v. 2, n. 4, 2022

International Journal of Biological and Natural Sciences

BIOREMEDIATION OF LEAD IN WATER BY TWO PURE MICROORGANISMS AND BACTERIA-YEAST CONSORTIUM

Marisol Espinoza-Castañeda
Universidad Autónoma Metropolitana
Departamento de Ciencias Básicas
Ciudad de México, México
https://orcid.org/0000-0002-1741-6569

Ana Victoria Selene Gómez Castelán
Universidad Autónoma Metropolitana
Departamento de Ciencias Básicas
Ciudad de México, México

María Rita Valladares Rodríguez
Universidad Autónoma Metropolitana
Departamento de Ciencias Básicas
Ciudad de México, México

Erasmo Flores Valverde
Universidad Autónoma Metropolitana
Departamento de Ciencias Básicas
Ciudad de México, México

María Teresa Castañeda Briones
Universidad Autónoma Metropolitana
Departamento de Ciencias Básicas
Ciudad de México, México

Resumen: La contaminación del agua por metales pesados, continúa siendo una problemática de interés ambiental, por lo que evaluar alternativas más eficientes para su remoción, es de gran importancia. En este artículo se presentan los resultados obtenidos al aplicar procesos de biorremediación microbiana mediante biosorción y bioacumulación para la remoción de plomo en agua mediante el uso de la bacteria *Pseudomonas putida* y la levadura *Saccharomyces cerevisiae*, en cultivos puros, en cuyo caso los procesos que resultaron más eficientes fueron aplicados en el consorcio bacteria-levadura. Las remociones más altas se alcanzaron con el proceso de biosorción, utilizando *Pseudomonas putida* inactivada en autoclave, ya que se obtuvo una remoción del 100 % en 24 horas, mientras que con *Saccharomyces cerevisiae* inactivada en baño María se obtuvo una remoción del 90.23 %. Posteriormente se realizó la evaluación de los microorganismos en consorcio, obteniéndose una remoción de plomo del 52.11 %, demostrando que el uso de cultivos puros de dichos microorganismos inactivados son una alternativa eficiente, rápida, confiable y de bajo costo, ya que al usar los microorganismos inactivados se reduce el uso de medios de cultivo para su preservación.
Palabras clave: Plomo, biosorción, *Pseudomonas putida*, *Saccharomyces cerevisiae, biorremediación microbiana.*

INTRODUCTION

The continuous environmental pollution by heavy metals is a problem of high importance at international level, they are chemical elements that have a density greater than 5 g/cm^3 and are not biodegradable. They are found naturally in the earth's crust at low concentrations, some of which are nutrients for flora and fauna, but at high concentrations under certain conditions can severely degrade the environment, affecting people (Caviedes et al., 2015; Rigoletto et al., 2020; Shaban et al., 2016).

Mercury (Hg), arsenic (As) and lead (Pb) are some of the most studied heavy metals due to their abundance and high toxicity to humans (Caviedes et al., 2015; Zango et al., 2020). The problems arising from them are due to their continued use in the industrial and mining sectors, as well as in the manufacturing processes of paint, pesticides, electronic products, munitions, among others, so that in some cases it is impossible to do without them. As a result of bad handling, poor practices and discharges of untreated waste, these metals are incorporated directly or indirectly into the air and soil as well as into aquifers, severely altering the environment, resulting in be one of the most important problems because these metals are persistent and highly toxic and can be incorporated into the food chain (Uslu & Tanyol, 2006; Rodríguez, 2017).

In particular, the contamination of water by Pb causes serious health effects, since it is highly toxic, due to a blockage in the biological functions of living beings causing, in some cases, irreversible damages. Its main sources of emission to the environment are the smelting, recycling of batteries, paints and pigments, as well as the mining and electronics industry, among others (Boskabady et al., 2018). Upon entering the environment is mobilized in it being able to bioaccumulate in various species of flora and fauna, on the other hand, generates affectations in the nervous, immune, reproductive and cardiovascular systems of the human being, where the effect is proportional to the amount of metal present in the body; Children usually have a higher absorption of Pb compared to adults. Once absorbed, most of it remains in the blood and spreads to the liver, kidney, bone marrow and central nervous system, as well as to the

bones (Wani et al., 2015; Beltrán-Pineda & *Gómez-Rodríguez, 2016;* Medfu et al., 2020).

