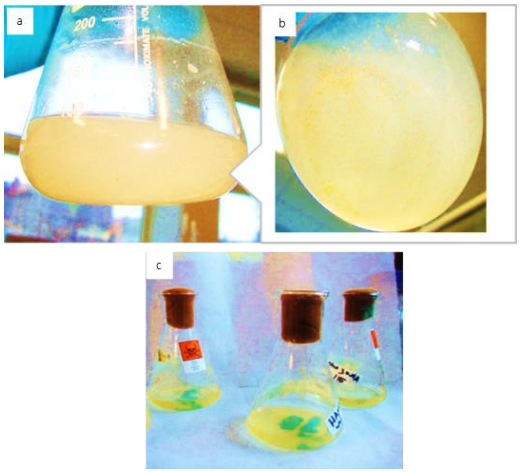

Figure 6. The observation of cadmium precipitation in cultural growth (a, b and c) in 50 ml of 17 mg/L Cd incubated at 37 °C and shaken at 150 rpm for 72 hours.

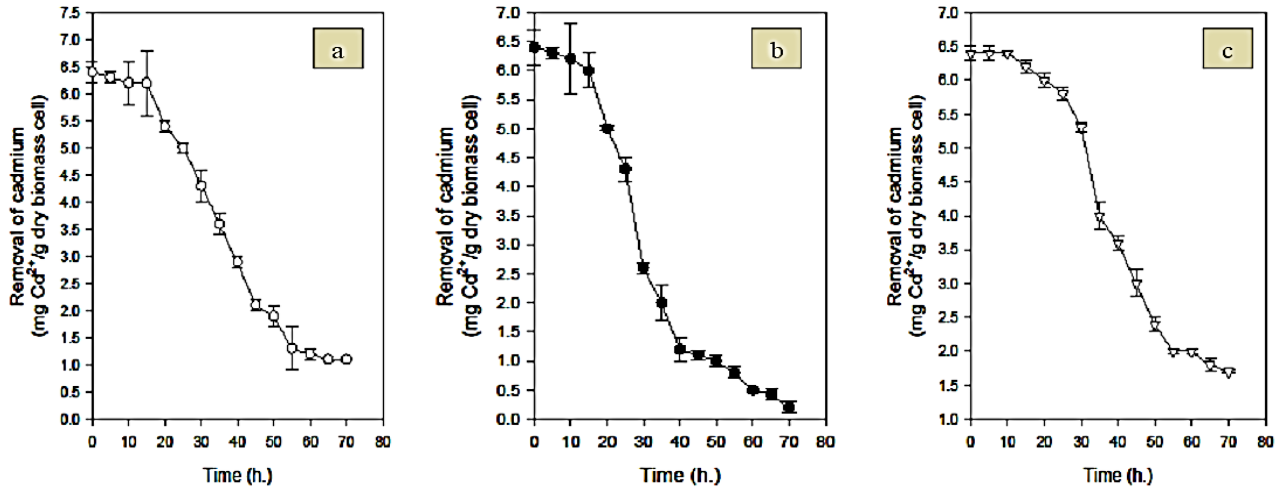

Figure 7. Removal amount of cadmium by (a) *E. coli* K12 Cd3, (b) mutant *E. coli* K12 Cd3^{R} and (c) *B. subtilis*168 Cd2 at cell concentration 2.5 g/L from solution contains 50 ml of 17 mg/l Cd in 250 mL flasks and incubated at 37 °C and shaken at 150 rpm for 72 hours. Error bars represent the standard error of the mean (*n* = 3).

