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Biological Treatment of Heavy Metals Removal from Sewage Using Saccharomyces Cerevisiae

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Abstract. A sample of the bread yeast Saccharomyces cerevisiae strain was taken from the local markets in the city of Mosul and grown on the appropriate medium for it in order to test its ability to remove some heavy metals. Concentrations for each metal ph and three temperatures The grown bread yeast was able to remove both zinc and lead alone and remove them both. The highest removal of zinc was 1.99 ppm , and lead was 0.5 ppm. It was noted that the removal rate increased according to the two minerals together, as the temperature was 10 and 25 The best value for pH is 4 and 9

Keywords. Saccharomyces cerevisiae , Biological treatment , heavy metal , Sewage.

Introduction

Sewage are very dangerous pollutants because a large number of them have toxic effects. Some of them contain heavy metals, dangerous pollutants that can accumulate in human tissues, animals and plants, as well as in surface and ground water[1]. Data on the distribution of heavy metals in the middle of the ocean, their absorption by plants and their relationship to health are still few, and some of them were toxic to humans even in small concentrations: others were toxic to plant Heavy metal pollution is one of the worrisome problems For many reasons, such as the harmful effect of some of these elements on living things, even if they are found in few concentrations , and some of them have toxic and carcinogenic effects[2]. Heavy metals accumulate in the bodies of animals that live in the aquatic environment and may lead to their death under the influence of high concentrations. As well as heavy metals are transmitted to human in food chains by fish, algae and plants, and these pollutants can reach agricultural soil with polluted water.

The scientists exerted a great effort through the studies they conducted based on the methods of treating sewage which come at the forefront of importance of Biological Treatment which mean the use of different organisms such as bacteria, fungi, algae and plants in the treatment and removal of toxic pollutants (organic and inorganic substances and heavy metals) from polluted water as one of the best modern methods for treating pollutants[3], and therefore the removal of this heavy metals in water resourses must be improved, especially those who depend basicly on microorganisms and yeasts.

Determination of heavy metals from sewage samples

100 ml of the sample was taken and placed in the digestion flasks, and 6 ml of nitric acid was added to it HNO_3The concentrate was placed in water bath at a temperature of 70°C for 30 minutes, then the samples were left to cool and 6 ml of concentrated nitric acid was added to it on Hot plate At a temperature of 70°C until it reaches a temperature before drying, then 2 ml of hydrochloric acid is added to it Hcl The concentrate is 50 ml of

distilled water and then separated using a centrifuge at 3600 rpm for one minute After the separation process was completed, it was taken from the filtrate and 100 ml of distilled water was added to it[3]. The samples (lead and zinc) were examined with a device Atomic Absorption In the central laboratory affiliated to the College of Agriculture at the University of Nineveh, the number of treatments was two, the first was the treatment of using chitosan and lobster, and the second was the treatment of shrimp chitosan with a comparison with the control treatment (control) sewage with three replicates for the purpose of statistical analysis The research aims to use bread yeast cells in biological sewage treatment of heavy metals[4]

Research materials and methods

materials and tools

Yeast Extract Peptone Glucose (YEPG) Medium The modified liquid consists of yeast extract, malt extract, peptone water, glucose, distilled water

The modified solid medium (YEPG) consists of the same components of the previous medium with the addition of agar-agar to solidify the medium, Standard solution of zinc metal in the form of aqueous zinc nitrate salt $Zn(NO3)2.6H2O$

Standard solution of lead metal in the form of aqueous lead nitrate salt $Pb(NO3)2.3H2O$.An 4% NaOH sodium water solutions to raise pH from 5.5 to 7 or 9 . And Glacial Acetic Acid to scale down to 4.

A 50ml capacity giass flasks ,Sterile plastic clarifier tubes of 13ml capacity, Sterile plastic bottles of 60ml capacity, PH meter, Parafilm paper,petri dish, Shaker, Incubator, Centrifuge, Autoclave and shker Incubator and Atomic Absorbtion spectroscopy GBC932 AA[1][5].

Application of the effect of heavy metals in yeast cells

After the growth of pure colonies on the solid medium, The liquid modified mediums were prepared (YEPG) to add heavy metals to it according to the following: Prepared 600 ml of the mentioned liquid medium and distribute it to three replicates of 200 ml each. The metal was added to the three replicates with a concentrations of 0.5, 1, 2 ppm and the amount of added metal was determined based on the concentration law

x1Volume 1= Volume 2* Focus2

$V*N=n*v$

N= The concentration of the metal in the standard solution is 1000 parts per million

V= The volume of the metal solution to be added to the medium to obtain the required concentration

n= The concentration of the required metal is equal to 0.5 or 1 or 2 ppm. in this research

v= The volume of the nutrient medium to which the mineral is added is equal to here 200ml.

