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Bioadsorption of Heavy Metals From Industrial Wastewater Using Some Species of Bacteria

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Abstract:

Three isolated bacteria were examined to remove heavy metals from the industrial wastewater of the Diala State Company of Electrical Industries, Divala-Iraq. The isolated bacteria were identified as Pseudomonas aeruginosa, Escherichia coli and Sulfate Reducing Bacteria (SRB). The three isolates were used as an adsorption factor for different concentrations of Lead and Copper (100, 150, and 200 ppm.), in order to examine the adsorption efficiency of these isolates. In addition, the effect of three factors on heavy metals adsorption were examined; temperature (25, 30, and 37 °C), pH (3 and 4.5) and contact time (2 and 24 hrs). The results showed that the highest level of lead adsorption was obtained at 37 °C by E. coli, P. aerugenosa and SRB with percentage of 95, 95.3 and 99.7 % respectively, whereas, E. coli, P. Aerugenosa and SRB gave a copper adsorption percentage of (40.63, 50.51 and 80.57%) respectively at 37 °C. Moreover, *E. coli* showed different percentage of metal adsorption ranged from 6.4% to 95 % with lead concentration of 100 and 200 ppm at pH4.5 and for each of 2 and 24 hrs contact time, whereas, it exerts percentage of copper adsorption ranged from 3.5 % to 40.63 % at 100 and 200 ppm and pH value of 4.5 for similar contact time. P. aerugenosa was also shown to be involved in metal adsorption with percentage ranged from 1.39 % for lead concentration of 150 ppm to 97.9 % for 200ppm under pH of 3 and contact times of 2 and 24 hrs. Interestingly, SRB exhibits significant differences in metal absorption values ranged from 14.97 % for lead (100 ppm) to 99.32 % at 200 ppm with a pH value of 3 and contact times of 2 and 24 hrs and under different temperatures.

Key words: Bioremediation, Bioremoval, treatment plant, *E. coli, P. aeruginosa* and SRB, Idustrial wastewater

Introduction:

Water pollution occurs when wastewater is introduced into

environments without treatment, which in turn changes the water quality and have a negative effect on the existent microorganisms [1]. Water quality refers to the concentrations of both organic and inorganic pollutant in water and changes in water properties [2]. The chemical and physical changes in water and their effect on organisms became a major problem worldwide, which is potentially due to the massive industrial developments. Generally, water pollutants are either natural like fires and volcanoes and degradation of organic and inorganic materials, or others resulted from human nature like discarded the industrial waste in water deforestation. environment. mining. treatment of waste water and Fertilizers Industry [3].

Industrial waste is water that carries heavy metal residues resulted from manufacturing processes and caused environmental problems, therefore, several physical and chemical treatment units were used to remove these pollutants from the industrial wastes, however, these methods are highly cost and not applicable [4].

Recently, the treatment of heavy metal has gained more attention due to the significant increase in pollution by these elements, as a product of industrial and agricultural process and mining [5]. Furthermore, because of the industrial development, heavy metal became widely distributed and is considered to be the most harmful type of pollution as it cannot be degraded naturally, and it accumulates in the organisms and transmitted to human through the food chain [6, 7]. These elements are harmful for human by direct uptake or by accumulation in the tissues of some organisms that consumed by human [8]. Several studies were focused on the importance of heavy metals that exsisting in the environment such as mercury, lead, zinc and chromium. Many scientists classified these elements according to their importance of and their effect in nature. Generaly, heavy metals are shared specific properties like; the heavy metals are poisonous and not able to degrade in nature, they are able to transform from low poisoning to high poisoning in the environment.and their ability to accumulate in the food chain and their activity effect on the of the physiological functions in human and other organisms [9].

The importance of some bacteria resides in the presence of the cell wall; the external component of bacterial cell composed structure: that is of peptidoglycan and is located under the plasma membrane [10]. Bacterial cell wall plays an important role in biosorption and heavy metal removal, due to the presence of variety of functional sites such as; carboxyl, amino, hydroxyl moiety, phosphate and sulphydryl [11]. Moreover, the bacteria are considered to be more efficient absorbent than other organisms for several reasons:

- They are able to grow under different conditions, and are tolerant to a wide range of environmental stress, in addition to their availability in nature and easy to collect [12]

- High surface-volume ratio [13].

- The cell wall properties exhibit anionic net charge to the surface [14].

- The bacterial cells are able to undergo to genetic modification in order to increase the adsorption efficacy [15].