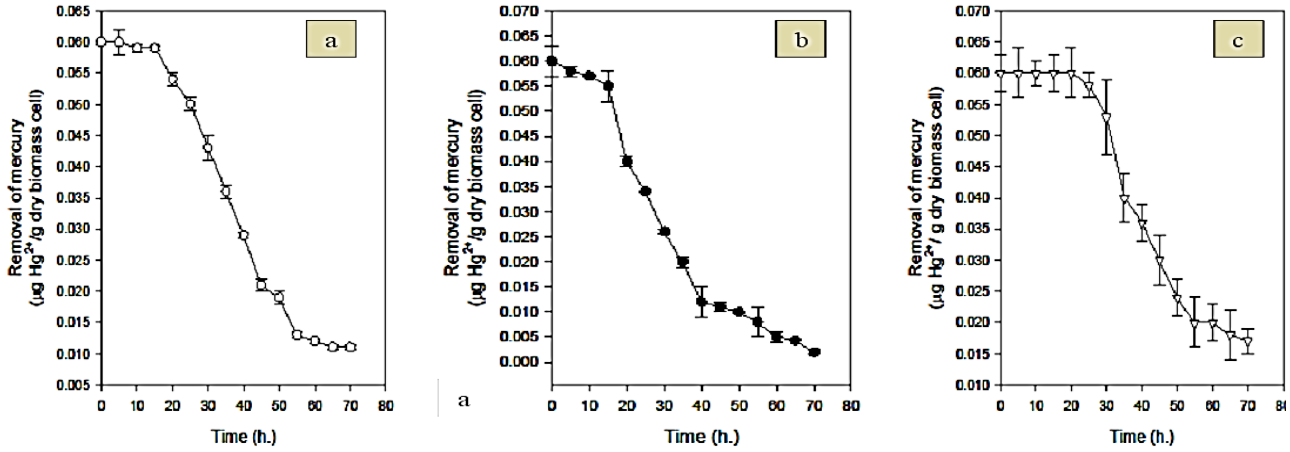

Figure 8. Removal amount of mercury by (a) *E.coli* K12 Hg20, (b) mutant *E.coli* K12 Hg20^{R} and (c) *B. subtilis*168 Hg20 at cell concentration 2.5 g/L from solution contains 50 mL of 0.15 µg/L Hg in 250 mL flasks and incubated at 37 °C and shaken at 150 rpm for 72 hours. Error bars represent the standard error of the mean (*n* = 3).

SEM analysis

The SEM images are shown in Fig. 9a, b, c and the interpretations of these images are presented in Tables 3 and 4. SEM showed that the change of the morphology of bacterial surface to irregularity shape due to extracellular biosorption of cadmium responded for cadmium removal (28). It showed that the cell-surface had the original size, rod-shape and clear surface in *E. coli* K12 Cd3 (Fig. 9a) and *B. subtilis* 168 Cd2 (Fig. 9c). In comparison, in mutant *E. coli* K12 $Cd3^R$ (Fig. 9b), the cell surface was bigger and shiny due to the presence of cadmium ions that appeared by SEM as metallic colour. In addition, it can be seen in Table 3 that there was variance in cell length and width between *E. coli* K12 Cd3 and mutant *E. coli* K12 $Cd3^R$ despite they belong to one genus and are because of their differences in removal abilities. While the SEM images of cells from batch flasks in media supplemented with mercury are shown in Fig. 9d, e, f and their line analyses data of their images are shown in Table 4. The comparison between *E.coli* K12 Hg20, mutant *E.coli* K12 $Hg20^R$ and *B. subtilis*168 Hg20 in SEM images and line analyses of these images showed nearly small differences in cell-shapes.

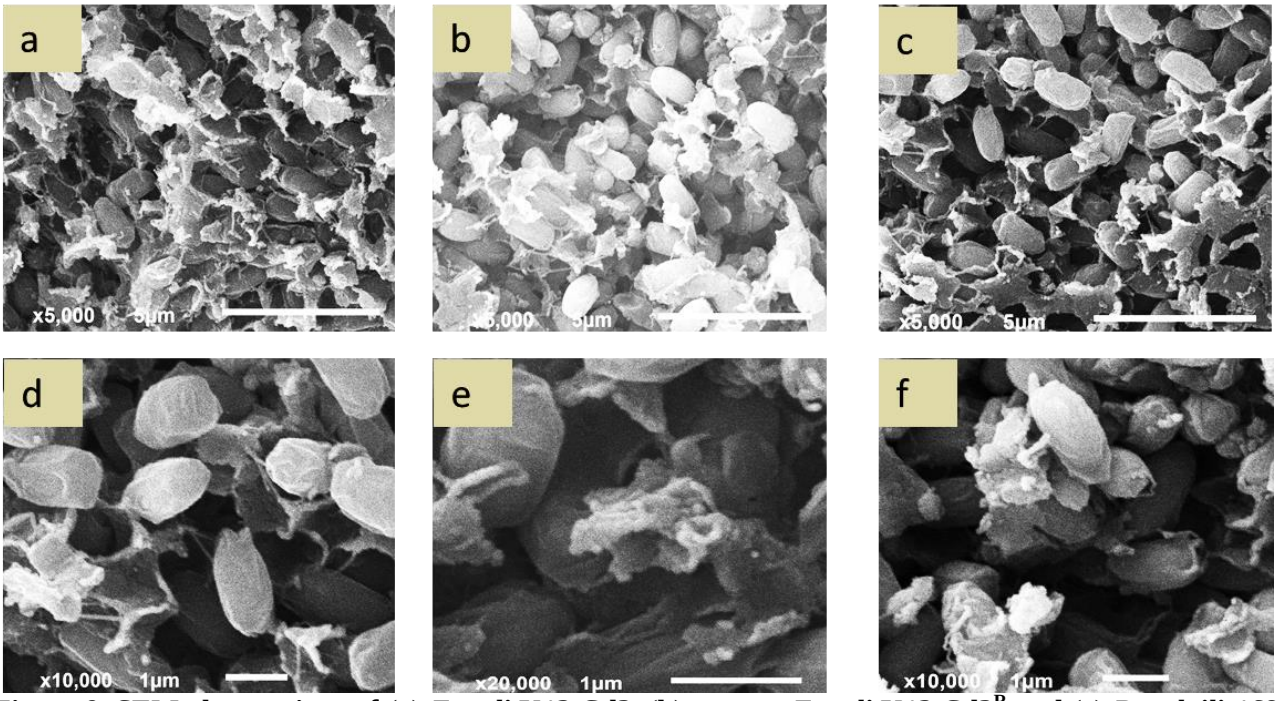

Figure 9. SEM observations of (a) *E. coli* K12 Cd3, (b) mutant *E. coli* K12 $Cd3^R$ and (c) *B. subtilis*168 Cd2 grown in cadmium 2 mM Cd and of (d) *E.coli* K12 Hg20, (e) mutant *E.coli* K12 $Hg20^R$ and (f) *B. subtilis* 168 Hg20 grown in 20 µM Hg in 250 mL flasks and incubated at 37 °C shaken at 150 rpm for 72 hours.