According to the previous law, 0.1 ml (100 microns) of the metal solution was added to the medium in which the concentration of the metal was 0.5 ppm and 0.2 mL (200 µm) of the metal were added to a concentration of 1 ppm and 0.4 mL (400 µm) of metal at a concentration of 2 ppm. 200 ml of the mentioned liquid medium was prepared for the control samples that did not contain metal or heavy metals distributed over three replicates.

The content of all of the above replicates was distributed among three replicates, the pH value of one of them was set on the value 4 using glacial actic acid and the second was set on the value 7 using concentrated NaOH by which the third was also set to a value of 9 by it.

The replicates of every particular pH was distributed into three replicates, one of them was incubated at the temperature of 5˚C and the second of 25 ˚C, while the third one was incubated at the temperature of 37˚C. These replicates were sterilized and then inoculated with a node-filling needle to culture the developing colonies of bread yeast grown on modified YEPG solid media and incubated at a temperature specific for each sample in a shaking incubator for 48 hours[6].

This research tested the effect caused by heavy metals according to the following: lead, zinc, lead and zinc[7].

Test the effectiveness of yeast in removing heavy metals from a liquid medium

Take 5 ml of the sample after incubation or subject it to the centrifugation process at a speed of 5300 rpm for two minutes. The residual which separated is the yeast cells and its content, and the other part containing the heavy metals, presence of metal in a sample is evidence of the effectiveness of yeast in removing this metal from the aqueous solution under the conditions in which the sample was incubated[8].

Statistical Analysis

The statistical program was used SPSS17 to test correlation coefficient between the different variables in the total samples.

Results and Discussion

The results of the effectiveness of yeast in removing metal or heavy metals from the liquid media after calculating the amounts of zinc and lead removal in samples subjected (each separately) and studying their relationships with metal concentration at incubation temperature and pH The results were as shown in the tables and diagrams below:
Table (1) shows that the minimum amount of the removal of 0.5 ppm zinc in a single form was equal to 0.21 ppm at a temperature of 37 C° and PH = 7, while the value of removal amounted to 0.40 ppm at a temperature of 10 C° PH = 4, as shown in Figure 1.

Table 1. The removal amount of 0.5 ppm of zinc.

Amount of removal (ppm)			Temperature C°
	PH		
9	7	4	
0.38	0.29	0.40	10
0.36	0.24	0.37	25
0.35	0.21	0.38	37

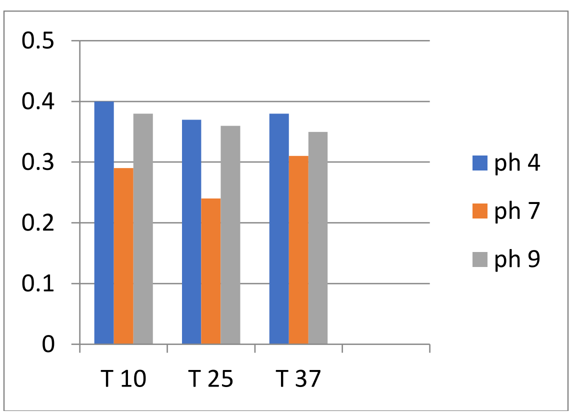

Figure 1. value of removal amounted to 0.40 ppm at a temperature of 10 C° PH = 4

Table(2) showed that the lowest value of the removal amount of 1ppm of zinc in a single form was 0.56 ppm in a degree 25 C° and PH = 9, while the highest value for removal is 0.69 ppm at 25 C° and PH = 4 , as explained in Figure 2.

Table 2. The removal amount of 1 ppm of zinc.