- It has been reported that some bacterial species produce proteins that is induced by heavy metals Methalothionine, which are able to bind metallic cations and remove their effects [16].

The treatment plant of the Diala State Company of Electrical Industries, Diyala-Iraq is lacking the biological treatment and not efficient to remove heavy metals from the industrial wastewater [17]. These reasons are justified by this study. The objective of this study was to isolate bacteria from the plant and examine their ability to adsorb the heavy metals.

Material and Methods: Sample collection

Bacterial samples were collected industrial and household from wastewater plant of the Diala State Company of Electrical Industries and in both cases of after and before treatment. Samples were collected in sterile containers of 1L and stored in cooled box, and transformed directly to the The current study laboratory. was performed from November and December 2014 to January, February and March of 2015.

Heavy metal stock solutions

A 1.5985 mg of Pb (NO₃) ₂ was dissolved in 1 l of deionised distilled water (ddH₂O) to get a stock solution of 1000 ppm. The stock solution was filter sterilized (0.45 μ m) and different concentrations were prepared and used. A 2.115 mg of copper chloride CuCl₂was dissolved in 1 l of ddH₂O to get a final concentration of 1000 ppm stock solution. The stock was filter sterilized (0.45 μ m) before serial concentration were being prepared APAH [18].

Isolation and identification of bacteria Identification of *E. coli*

E. coli was identified by their growth properties on MacConkey agar. Bacterial colonies appeared to be small, smooth and dry, spherical shape and red colour (lactose fermenting). The Microscopic examination was conducted and confirmed that *E. coli* was Gramnegative and rod shape. Biochemical tests were also performed for further identification steps according to [19].

Identification of *P. aeruginosa*

P. aeruginosa was identified by subculturing the bacteria on nutrient agar at 37 °C for 24 hrs. The colonies appeared to be large, spherical with smooth shape, concave and have undesired smell. Bacteria produce pale colonies when grown on MacConkey, and thus refers to the non lactose fermenting, whereas, it produces the pyocine stain when grown on King B agar. *P. aeruginosa* was also shown to be positive to the oxidase test. Microscopic examination was done in order to confirm the identity of this type of bacteria, and showed that bacterial cells are Gram-negative, rod shape and non spore forming. The isolate was subjected to biochemical test to confirm the identity, according to [19].

Identification of Sulfate reducing bacteria SRB

A total of 20 isolates of SRB were obtained from industrial waste water at 20 cm depth, the identification of SRB was performed according to [20]. The SRB was isolated and purified under anaerobic conditions using N₂:CO₂ ratio at 20:80 % in the presence of oxygen reduction elements. Bacterial colonies appeared in black colour when it grown on API agar after 1-3 days at 37 °C. Microscopic examination showed that SRB was spherical shape, regular edges and large. After 3 to 5 days, the colonies turned to black, and this due to the presence of iron in the media which binds to the sulphur resulted from sulphate reduction to produce black ferrous sulphide which is an indication for SRB. SRB was further purified using API broth media which contains lactate that is considered to be an energy source, and suitable for growing up to 80 % of SRB. In addition, the broth was also contains sulphate source that is needed for SRB growth such as ammonium ferrous sulphate and magnesium sulphate, in addition to oxygen reduction elements. Moreover, using of sodium bicarbonate solution as a buffer solution and a source for carbon dioxide [21], and also includes reduction factors like Sodium dithionite and cystine, which is to be a selective medium for isolation and purification of SRB. Like *E. coli* and *P. aeruginosa*, SRB was subjected to biochemical tests.

Results and Discussion:

The physicochemical factors and the efficiency of treatment plant of the Diala State Company of Electrical Industries were studied by Hassan et al. [17]. The evaluation study of the treatment plant shown that the heavy metals concentrations were not affected by the treatment process in the plant.

The effect of different factors on bioremoval process

Temperature

Temperature is considered to be the most effective factor on adsorption Turan et al. [22] mentioned process, that the adsorption process is endothermic. Results showed that the adsorption level was increased in high temperatures (Fig. 1), the highest level of lead adsorption was recorded at 37 °C, for *E. coli* which gaves a percentage of 95 % (LSD =7.025 at p<0.05). P. aerugenosa was showed high level of lead adsorption for about 95.3 % (LSD = 6.33 at p< 0.05), whereas, SRB exhibits the highest level of lead adsorption of 99.07 % at the same LSD value, in comparison to E. coli and P. aeruginosa.

