Plant Archives Vol. 19, Supplement 2, 2019 pp.1751-1755 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

REMOVAL OF LEAD FROM AQUEOUS SOLUTIONS USING THE BIOFILM FORMED BY *LEUCONOSTOC MESENTROIDES* AND *LACTOBACILLUS CASEI*

Ahmed S.A. Salman[1] and Asmaa Sabah Ahmaed[2]

[1] General commission for Irrigation and Reclamation projects, Ministry of Water Resources, Iraq

[2] Department of Food Sciences, College of Agricultural Engineering Sciences, University of Baghdad, Iraq

Abstract

In this study, the efficiency of the biofilm formed by the two bacterial isolates *Leuconostoc mesentroides* and *Lactobacillus casei* were measured separately in the removal of lead from aqueous solutions, and the effect of temperature, contact time and the concentration of lead in the solution on the process of removal by the biofilm were studied .Maximum removal rate of lead were measured at contact time 2 hrs. for *Leuconostoc mesentroides* and 4hrs. for *Lactobacillus casei* at 25^{o}C ,10mg\L and 14 days incubation time to form the biofilm, then the biofilm formed by the bacterial isolates can be hyper- bioremediator and reduced the lead pollution. The aims of this research is to obtain the highest removal rate of the lead from aqueous solutions, and the possibility of applying this technique in laboratory biofilter.

***Keywords*:** Lead, *Leuconostoc mesentroides , Lactobacillus casei,* biofilm, biofilter

Introduction

Pollution could be defined as the destruction of the natural purity of the living or non-living habitat by many factors like the addition of undesirable material transmitted through the air, water or soil into the environment as a result of human and natural activities which might cause a serious threat to both life and environment (Shrivastava, 2008).

Water pollution is a worldwide issue and one of the most serious environmental problems. It is defined as the addition of materials to the aqueous environment into a levels where harmful effects to the human health, other living sources and the ecosystem as well (Fereidoun *et al*., 2007).

Water quality is just as important as water quantity for satisfying basic human and environmental needs, yet it has received far less investment, scientific support and public attention in recent decades, even though the two issues are closely linked (Biswas and Tortajada, 2011).

Over the last few years, the contamination level of heavy metals in water ways and soils have increased at an alarming rate, and as a consequence, concentrations of toxic metals in vegetables and grains have increased. This poses a significant threat to humans, other living organisms and the environment (Khan *et al.*, 2015)

Lead (II) is heavy metal poison which forms complexes with Oxo groups in enzymes to affect virtually all steps in the process of hemoglobin synthesis and prophyrin metabolism. (Ademorati, 1996).

The main sources of human exposure to lead include the uses of leaded gasoline, industrial sources such as lead mining, smelting and coal combustion, the use of lead-based paint and lead containing pipes in water supply systems. According to the World Health Organization, the accepted range of (Pb) in water is (0.01 ppm) (Okoye *et al.*, 2010).

Lead is one of the very dangerous heavy metals, which causes many dysfunctions in all human beings. In children, excess amounts of this metal, would probably lead to a decrease in their intelligence quotient (IQ) score, and an impairment of learning, hearing, physical growth, the circulating system, the reproductive system, nervous system especially the brain, kidney functions and some mental disease such as dementia, depression, madness, as well as problems in metabolism system, deficiency of vitamin D, also increasing level of this toxic metal may lead to death (Okoro *et al.*, 2007).

In view of all the damage caused by heavy metals pollution to the environment in general and to human health in particular , effective treatment methods had to be found to eliminate or reduce the risk of heavy metals harmful concentrations in the ecosystem, so the scientists went to experiment and development the biological treatments because of their advanced characteristics comparing with the physical and chemical methods, these features include high efficiency of removal at extreme concentrations, low power consumption and low coast (Halttunen *et al.*, 2007).

One of the vital methods that used in the decontamination of heavy metals from aquatic environments is the use of biofilms formed from different microorganisms such as some algae, yeasts and bacteria and attempt to study and develop the factors that affect the processing of biological treatments using the biofilm (Moga *et al.*, 2018).