Amount of removal (ppm)			Temperature C°
	PH		
9	7	4	
0.66	0.67	0.65	10
0.56	0.64	0.69	25
0.57	0.61	0.68	37

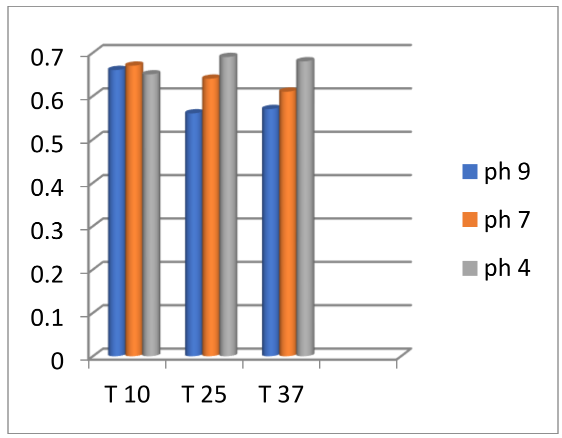

Figure 2. highest value for removal is 0.69 ppm at 25 C° and PH = 4

Table 3 showed that the lowest value of the removal amount of 2ppm of zinc in a single form was equal to 1.27 ppm in a degree 25°C and PH = 9 and 4, while the highest value for removal was 1.42ppm at a temperature of 10 and PH = 4 Figure 3.

Table 3. The removal amount of 2 ppm of zinc.

Amount of removal (ppm)			Temperature C°
	PH		
9	7	4	
1.38	1.36	1.42	10
1.27	1.33	1.31	25
1.28	1.30	1.27	37

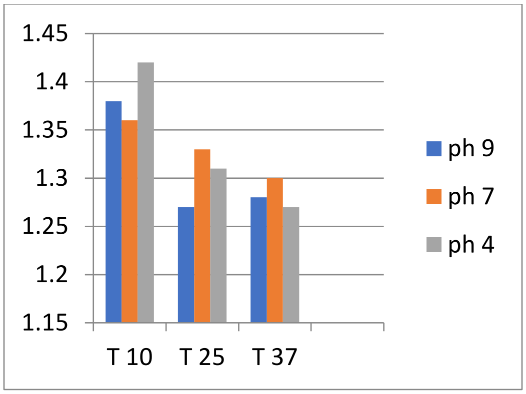

Figure 3. highest value for removal was 1.42ppm at a temperature of 10 and PH = 4

We notice in the previous tables that the amount of single zinc removal increased clearly with the increase of the concentration, and that the best value of pH to remove the metal was 4. It was sometimes observed that the amount of removal decreased at the temperature of 37 C°.

Table (4) showed that the minimum value of the removal amount of 0.5 ppm of lead in a single form was 0.03 ppm in a degree 37˚C and PH = 4, The highest value for removal is 0.42 ppm at a temperature of 10 and PH = 4. Figure 4.

Table 4. The removal amount of 0.5 ppm of lead

Amount of removal (ppm)			Temperature C°
	PH		
9	7	4	
0.35	0.18	0.42	10
0.15	0.08	0.35	25
0.12	0.32	0.03	37

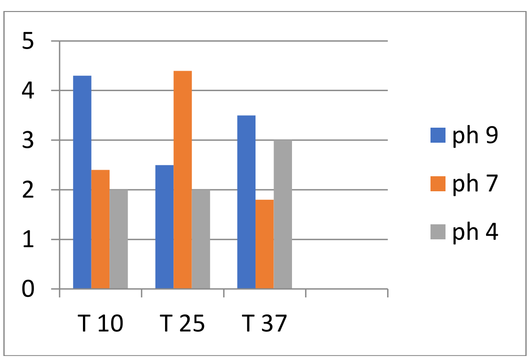

Figure 4. highest value for removal is 0.42 ppm at a temperature of 10 and PH = 4

Table 5 showed that the minimum value of the removal of 1 ppm of lead in a single form was 0.03 ppm in a temrature 37C° and PH = 7 The highest value for removal was 0.87 ppm at both temperatures 25 and 37C° and PH = 4, as in Figure 5.

Table 5. The removal amount was 1 ppm of lead

Amount of removal (ppm)			Temperature C°
	PH		
9	7	4	
0.58	0.66	0.82	10
0.49	0.71	0.87	25
0.76	0.48	0.87	37

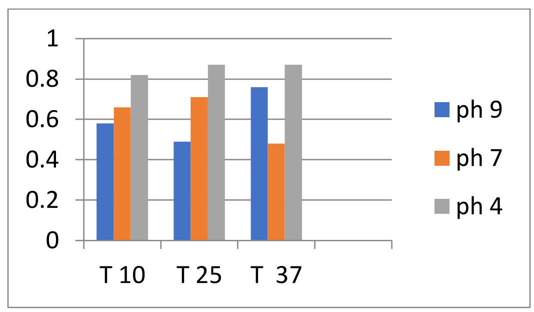

Figure 5. highest value for removal was 0.87 ppm at both temperatures 25 and 37C° and PH = 4

Table (6) showed that the minimum value of the removal amount of 2 parts per million of lead, in its single form was 0.89 ppm in temperature 37˚C and PH = 4. The highest value for removal was 1.99 ppm at a temperature of 25 and PH = 4, Figure 6.