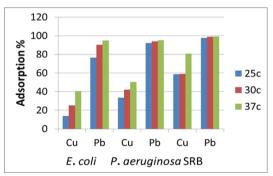


Fig. 1: The percentage of adsorption by the studied bacteria under different temperatures

In this study the results were clearly showd that the temperature has a great effect on copper adsorption, and 37 °C is the ideal temperature for copper

adsorption by the isolated bacteria, as E. coli was able to adsorbe for about 40.63 % (LSD = 8.52 at p<0.05), and 50.51 % and 91.36% (LSD = 7.31 at p < 0.05) and 8.44 were obtained from *P*. and SRB, respectively aeruginosa (Table1-3). Paranthaman and Karthikeyan [23] has mentioned that the range of lead adsorption by P. aeruginosa was increased significantly under temperatures ranged from 25-30 °C. Another study was mentioned that chromium uptake was increased with increasing the temperatures below 40 °C, when E. coli and P. aeruginosa where used [24]. In contrast, the chromium adsorption decreased when temperatures increased to 50 °C. It has been reported that the adsorption of lead and chromium was increased by increasin the temperature to 50 °C by P. aeruginosa [25]. In another study of using algae for some elements adsorption, it is found that the best temperature for adsorption is ranged from 15-35 °C at concentration of 50 Furthermore, AbduSattar ppm [26]. [27] was suggested that 30 °C is the ideal temperature for cobalt adsorption by using orange peels. Moreover, Vijayaraghavan and Yan [28] was reported that the sharp increase or temperatures decrease in caused shrinkage of adsorption cells, and in turns lead to reduce the adsorption surface area and therefore reduction in adsorption levels.

pН

The results of this study clearly demonstrate that the best pH value for copper and lead adsorption is at pH 4.5 by using of *E. coli* and *P. aeruginosa*, compared to pH 3 (Figure 2). It showed that *P. Aeruginosa* exert a high level of copper and lead adsorption percentage of about 95 % and 50.51%, whereas, it was 95 % and 40.36 % for copper and lead respectively by using of *E. coli* (Table1-3). The SRB was showed a significant adsorption percentage of 91.36 at pH 3 in comparison to pH 4.5, whereas, there is no different in lead adsorption at pH 3 (99.06 %) and pH 4.5 (99.07 %).

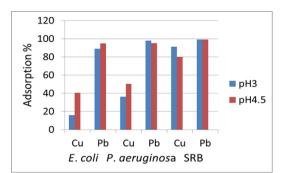


Fig. 2: The adsorption percentage of copper and leads by the studied bacteria at different pH

 Table 1: The percentage of heavy metals adsorption by E. Coli at different temperature, pH and contact time

 pH3 and 2hrs

pH3 ar	<u>1d 2hr</u>	s				_	pH3	ar	1d 24	4hrs			
		1	30		LSD					25	30	37	LSD
п	100	30.46	39.72		9.68*		mdd		100	2.39	12.41	28.33	7.943*
Cn bbm Cn	150	63.75	64.25		9.47*	1	Pb1		150	8.39	16.94	44	9.103*
Pb	200	84	85.86	89	10.52NS	1		2	200	10.06	15.14	55.4	8.475*
LSD		11.59*	9.75 [*]	9.02*		1	LS		D	5.488*	3.041*	7.812	
pH4.5	and 2				pH 4	.5	and 2	24ł	nrs				
		Tempe	rature °(0		Γ				Temper	ature °C		
ι		25	30	37	LSD	Γ				25	30	37	LSD
udc	Temperatur 25 3 100 30.46 39. 150 63.75 64. 200 84 85. LSD 11.59* 9.7 14.5 and 2hrs Temperatur 100 10.08 28. 100 10.08 28. 150 70.14 71. 200 76.53 90. 3D 9.021* 8.5 I3 and 2hrs Temperatur 200 76.53 90. 300 9.021* 8.5 13 and 2hrs Temperatur 200 7.12 8.7 100 3.87 6.9 150 7.12 8.7 200 5.67 11.	28.62	77.21	9.431		udd	10	00	6.4	6.92	8.08	3.41NS	
LSD pH4.5 an	150	70.14	71.84	92.57	6.509*		Pb1	15	50	44.14	48.14	49.49	5.52NS
	200	76.53	90.24	95	7.025*			20)0	47.03	70.89	76.53	8.95*
LSD		9.021*	8.562*	6.305*		I	SD			8.32*	8.96*	7.68*	
pH3 aı	nd 2hr	s				_	pH3	an	nd 24	4hrs			
		Tempe	rature °(2						Temper	rature °C	2	
		25	30	37	LSD	Γ	_			25	30	37	LSD
udc	100	3.87	6.91	9.28	3.86*		uudc	10	00	2.07	2.63	2.4128.33 6.94 44 5.14 55.4 $.041*$ 7.812 re °C 37 2 8.08 14 49.49 89 76.53 $6*$ $7.68*$ Ine °C 30 30 37 63 3.87 27 5.71 87 7.85	1.97NS
Cul	150	7.12	8.75	14.27	5.29*		Cu ppm	15	50	3.36	5.27	5.71	2.66NS
	200	5.67	11.69	16.07	5.63*		-	20	00	3.56	6.87	7.85	2.75
LSD		2.035*	2.485*	2.511*			LS	SD		1.98NS	2.407*	2.156*	
pH4.5	and 2	hrs			pН	[4	.5 an	d 2	24hrs	8			