Table 3. Cellular dimensions of study strains under the initial Cd(II) concentrations 17 mg/L.

Strain	Length (µm)	Width (µm)
E. coli K12 Cd3	2.3	1.1
Mutant, *E. coli* K12 $Cd3^R$	2.7	1.4
*B. subtilis*168 Cd2	2.1	1.2

Table 4. Cellular dimensions of study strains under the initial Hg (II) concentrations 0.15 µg/L.

Strain	**Length (µm)**	**Width (µm)**
E.coli K12 Hg20	1.4	0.8
mutant *E.coli* K12 $Hg20^R$	2.1	0.9
*B. subtilis*168 Hg20	1.6	0.7

Overall, *E. coli* K12 and *B.subtilis*168 were used to remove more than 97 % of Cd and Hg ions in the treatment of the solution 17 mg/L Cd and 0.15 µg/L Hg within 72 hours of incubation at 37 °C (pH=7) *in vitro* assays. The development process of cadmium and mercury removal was carried out using a biological system in *E. coli* K12, environmental biotechnology. The mutant *E. coli* K12 $Cd3^R$ and mutant *E. coli* K12 $Hg20^R$ were also effectively developed the process in cadmium and mercury removal because of their high MIC. These strains were considered as a potential agent for cadmium and mercury bioremediation as increasing the MIC of bacteria led to improve the bioremediation in terms of removal amounts. This process was lower cost and higher efficiency, which

depends on different factors including bacterial species, strains, resistant abilities and environmental conditions. It is recommended that further studies could be conducted using the mutants, *E. coli* K12 $Cd3^R$ and E.*coli* K12 $Hg20^R$ in laboratory and pilot-scale- reactors to confirm the development of bioremediation processes of cadmium and mercury by environmental biotechnologies of this work. It is possible to study the surface complexation of Cd or Hg in strains for determining the model adsorption reaction (27). Also, more analysis, such as transmission electron microscopy (TEM) could be used to identify whether the removals were by adsorption or bioaccumulation mechanisms. In addition, as shown in Fig. 5 that the cadmium was removed by bacterial cultures as precipitation of CdS as shown the yellow colour so, it is important to investigate the forms of precipitated cadmium.

Conflicts of Interest: None.

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المعالجة الحيوية المختبرية: عملية تطوير لإزالة الكادميوم والزئبق بواسطة التقانات الحيوية البيئية ل *Escherichia coli* K12 و*Bacillus subtilis168* المطفّرة بالأشعة فوق البنفسجية

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الخلاصة :

تم فحص بكتريا*E. coli* K12 و *B. subtilis168* لقدراتهم التحمليه للكادميوم والزئبق .قد طورت بواسطة تقنية الطفرات فوق الاشعه البنفسجية لزيادة تحملهم، ولقد سميت البكتريا اعتمدا على اسم / تركيز المعدن. أظهرت الدراسه المطفره قادرة *E. col*i K12 $Cd3^R$ من المعالجة البيولوجية مقدار (*E. coli* K12 Cd3) كانت قادرة على إزالة 5.2 Cd mg / g dry biomass cell في معالجة 17mg / L Cd في غضون 72 ساعة من الحضانة عند 37 درجة مئوية (الرقم الهيدروجيني = 7) في فحوصات المختبر. أظهرت النتائج أن *E.coli* K12 Hg20 كانت قادراً على إزالة 0.050 Hg µg وزيادة الإزالة بواسطة *E.col*i K12 $Hg20^R$ الطافر إلى 0.060 Hg µg / g dry biomass cell في معالجة µg /L 0.15 ، بينما كان *B. subtilis* 168 Cd2 قادرًا على إزالة أقل كمية من الكادميوم 5g Cd mg/ biomass cell ومن الزئبق إزالة 0.045 Hg µg / g dry biomass cell تحت نفس الظروف التي استخدمت في *E. coli* K12 . أيضا ، كما تم التأكيد على إزالة كل من المعادن عن طريق مسح تحاليل المجهر الإلكتروني (SEM) التي تبين تأثير الكادميوم والزئبق على الكتلة البكتيرية وأيضا أظهرت الصور أن كميات الإزالة كان لها علاقات في تغيير الشكل المظهري للخلايا تحت التجارب المختبريه.

الكلمات المفتاحية: *B. subtilis*، *E. coli*، كادميوم ، الزئبق، المعالجة البيولوجية.