Table 6. The removal amount is 2 ppm of lead

Amount of removal (ppm)			Temperature C°
	PH		
9	7	4	
1.14	1.29	1.60	10

1.28	1.25	1.99	25
1.55	1.45	0.89	37

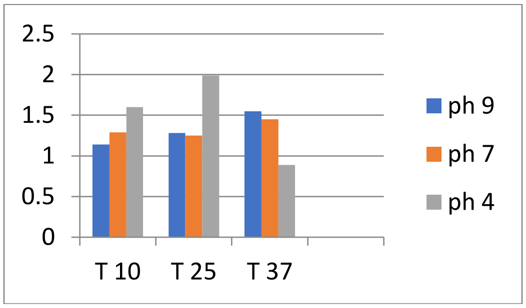

Figure 6. highest value for removal was 1.99 ppm at a temperature of 25 and PH = 4

We note from the previous tables that the amount of single removal of lead increased clearly with its high concentration and the best value of pH for removal was 4. It was also noted that the removal amount sometimes decreased at a temperature of 37 C° [10].
Calculating the amounts to remove zinc and lead together in the subjected samples and studying their relationship with concentration, temperature, incubation and pH results were as shown in the tables and diagrams (7-9).
Table (7) showed that the lowest value of the removal amount of 0.5 ppm of zinc combined with lead was 0.25 ppm at temperature 37 C° and PH = 9. As for the highest value was 0.48 ppm at a temperature of 25 C° and pH = 4. On the other hand, the lowest value for the removal of 0.5 ppm of lead was equal to 0.15 ppm at the temperature of 37 C° and pH = 4 and the highest value = 0.48 ppm at 25 C° degrees and PH=9[11] Figure 7.

Table7. The removal amount is 0.5 ppm of lead + 0.5 ppm of Zinc

Amount of removal (ppm)						Temperature C°
PH						
9		7		4		
Zn	Pb	Zn	Pb	Zn	Pb	
0.35	0.44	0.42	0.16	0.25	0.46	10
0.48	0.48	0.42	0.33	0.48	032	25
0.25	0.32	0.41	0.31	0.26	0.15	37

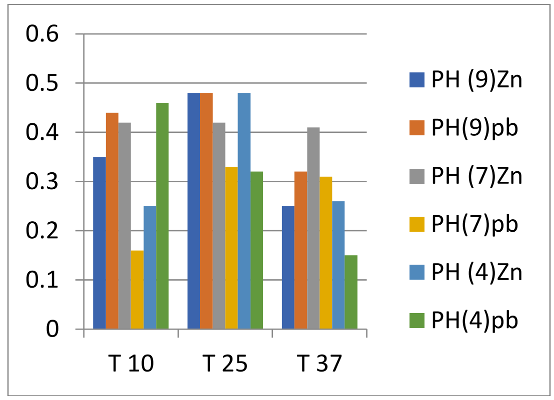

Figure 7. highest value = 0.48 ppm at 25 C° degrees and PH=9[11].

Table(8) showed that the lowest value of removal of 1 ppm of zinc combined with lead was 0.25 ppm in degree 37°C and PH = 7. The highest value for the removal amount was 0.85 ppm at temperature 25 C° and pH = 4, while the lowest value for the removal amount of 1 ppm of lead was 0.01 ppm at temperature 37 C° and pH = 4 and the highest value was 0.95 ppm at temperature 25 C° and PH=9.

Table 8. The removal amount is 1 ppm of lead + 1 ppm of Zinc

Amount of removal (ppm)						Temperature C°
PH						
9		7		4		
Zn	Pb	Zn	Pb	Zn	Pb	
0.48	0.22	0.17	0.32	0.81	0.25	10
0.55	0.95	0.29	0.90	0.85	0.56	25
0.52	0.10	0.25	0.13	0.80	0.01	37