		Temper	ature °C								
		25	30	37	LSD			25	30	37	LSD
bpm	100	7.89	13.89	17.22	5.093*	mqq	100	3.5	4.83	5.53	2.33NS
CuJ	150	9.07	14.21	14.33	4.69*	CuJ	150	6.5	6.73	7.89	2.88NS
	200	13.87	25.31	40.63	8.52*		200	8.25	11.03	13.82	3.594*
LSD		4.522*	6.85*	6.53*		LS	D	2.65	3.77*	3.91*	

* (P<0.05), NS= Not Significant

Table 2: The percentage of heavy metals adsorption by P. aerugenosa at different temperature , pH and contact time

	S				pn5 a	and 24	nrs					
	Temp	erature	°C				Temperature °C					
	25	30	37	LSD			25	30	37	L	SD	
100	42.16	68.5	73.5	9.63*		100	4.35	23.8	35.52	7.3	3*	
150	57	80.1	93.28	7.97*	udd	150	1.39	36.98	53.5	9.0	9.08*	
200	85.22	88.28	97.9	7.44*	Pb	200	15.02	15.52	56.66	7.42*		
	13.49*	9.53*	8.94*		LS	SD	4.623*	523* 6.549* 6.98*				
and 21	nrs				pH 4.5	and 24	4hrs					
	Temp	erature	°C			Temperature °C						
	25	30	37	LSD			25	30	37	L	SD	
100	68.98	72.58	75.56	7.02NS	udd	100	46.72	66.87	71.42	8.3	16*	
150	80	85.82	91.25	7.54*	Pb1	150	57.86	72.79	77.53	8.0	27*	
200	92.09	94.04	95.3	6.33NS		200	72.79	76.66	83.34	7.1	75*	
SD	8.943*	7.16*	7.81*		LS	LSD 8.925* 6.512* 6.967						
nd 2hrs	S				pH3 aı	nd 24h	rs					
	Temp	erature	°C			Temperature °C						
	25	30	37	LSD			25	30	37	L	SD	
100	7.44	17.64	18.84	5.96*	udd	100	0.3	0.3 0.72		2.4	-2*	
150	7.67	18.06	21.27	7.02*	Cup	150	5.07	7.48	9.86	2.9	8*	
200	10.67	33.49	36.23	7.44*		200	7.7	7.91	10.66	2.67*		
SD	4.32NS	S 6.59*	6.42*		LS	SD	2.45*	2.55*	3.08*			
and 21	ırs			pH 4	.5 and 2	24hrs						
	Tem	perature	°C				Temperature °C					
	25	30	37	LSD			25	30	37		LSD	
100	9.95	26.36	47.72	7.54*		100	0.6	2.42	7.01	1	5.32*	
150	11.98	47.86	40.08	9.36*		150	6.14	9.81	10.18		4.92NS	
200	33.47	42.18	50.51	7.31*	_l _	200	5.41	9.78	36.9	ľ	7.43*	
SD	7.63*	6.02*	4.389NS		I	LSD 3.29* 4.053* 7.22*						