Biofilm is a community of microorganisms that develops in moist environments. The organic and inorganic materials that comprise a biofilm can range from decaying products in wastewater, to the millions of species of microorganisms in a lake (Deibel *et al.*, 2003)

Biofilms have the ability to grow on natural materials above and below ground on metals, plastics, medical implant materials, and plant and body tissue. Over time, a biofilm in the appropriate environment will grow and become strongly attached to the surface it lives on (Deibel *et al*., 2003).

The biofilm matrix provides bacteria with a physical barrier against toxic compounds, it is also provides protection against many environmental stresses including U.V. radiation and pH changes (Zaidi, 2011).

Materials and Methods

Activation of bacterial isolates

The bacteria isolates were activated by cultivation in sterilized tubes contains (10 ml) of MRS broth media and incubated at 37^{o}C for 48 h.

Preparation of bacterial cultures to forming biofilms:

The flasks were cultivated with 2% of the bacterial isolates at sterilized conditions, each (1ml) contains (10^8 CFU) and incubated at (37°C) for (14 days) to forming the biofilm.

The plastic biofilm carrier:

It was used as a hard surface to attached by bacterial cells to form the biofilm (Mcquarrie and Boltz, 2011).

It is made from plastic named (Active cell 450) 15mm depth and 22mm diameter. It was obtained from the Ministry of Science and Technology.

Before using it was soaked with deionized water overnight.

Used by immersing about 50 pieces (18 gm.) in 100 ml of MRS broth in each flask in this study.

The flasks sterilized by autoclave at 121°C (15 lbs./inch^2 pressure)for 15 min.

Lead nitrate $Pb(NO_3)_2$: Manufactured by (Merck/ Germany) used as a lead source with concentration of (1000mg\L) as a stock solution to prepare the different concentrations that used in this study .

Determination of residual concentration of lead:

It has been taken about (8-10 ml) from each flask and it was purifying by Millipore filter (0.45µm) for measuring the residual concentration of heavy metals with Atomic absorption spectrophotometer.

The percentage of removal is calculated according the equation below:

The percentage of removal=[concentration of metal before removing - con. After removing /con. Before removing] * 100%

Medium ingredient's effect in lead removal:

For the possibility of removing heavy metals by MRS broth media components. A flask was prepared contained 100 ml of MRS broth, lead was added to the flask and the test was done by Atomic spectrophotometer.

Studying the optimum conditions for lead removal:

To determine the optimum conditions for the removal of heavy metals by the bacterial isolates *(Lactobacillus casei* and *Leuconostoc mesenteroides*) included contact time, temperature and heavy metal concentration,

Contact time:

The lead was added at a concentration of (10mg\L) at different contact times (2, 4, 8, 16 and 24 hrs.).

Temperature:

The lead was added at a concentration of (10mg\L) at different temperatures (15, 25, 35, and 45) °C .

Heavy metals concentration:

Different concentrations of lead have been taken (10, 20, 50, 75 and100) mg\L.

Expanding the removal of lead(Biofilter experiment):

The combined bacterial culture had been used to study the ability of expanding heavy metal removal and apply the removal process by making a biofilter, where a 2litter glass column containing (1500 ml.) of MRS broth was used in the experiment, with the addition of the plastic biofilm carrier to the column and 2% of the bacterial cultivation ,and then the incubation was done at 37℃ for 14 days, after the end of incubation time the lead has been added at a concentration of 10mg\L under the optimum removal conditions and the removal ratio was measured by the atomic absorption spectrophotometer.

Results and Discussion

Effect of cultivation media components in the removal process:

To ensure that the removal processes in the different experiments in this study are due to the biofilm used for the biological treatment and to determine the possibility of holding lead by the components of MRS broth media, this experiment was conducted.

The results showed that the percentage of lead removal by MRS broth components was (0.01%), the percentage did not affect the results of the biological treatments by using the biofilm in the experiments of this study.

The optimum conditions of lead removal:

In order to obtain the highest efficiency of the biofilm in the removal of lead, it is necessary to study some of the conditions that affecting in this process.

Contact time:

The contact time between the ions of lead to be removed and the biofilm is considered as an important factor in determining the efficiency of the removal of the heavy metals ions.

The figure (1) shows the results that obtained from testing different contact times (2, 4, 8, 16 and 24) hrs. The removal rate for the isolation *Lactobacillus casei* for lead were (90%, 100%, 92%, 87% and 85%) respectively, and the results for the isolation *Leuconostoc mesenteroedes* were (99%, 81%, 78%, 69%and 64%) respectively.