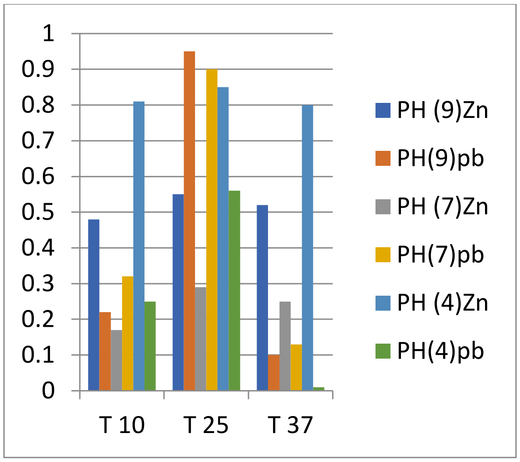

Figure 8. pH = 4 and the highest value was 0.95 ppm at temperature 25 C° and PH=9

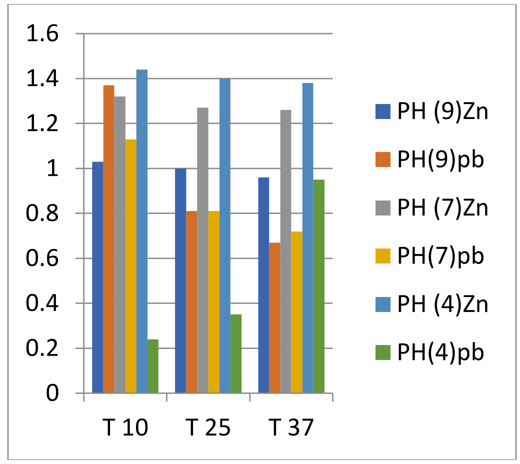

Figure 9. highest value = 1.37 parts per million at temperature 10 C° and PH = 9

Table (9) showed that the lowest value of removal of 2 ppm of zinc combined with lead was 0.96 ppm in temperature 37 C° and PH = 9 The highest value for the removal amount was 1.44 ppm at 10 C° temperature and PH = 4. In contrast, the lowest value for the removal amount of 2 parts per million from lead was 0.24 ppm at 10 and pH = 4 and the highest value = 1.37 parts per million at temperature 10 C° and PH = 9.

Table 9. The removal amount of 2 ppm of lead + 2 ppm of Zinc

Amount of removal (ppm)						Temperature C°
PH						
9		7		4		
Zn	Pb	Zn	pb	Zn	pb	
1.03	1.37	1.32	1.13	1.44	0.24	10
1.00	0.81	1.27	0.81	1.40	0.35	25
0.96	0.67	1.26	0.72	1.38	0.95	37

We note from the previous tables that the bilateral removal of zinc and lead increased clearly with the increase in their concentrations, and the removal of zinc and lead was approximately equal, and no increase in the amount of one of them was observed over the other, but a decrease was observed in the amount of lead removal compared to removing it alone, The best pH value for zinc removal was equal to 4, but the best of lead removal was equal to 9[12][15].

Incubation temperature did not have a clear effect on the removal amount, but it was noted that most of the minimum values for the removal of the two metals were at a temperature of 37C°, meaning that it was less preferable to the removal than the temperatures 10 and 25 C°.

Finally, the removal amounts of the single and bilateral zinc and lead increased with the increase in their concentration, and this is agree with the results of other researches, as the higher the concentration of the initial heavy metal in the solution, the greater amount of its removal. And that can be explained that metallic bind with the surface of the cells forming metallic ions complex and this in turn bind a new metallic ions.

The results of the research, which showed a decrease in the amount of removal at high temperature of 37C°, agree with the results of other researches, and this is attributed the dependence of removal on enzymatically regulated metabolic reactions. As the enzymes sensitive to thermal changes are destroyed at high temperatures, a decrease in the level is observed at removal from that it is at the lower temperature.

And the preferred pH value for removal was equal to 4 for zinc and lead in the single removal and of zinc in a binary removal[13]. It agreed with the findings of 2018 because in the acidic medium the minerals are in the form of free positively charged ions that can bind to the cell, but in alkaline media they bind with hydroxyl ions. for the formation of non-dissolved metals hydroxylates of metals. The value of pH preferred to remove lead when mixing with zinc was equal to 9. This agreed with the results, which were explained by increase amount of removal in the alkaline medium by the increase in the places of introduction of metal ions into the cell after structural destruction due to the alkalinity of the medium[14].

Conclusion

Biotechnoligy can be applied by using local bread yeast in removing zinc and lead from sewage when adjusting the appropriate conditions of temperature and pH to reach a new level of removal of these metals .we need to applicate this technology noticing the problems and changes in suitible conditions in order to correct it and control until reaching certain steps to be relied upon in applying this technology on a permanent basis to reach the desired results of reducing heavy metals for sewage and reducing their toxicity with the lowest possible energy and cost[16].

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