	150 200 and 21 100 150 200 SD and 2hrs 100 150 200 SD and 21 100 150 200	25 100 42.16 150 57 200 85.22 13.49* and 2hrs Temp 25 100 68.98 150 80 200 92.09 SD 8.943* nd 2hrs Temp 25 100 7.44 150 7.67 200 10.67 SD 4.32NS and 2hrs Temp 25 100 9.95 150 11.98 200 33.47 7.67* 200 33.47	25 30 100 42.16 68.5 150 57 80.1 200 85.22 88.28 $13.49*$ $9.53*$ and 2hrs Temperature 25 30 100 68.98 72.58 150 80 85.82 200 92.09 94.04 $5D$ $8.943*$ $7.16*$ nd 2hrs Temperature 25 200 92.09 94.04 $5D$ $8.943*$ $7.16*$ nd 2hrs Temperature 25 200 10.67 18.06 200 10.67 33.49 $5D$ $4.32NS$ $6.59*$ and 2hrs Temperature 25 30 100 9.95 26.36 150 11.98 47.86 200 33.47 42.18 $7.67*$ $12.95*$ 30	100 42.16 68.5 73.5 150 57 80.1 93.28 200 85.22 88.28 97.9 13.49* 9.53* 8.94* and 2hrs Temperature °C 25 30 37 100 68.98 72.58 75.56 150 80 85.82 91.25 200 92.09 94.04 95.3 3D 8.943* 7.16* 7.81* nd 2hrs Temperature °C 25 30 37 100 7.44 17.64 18.84 150 7.67 18.06 21.27 200 10.67 33.49 36.23 3D 4.32NS 6.59* 6.42* and 2hrs Temperature °C 25 30 37 100 7.44 17.64 18.84 150 7.67 18.06 21.27 200 10.67 33.49 36.23 37 100	25 30 37 LSD 100 42.16 68.5 73.5 9.63^* 150 57 80.1 93.28 7.97^* 200 85.22 88.28 97.9 7.44^* 13.49^* 9.53^* 8.94^* $and 2hrs$ Temperature °C 25 30 37 LSD 100 68.98 72.58 75.56 $7.02NS$ 150 80 85.82 91.25 7.54^* 200 92.09 94.04 95.3 $6.33NS$ SD 8.943^* 7.16^* 7.81^* $nd 2hrs$ Temperature °C 25 30 37 LSD 100 7.44 17.64 18.84 5.96^* 150 7.67 18.06 21.27 7.02^* 200 10.67 33.49 36.23 7.44^* SD $4.32NS$ 6.59^*	25 30 37 LSD 100 42.16 68.5 73.5 9.63^* 150 57 80.1 93.28 7.97^* 200 85.22 88.28 97.9 7.44^* LS 13.49^* 9.53^* 8.94^* LS and 2hrs Temperature °C ISD $H4.5$ 100 68.98 72.58 75.56 $7.02NS$ 150 80 85.82 91.25 7.54^* A 200 92.09 94.04 95.3 $6.33NS$ A SD 8.943^* 7.16^* 7.81^* LS ad 2hrs $PH3$ at IS $PH3$ at $Temperature °C$ IS $A.32NS$ 6.59^* 6.42^* IS 100 7.44 17.64 18.84 5.96^* IS and $2hrs$ $pH4.5$ IS IS and $2hrs$ $pH4.5$ IS IS and $2hrs$	$\begin{array}{ c c c c c c c c c c c c c c c c c c c$	$\begin{array}{c c c c c c c c c c c c c c c c c c c $	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	$ \begin{array}{ c c c c c c c c c c c c c c c c c c c$	