Due to the innumerable cases of water contamination by heavy metals, various physicochemical techniques have been implemented for their decontamination, such as chemical precipitation, membrane filtration, ion exchange, coagulation and flocculation, among others, which are able to eliminate pollutants, but have shown some deficiencies such as high energy consumption, toxic sludge generation and high maintenance costs (Abdel-Raouf & Abdul-Raheim, 2017) reasons why other types of effective technologies based on biological decontamination have been contemplated, such as bioremediation, which has proven to be very efficient, since it allows the reduction of costs in the process and does not generate as many negative impacts on the environment (Kanamarlapudi et al., 2018).

In recent years, microbial bioremediation and biosorption/bioaccumulation processes have been considered as alternative technologies for novel, economical, efficient and ecological treatments, for the elimination of heavy metals in waste water contaminated by the industrial sector (Kanamarlapudi et al., 2018), in addition have demonstrated superior removal capacity to conventional physicochemical processes (Beltrán-Pineda & *Gómez-Rodríguez, 2016). Algae, fungi and bacteria are the most commonly used microorganisms in microbial bioremediation as they are able to tolerate extreme conditions of pH, temperature and high concentrations of metals, making them viable either as active (living) or inactive (dead) biomass, for the various bioremediation processes (*Ayangbenro & Babalola, 2017; Vendruscolo et al., 2017; Zango et al., 2018).

Microorganisms as biosorbents have the ability to effectively remove metal ions in the solution, in addition to reducing the concentration of ppm to ppb efficiently, therefore they are considered ideal for the treatment of complex waste water of high volume and with low concentration of metallic ions (Covarrubias et al., 2015). Bioaccumulation is a mechanism dependent on the metabolism of the microorganism to be used, which means that biomass must be active; This process consists of a first stage, which is the surface adsorption of the metal ion in the cell wall, to be later internalized (bioaccumulated) in the cell, where the metal in some cases is reduced, thus decreasing its toxicity. This process occurs by cultivating the microorganism with the metal to be accumulated, both in the same culture medium since in this way the metabolic process of the microorganism is activated and the intracellular transport systems are activated for the accumulation of metal ions. Biosorption is a metabolically passive process, consisting of the binding of metal ions to the surface of the microorganism. It has several advantages, such as reducing the maintenance costs of microorganisms, since it is a non-invasive passive process, where microorganisms are inactivated, therefore no energy or use of culture media are required for long-term preservation, in addition to a low operational cost. In order for biosorption to be carried out, the microorganism must be suspended in the same solution containing the ions of the metal to be removed, there should be a process of incubation during a certain time so that the equilibrium is reached and biosorption begins, a surface adsorption occurs, in which the cell wall captures the ions, avoiding their availability (Kanamarlapudi et al., 2018; Soares & Soares, 2013; Gupta et al., 2016; Hansda et al., 2016; Zango et al., 2020).

Among the various microorganisms with capacity to bioremediate heavy metals, are the bacteria *Pseudomonas putida* and the yeast *Saccharomyces cerevisiae,* among

others, which have the advantage of being good biosorbents in processes of biosorption and bioaccumulation (Massoud et al., 2019; Vimalnath & Subramanian, 2018).

PSEUDOMONAS PUTIDA

It is an aerobic bacterium, Gram negative, with short bacillus morphology, which is capable of metabolizing various organic and inorganic compounds, as well as showing great resistance to heavy metals and organic solvents. This bacterium plays an important role in the removal processes due to its thin cell wall, as it contains a smaller amount of peptidoglycan, However, the presence of an additional outer layer composed of phospholipids and lipopolysaccharides gives this type of bacteria a negative charge, which facilitates the binding of metals. In addition, due to its reduced size the surface/volume ratio it raises, present in the microscale, which increases the amount of active sites available for absorption, being excellent for sequestering metal ions from industrial effluents (Kanamarlapudi et al., 2018; Bedoya et al., 2019; Poblete-Castro et al., 2012; Timmis, 2002). Great efficiency in the removal of heavy metals has been demonstrated, as reported by Mendoza-Hernández et al. (2010), who demonstrated that *P. putida* presents high resistance to high concentrations of As, Cr and Pb in aqueous solutions, bioaccumulating between 50 and 80 ppm of Pb.

SACCHAROMYCES CEREVISIAE

Fungi are considered economic and ecological biosorbents due to their ease of cultivation and high biomass yield, among which are yeasts, that are single-celled systems. Most of the yeast biomass biosorbs a large variety of metals; in some cases, may have affinity to a single metal ion. *S. cerevisiae* biomass has ellipsoidal elongated cell morphology; is one of the most studied yeasts for the removal of contaminants, being among the best to decontaminate water, due to its great capacity for biosorption and bioaccumulation. This yeast has proved to be a good biosorbent for Cd, Co, Cr, Hg, Ni, Pb, U and Th (Labuto et al., 2014; Moreno-Rivas et al; 2018). Thippeswamy et al. (2014), reported a removal efficiency of Pb of 61 %, better than that obtained by Deborah and Raj, (2016), of 59 %.