Thus the best contact time is 4hrs. for the isolation *Lactobacillus casei* and 2hrs. for isolation *Leuconostoc mesenteroedes*, Sabae *et al.*(2016) mentioned that the contact time is an important factor affecting the process of adsorption heavy metals and the removal ratios reached their highest levels at the beginning of the contact time, while Monachese (2012) found that the contact time of 5hrs. for *Lactobacillus* was effective to remove lead from metal solutions.

As well Halttunen (2007) pointed out that the local isolation is better in the removal of metals than the lyophilized isolation, because of the possibility of damage to the cell wall in the peptidoglycan, resulting weak removal of metals such as iron.

The differences in the structural nature and the proportion of the components of the two biofilms that formed from the bacterial isolates is the reason of the difference in the optimum contact time (Kirillova *et al.*, 2017).

Tunali *et al*. (2006) explained the stability of the removal at a certain point is due to the lack of additional binding sites on the surface of the cells, no matter how long the contact time is.

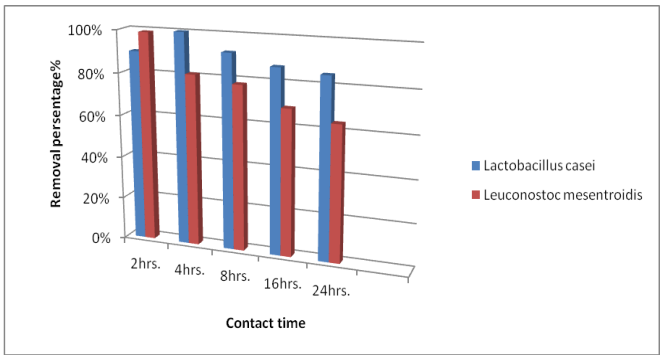

Fig. 1 : Effect of different contact times on lead removal ratio by the biofilm formed from the bacterial isolates

Temperature

The figure (2) showed the results obtained from conducting lead removal experiment after the optimum conditions for previous experiment were established, at a range of temperature (15, 25, 35 and 45)^{o}C. This range has been chosen to comply with the overall maximum temperature during the year in Iraq.

The biofilm of the two bacterial isolates showed the highest removal rates for lead at 25^{o}C.

The removal ratio of lead by bacterial isolation *Lactobacillus casei* were (60%, 100%, 55% and 36%) respectively .While the isolation *Leuconostoc mesenteroedes* the ratio of lead removal were (48% , 99% , 52% , 40%) respectively.

The effect of temperature variation is evident in the results of the experiment, which showed that the optimum temperature of removal is 25^{o}C for the both isolates and this is consistent with Ozturk *et al.* (2004) they mentioned that the optimum temperature for nickel removal is 25^{o}C.

The relative increase in temperature leads to damage to the sites of binding of the metal ions with the surfaces of the biofilms, and opening polysaccharides sites leading to poor link between them (Ozer and Ozer, 2003).

Congeevaram *et al.* (2007) pointed that the temperature of the medium of removal is necessary for the energy on which the mechanism of heavy metals removal by the microorganisms based on, as the temperature affects the stability and the shape of the cell wall in addition to its impact in the ionization status, and these factors may affect the binding sites of different bacterial species, leading to reduction in the removal of heavy metals.

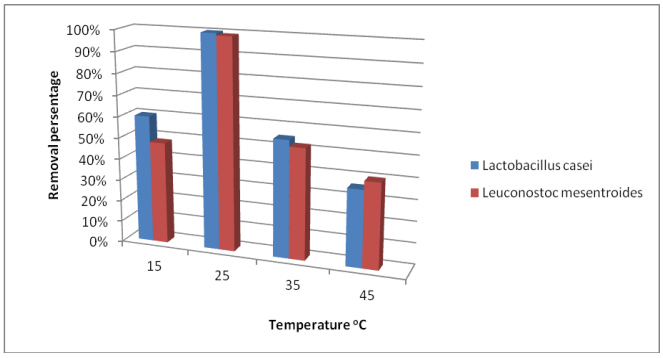

Fig. 2 : Effect of temperature in lead removal efficiency by the biofilm formed from two bacterial isolates(L.C andL.M1)

Heavy metal concentration:

Different concentrations of lead were used in this experiment (10, 20, 50, 75, and 100 mg\L) , to study the effect of these concentrations on the efficiency of the removal process by the biofilm.