* (P<0.05), NS= Not Significant

-							-							
		perature				Temp	erature	°C						
_		25	30	3	7	LSD			25	30		37	LSD	
udd qd	100	54.16	60.09	60.6		8.02NS	Pb ppm	100	0.72	10.17	53.2	21	11.39*	
	150	67.05	78.58	89.5		7.98*	Pb I	150	5.59	57.25	59.8	85	10.83*	
	200	76.48	87.92	91.36)	8.44*		200	56.75	58.77	66.0	01	8.25*	
	SD	9.33*	8.65*	9.72*	:		LS	_	12.07*	10.86*	7.4	1*		
pH4.5	and 2	hrs				-	pH 4.5	and 2	24hrs				-	
		Temperature °C						Temperature °C						
Pb ppm		25	30	3	7	LSD			25	30		37	LSD	
	100	39.09	36.84	51.87	'	5.56*	Pb ppm	100	0.4	1.11	31.3	39	10.54*	
	150	41.12	55.85	60.25	i	11.02*	Pb	150	0.6	31.37	40.0	09	9.32*	
	200	58.77	59.05	80.57		8.76*		200	2.25	40.09	41.8	84	9.59*	
	SD	7.09*	7.81*	9.32*	:		LS	LSD 2.25NS 7.91* 7.22*						
oH3 a	nd 2hr	s					pH3 a	nd 24	hrs					
		Temp	erature	°C										
_		25	30		37	LSD			25	30)	37	LSD	
Cu ppm	100	90.24	98.84	99	.14	7.54*	Cu ppm	100	14.97	17.2		17.9	5.74NS	
CuJ	150	93.67	99.02	99	.06	8.02NS	Cuj	150	24	26.7		30.24	5.13*	
	200	98.81	99.03	99	.32	7.15NS		200	25.21	28.91		31.01	5.09*	
Ľ	LSD 7.31* 6.44NS 6.02N					L	SD	6.73*	6.59*		6.82*			
oH4.5	and 21	hrs					pH 4	.5 and	24hrs					
		Temperature °C							Temp	erature	°C			
_		25	30	Ĩ	37	LSD] _		25	30)	37	LSD	
Ξ	100						4 8							

īdd

Cun

100

150

200

LSD

14.97

26.69

26.71

6.45*

19.5

28.1

30.01

7.56*

21.01

28.79

30.78

6.13

5.94*

5.31NS

5.63NS

Table 3: The percentage of heavy metals adsorption by SRB at different temperature, pH and contact time

pH3 and 2hrs

pH3 and 24hrs

6.74 NS LSD * (P<0.05), NS= Not Significant

92.65

93.36

97.69

97.85

98.65

98.78

5.41 NS

98.13 7.98 NS

7.87 NS

6.33 NS

98.84

99.07

5.03

nqq

G

100

150

200

pH values play a great role in the adsorption, it is related to heavy metal removal using the microorganism. Its effect relies on the number of functional sites on the surface of the cell, and involve in diversity of heavy metal compounds [29]. Pardo [30] reported the effect of pH on the functional sites and metal in a solution, so, at low pH value, hydronium ions (H₃O) was able to bind to the cell wall, and thus restrict the binding of metal ions from the adsorption surface, because of charge dissimilarity. At high pH value, the functional sites such as carboxyl. phosphate, and amino group were increased and the overall charge net became anions, which binds to the cationic metals by electrostatic interaction at the surface of the cell.

Lead adsorption was noticed to be inhibited at alkaline pH values, because of forming of peroxides, hydroxyls and non-soluble carbons [31]. It has been reported that the increased in pH values is lead to forms hydroxyl complexes that compete with the functional sites to bind with metals ions and therefore reduce the level of adsorption.

Oves et al. [32] referred to the most suitable pH value for copper adsorption was at 6, and they reported that the increase of pH value above 5 caused precipitation of lead ions. Furthermore, it has been determined that pH 5 is an ideal value for lead adsorption by Stenotrophomonas maltophilia and B. subtilis [33]. While, another study has mentioned that the best pH value for lead adsorption by Bacillus megaterium was around 7 [34]. The study of Aloosh and AL-Azawi [35] determined the appropriate pH value for bacterial growth and survival. They found that the ideal pH value for C. freundii was 6, and 7 for C. kosari for adsorption of lead and chromium. It has also demonstrated that the best pH value for lead adsorption was 5 by using of lyophilised P. aerugenosa [36]. It was also recorded that the ideal pH for copper adsorption was at 4 when using algae [26].

Contact time

The results of a current study referred to the best contact time for lead and copper adsorption using of E.coli, P. aeruginosa and SRB for all concentrations were 2 hrs (Figure 3, Table1-3). The contact time is one of the important factors affecting the heavy metal adsorption because the fast saturation of the functional sites [37], as well as the capability of empty functional sites to bind to metal ions [11]. Some of related studies were agreed that the adsorption occurs in the first hour of the contact between the metal ion and the adsorption surface [27, 38]. Other studies revealed that the adsorption will take place in less than one hour and approximately 20 min [11,39]. In contrast, other studies were reported that the adsorption occurs in more than one hour [40, 41].

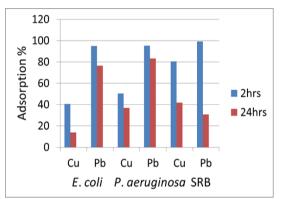


Fig. 3: Lead and Copper adsorption percentage by the studied bacteria during two contact times.

Heavy metal concentration

The increasing of heavy metal concentration leads to increase the kinetic energy in one side, and the capability of metals to bind to the solutions or solid surfaces in another side, as well as accelerate the contact and clash between the metals and the adsorption surfaces which increase the uptake of metals [33]. At low concentrations, the available ions that ready for adsorption are low, unlike the high concentrations that lead to increase the availability of ions that ready to be saturated [42].

Kirova et al. ([43] reported that the increasing of lead concentrations is due to increase the adsorption process, when lead concentration of 25 ppm around 24.70 mg/g was adsorbed, whereas, at 50 ppm about 48.84 mg/g was adsorbed. At 100 ppm the adsorption portion of lead was increased to about 89.23 mg/g by Streptomyces fradiae. Furthermore, Wierzba and Latala [44] were mentioned in their study that the and increasing of nickel lead concentrations enhance the binding of functional sites with the adsorption surface. In addition, they reported that the adsorption in the life cells is better than that of dead cells, because of in the former the elements were up taken not only by adsorption but also by the intracellular process like accumulation. The results of this study referred to

increase the range of adsorption with increasing the concentration of the heavy metal, as it showed that the highest adsorption for lead was 99.07 % at 200 ppm and pH 4.5 and under 37 °C for 2 hrs by SRB (Table 3). The lowest adsorption percentage (2.39 %) for lead was observed at 100 ppm, pH 3, 25 °C for 24 hrs by *E. coli*.

Like in lead adsorption, the best adsorption percentage (91.36 %) for copper was obtained at 200 ppm, 37 °C, pH 3 for 2 hrs by SRB (Figure 4), whereas, the lowest percentage (2.07 %)was at 100 ppm, pH 3, 25 °C for 24 hrs by E. coli. The above results were in agreement with the result of other studies [35]. However, Mohammed [37] was reported that the decrease in the adsorption percentage of lead from 85.5 % to 68.25 % and for cadmium from 97.5 % to 61.3% was due to increase the concentrations from 10 mg/l to 80 mg/l by using the crust of sunflowers as an adsorb surface.

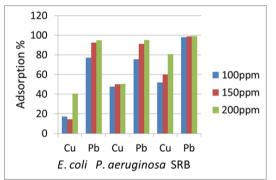


Fig. 4: The adsorption percentage of copper and lead by the studied bacteria at different concentrations

Taken into account these results, it can be clearly seen that the adsorption percentage of lead is better than of copper adsorption, and thus is due to several reasons; radius ionosphere and high electricity of lead [39]. In the study of [45], it was recorded that several changes could occur in the rate of permeability and formation of lead ligand, compared to copper. In addition to the ionization power which is increased by decrease the radius atomic radiation, and the solubility of lead nitrate in water is less than that of copper chloride, which make it more vulnerable for adsorption [26].

The results of the current study clearly showed that the ability of P. aeruginosa to adsorbe and remove heavy metals is better than that of E. coli, because it has a system that works as a regulator for heavy metal uptake by using of ATPase type technique, by which it use the ATP as a pump for metal ions. Moreover, the bacteria have a complex enzymatic system which is able to degrade more than 3000 organic materials that available in the environment. In addition to their ability to produce dimethyl disulfide (DMDS), which plays a major role in heavy metal precipitation [46]. Mohammed [47] also mentioned to the preferential capability of P. aeruginosa for breaking down the hydrocarbons, in comparison to B. subtilis and B. cereus. The reason behind that is due to the efficiency of *P*. aeruginosa to reduce the hydrocarbons group because of their high surface tension as a result of bioprocess on the cell surface.

SRB is distributed widely in natural and artificial environment that have sulphur [48, 49]. SRB have many applications such as adsorption process and heavy metal uptake from contaminated water. The latter is considered as a distinguisher between sulphate and sulphide, when sulphate high solubility compared has to sulphide, so the reduction of sulphate and oxidation of residual sulphide were developed to remove heavy metal [48]. The adsorption results of SRB showed high capability to adsorb lead and copper compared to the others (Table 1-3). This bacteria reduce sulphur element which then act as a final ion receptor, in the study of [50], SRB played a role in breaking down or substitute cadmium compound to be easy to adsorbe by plant, with percentage of 70 %. In another study, a zero valent iron (ZVI) was used by which bacteria can be prepared with the iron column, and the heavy metal acids of lead and copper were passed in the column. The use of this technique increased the efficiency of bacteria in metal remover to a percentage of 99.7 % [51]. In a similar study the same bacteria were used to remove copper after addition of iron, thus, lead them to suggest that SRB+Fe system will increase the bacterial role to 95 % [52]. Another study was mentioned that copper remover is well done in the presence of iron sulphate [53]. In addition. column some researchers mentioned to use the Electron Micropial Analysis (E M P A) to decrease the data and obtained of good results for metal removal [54]. The RSB plays a great role in the chromium adsorption because the presence of iron oxide on the surface of the cell [55].