The figure (3) showed a reduction in the removal efficiency by increasing the concentration of lead.

The removal ratios of isolation *Lactobacillus casei* for lead were (100%, 93%, 82%, 70% and 58%) respectively , and for isolation *Leuconostoc mesenteroedes* the removal ratio of lead were(99%, 89%, 76%, 65% and52%) respectively

It is clear that the highest removal rate by the two bacterial isolates for lead and nickel is at (10mg\L) and this corresponds to what Yee and Fein, (2003) and Ibrahim *et al.* (2006) mentioned and confirmed that the binding of *Lactobacillus* bacteria with heavy metals depends on the type and concentration of the metal ,also depends on the bacterial strain and its resistance to the metal itself .

The reduction in the removal ratio is due to the insufficient binding sites of the metal ions with the biofilm to complete the removal process, the higher the concentration of the element , the greater the competition for binding to the link sites in the biofilm, therefore the relationship is inverse between the concentration of the element and the rate of the heavy metal removal from the media (Elsanhoty *et al.*, 2016).

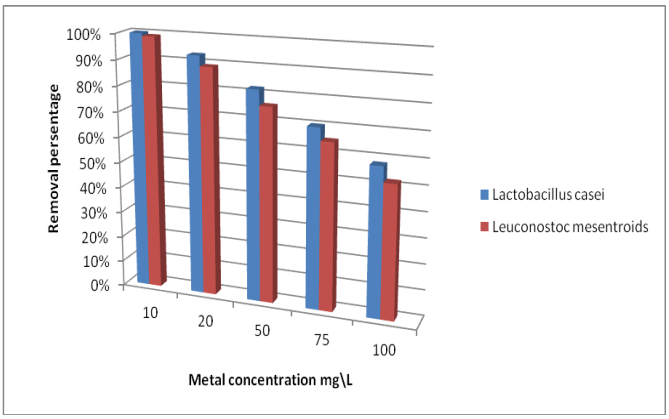

Fig. 3 : Effect of different concentrations of lead in the removal efficiency of the biofilm

Lead removing under the optimum conditions:

The removal experiment was performed under conditions that obtained from previous experiments, the bacterial isolates were incubated for 14days to form the biofilm, and the lead added at a concentration of 10mg/L at 25^{o}C and 2 hrs. contact time for the isolation *Leuconostoc mesenteroedes* and 4hrs. for the isolation *Lactobacillus casei*

The removal rates ranged about (99-100%). It was observed that the biofilm that formed from the above two bacterial isolates is efficient in removing lead.

Expanding the removal process (Biofiltration):

An experiment was conducted by using a biofilter from the combination of two bacterial isolates *Lactobacillus casei* and *Leuconostoc mesenteroedes* in the form of a glass column, containing the MRS broth media and the biofilm that formed by the growth of the two bacterial isolates above, the lead added at a concentration of (10mg/L) under the optimum

conditions from the previous experiments, the removal rate was (90%).

The reduction in the removal ratio compared with the individual bacterial cultures (the removal rate 99-100%) is due to the variation in the contact time between the two bacterial isolates, in this experiment an average of 3 hrs. of contact time was adopted, so it may be the reason for the relatively low lead removal rate (Elsanhoty *et al.*, 2016).

The ease and efficiency of using the initial design of the biofilter was observed in this experiment, where the heavy metal ions pass the layers of the biofilm on the same moving beds bioreactors (MBBR) way, after entering the samples that contaminated with heavy metal from the top of the glass column, then extracted after the biofiltration process is done from the bottom of the column, as shown in figure (4).

In the case of design a field biofilter, the flow of polluted samples can be considered as in this experiment.

Fig. 4 : The experiment of biofilter in the laboratory

References

Ademorati, C.M.A. (1996). Case studies of bioaccumulation of heavy metals. Environmental chemistry and Toxicology. Foludex press Ltd. Ibadan, Nigeria, 130-136.

Biswas, A.K. and Tortajada, C. (2011). Water Quality management: An Introductory Framework', International Journal of Water Resources Development, 27: 1, 5–11.

Congeevaran, S.; Dhanarani, S.; Park, J.; Dexilin, M. and Hamaraiselvi, K. (2007). Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates. J. of Hazardous Material. 146: 270-277.