Conclusion:

The results recorded the significant differences in the temperature when sulfur bacteria was used in the adsorption of lead, and recorded a significant difference in the adsorption of copper. For heavy metals concentration, the results indicate that the highest adsorption percentage was at 200ppm concentration for both elements, and results were also showed that the type of bacteria were able to adsorb lead at higher rates than copper. Moreover, increased adsorption of metals was observed at high temperature and pH4.5 for a 2hr contact time by the studied bacteria. The three isolated bacteria were varied in their abilities to adsorb elements. the results demonstrated that the adsorption efficiency of sulfur bacteria was higher than that of *P. aeruginosa* and *E. coli*, while, the adsorption efficiency of P. aeruginosa was higher than in E.coli.

The study concluded that SRB is the most efficient bacteria to remove the Pb and Cu from the wastewater treatment plant.

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الادمصاص الحيوي للعناصر الثقيلة من مياه الفضلة الصناعية باستخدام بعض الانواع البكتيرية

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الخلاصة:

اختبرت ثلاثة أنواع من البكتريا المعزولة وهي Pseudomonas aeruginosa و Escherichia coli و Sulfate Reducing Bacteria. لازالة العناصر الثقيلة من مياة الفضلة الصناعية لشركة ديالي للصناعات الكهربائية، ديالي – العراق وأستخدمت في المختبر كعامل امتزاز ، كما استخدمت تراكيز مختلفة من الرصاص والنحاس(100و 150 و200) جزء بالمليون الاختبار كفاءة الامتزاز للبكتريا المعزولة. وبالإضافة إلى ذلك، تم اختبار تُاثير ثلاث عوامل على امتزاز الرصاص والنحاس وهي الحرارة (25و 30و 37) م° ، ومستويات مختلفة من الأس الهيدر وجيني (3و 4.5)، بالاضافة الى وقت الاتصال استخدمت فترتين (2 و 24) ساعة. تم الحصول على اعلى مستوى لامتزاز الرصاص وبالنسب 95% ، 95.3% و 99.7% للبكتريا E.coli و P. aeruginosa و SRB عند37م° على التوالي بينما كانت نسب امتزاز النحاس 40.63% و % 50.51 P. aeruginosa · E.coli و SRB عند درجة حرارة 37م°، على التوالي. قد اظهرت E.coli نسب مختلفة من الامتزاز للمعادن تراوحت من 6.4% إلى 95% في تركيز الرصاص من 100و 200 جزء بالمليون وقيمة الاس الهيدروجيني 4.5 لكل من فترات الاتصال 2 و 24 ساعة على التوالي في حين تراوح امتزاز النحاس من3.5% إلى 40.63% في 100و 200 جزء في المليون في قيمة أس هيدروجيني 4.5 وخلال فترة اتصال2 و 24 ساعة ، على التوالي . حققت P. aeruginosa امتزاز على النحو التالي :تراوح امتزاز الرصاص من 1.39٪ في التتركيز 150جزَّء في المليون إلى 97.9٪ في 200 جزء في المليون عند قيمة الأس الهيدروجيني3 وبفترات اتصال 2 و 24 ساعه على التوالي. اظهرت SRB فعالية امتزاز اكبر من الانواع لاخرى من لبكتريا المستخدمة في الدراسة, وقد تراوح امتزاز الرصاص من 14.97% بتركيز 100 جزء من المليون وقيمة الأس الهيدروجيني ولفترة اتصال 24 ساعة إلى 99.32% في تركيز الرصاص 200 جزء في المليون وقيمة الأس الهيدر وجيني 3 وبفترة اتصال 2 ساعة. سجلت النتائج وجوَّد فروق معنوية في درجات الحرارة عند استخدام بكتيريا SRB في امتزاز الرصاص والنحاس.

الكلمات المفتاحية: المعالجة البايولوجية، وحدة المعالجة، E. coli, P. aeruginosa and SRB, ، الفضلة الصناعية