Deibel, P.H.D. and Jean Schoeni, PH.D. (2003). Develop a Defense Against Biofilms. Food Safety Magazine Jan. 2003.

Elsanhoty, R.M.; Al-Turki, I.A. and Raamadan, M.F. (2016). Application of lactic acid bacteria in removing heavy metals and aflatoxin B1 from contaminated water. Water Science and technology. 74(3): 625-638.

Fereidoun, H.; Nourddin, M.S.; Rreza, N.A.; Mohsen, A.; Ahmad, R. and Pouria, H. (2007). The Effect of Long-Term Exposure to Particulate Pollution on the Lung Function of Teheranian and Zanjanian Students, Pakistan Journal of Physiology, 3(2): 1-5.

Halttunen, T.; Salminen, S. and Tahvonen, R. (2007). Rapid removal of lead and cadmium from water by specific lactic acid bacteria. International journal of food microbiology, 114(1): 30-35.

Ibrahim, F.; Halttunen, T.; Tahvonen, R. and Salminen, S. (2006). Probiotic bacteria as potential detoxification tools: assessing their heavy metal binding isotherms. Can. J. Microbiol. 52:877-885.

Kirillova, A.; Maxson, R.; Stoychev, G.; Gomillion, C.T. and Ionov, L. (2017). 4D biofabrication using shape‐morphing hydrogels. Advanced Materials, 29(46): 1703443.

Khan, A.; Khan, S.; Khan, M.A., Qamar, Z. and Waqas, M. (2015). The uptake and bioaccumulation of heavy metals by food plants, their effects on plants nutrients, and associated health risk: a review. Environmental Science and Pollution Research, 22(18): 13772-13799.

McQuarrie, J.P. and Boltz, J.P. (2011). Moving bed biofilm reactor technology: process applications, design, and performance. Water Environment Research, 83(6): 560-575.

Moga, I.C.; Iordache, O.I.; Petrescu, G.; Pricop, F. and Dumitrescu, I. (2018). June. Polyethylene Based Materials for Biofilm Carriers Used in Wastewater Treatment. In IOP Conference Series: Materials Science and Engineering, 374(1): 012080. IOP Publishing.

Monachese, M.A. (2012). Sequestration of lead, cadmium and arsenic by Lactobacillus species and detoxication potential. Thesis. The University of Western Ontario. London, Ontario, Canada

Okoro, I.; Oriaku, C. and Ejike, E. (2007). Lead Characterization of street dust in some cities of south eastern Nigeria. Research Journal of Applied Sciences, 2(1): 39-42.

Okoye, A.I.; Ejikeme, P.M. and Onukwuli, O.D. (2010). Lead removal from wastewater using fluted pumpkin seed shell activated carbon: Adsorption modeling and kinetics. International Journal of Environmental Science & Technology, 7(4): 793-800.

Özer, A. and Özer, D. (2003). Comparative study of the biosorption of Pb (II), Ni (II) and Cr (VI) ions onto S. cerevisiae: determination of biosorption heats. Journal of hazardous materials, 100(1-3): 219-229.

Öztürk, A.; Artan, T. and Ayar, A. (2004). Biosorption of nickel (II) and copper (II) ions from aqueous solution by *Streptomyces coelicolor* A3 (2). Colloids and Surfaces B: Biointerfaces, 34(2): 105-111.

Sabae, S.Z.; Refat, B.M. and Tahoun, U.M. (2016). Biosorption of Copper, Lead and Cadmium using bacterial biomass of Pseudomonas putida isolated from El-Manzala Lake, Egypt.

Shrivastava, J. (2008). Managing water quality with aquatic macrophytes. Rev. Science and Biotechnology. 7: 255-266

Tunali, S.; Cabuk, A. and Akar, T. (2006). Removal of lead and copper ions from aqueous solutions by bacterial strain isolated from soil. Chemical Engineering Journal, 115(3): 203-211.

Yee, N. and Fein, J.B. (2003). Quantifying metal adsorption onto bacteria mixtures: a test and application of the surface complexion model. Geomicrobiol. J. 20:43-60.

Zaidi, A.H. (2011). Biofilm development and toxic compound resistance in *Lactococcus lactus*. University Library Groningen][Host].