In this work, the removal of Pb was evaluated using *P. putida* and *S. cerevisiae,* both in pure culture, and in yeast-bacteria consortium.

MATERIALS AND METHODS

REAGENTS AND CULTURE MEDIA

For the preservation and development of microorganisms was used for *P. putida* agar of soybean trypticasein (AST) and broth Luria Bertani (CLB), for *S. cerevisiae* agar and broth Sabouraud dextrose (ASD and CSD, respectively), all media were acquired at Comercializadora de Materiales y Reactivos Químicos Tomasa Hernández. For digestion nitric acid (HNO_3) and hydrochloric acid (HCl), concentrates and to prepare the synthetic water and standard solutions of Pb, lead nitrate salt ($Pb(NO_3)_2$) were used; All the reagents were obtained from Sigma-Aldrich.

EQUIPMENT

Automatic vertical autoclave (Lab Tech), atomic absorption spectrophotometer (GBC, Xplora model), Millipore Simplicity Smart water purification system for Milli-Q water, centrifuge (Benchmark Hermle Z206A) and HANNA potentiometer.

MICROORGANISMS

Two different microorganisms were used in this study: the *P. putida* bacteria from the culture collection of the Environmental

Microbiology Laboratory of the Universidad Autónoma Metropolitana-Azcapotzalco of Mexico City and the yeast *S. cerevisiae,* Saf-instant, LESAFFRE brand, freeze-dried.

Optimization of the pb removal process using pure cultures

Inactivation of microorganisms

For the inactivation of *P. putida* and *S. cerevisiae*, two physical treatments were evaluated:

- Inactivation in a water bath: 3 tubes with 5 mL of the microbial culture were placed in a water bath at 85 °C for 1 hour.
- Autoclave inactivation: 3 tubes with 5 mL of the microbial culture were placed in the autoclave at 15 psi and 121 °C, for 15 minutes.

Once these treatments were completed, the efficiency of the inactivation process was determined by reseeding in Petri dishes and tubes with sterile culture media. *P. putida* was resembled in Petri dishes with AST and CLB tubes, which were incubated for a period of 24 to 48 hours at 28 °C, while *S. cerevisiae* was resembled in ASD Petri dishes and CSD tubes, which were incubated for a period of 48 to 72 hours at 28 °C. If microbial development was observed at the end of incubation, that inactivation process was ruled out. On the contrary, the lack of development demonstrated the efficiency of the inactivation process.

From the tubes containing the efficiently inactivated microorganism, duplicate smears were prepared to verify its morphology, because it is of the great importance that microorganisms retain their original morphology so that there are sites where metal ions will bind.

Parameter optimization in the removal of Pb in pure cultures

For the optimization of the parameters for the maximum removal of Pb, the active and inactive biomass of each microorganism was used in pure culture to determine which of the two obtained a higher removal of Pb.

Glass bioreactors containing 7 mL of *S. cerevisiae* in CSD and 28 mL of *P. putida* in CLB were prepared, respectively, in addition to 4.2 mL of synthetic water (Pb solution in Milli-Q water at a concentration of 30 mg/L), which were rated at 70 mL with Milli-Q water, incubated at 30 °C. Table 1 shows the design of biosorption and bioaccumulation processes in bioreactors, with the parameters to be evaluated in each case.

Figure 1 shows the diagram of the 6 types of experiment in which the different values for the optimization of parameters described in Table 1 were evaluated. In each case, the concentration of Pb present in each bioreactor at the beginning and at the end of the experiment using atomic absorption spectrophotometry (EAA) in duplicate.

All experiments were performed in triplicate. As controls, the same number of bioreactors were prepared in the absence of microorganisms.

Process of removal of Pb by the consortium

According to the results obtained during the bioaccumulation and biosorption experiments of Pb with *P. putida* and with *S. cerevisiae* in pure cultures, the most efficient process was determined for each microorganism, to test the bacteria-yeast consortium, under the same conditions, for which the corresponding bioreactors containing the metal, the sterile culture medium, synthetic water and the consortium were prepared. The experiment was performed in triplicate; As a control, 3 bioreactors were prepared in the absence of the consortium.

Microorganism	Process	Parameters to optimize					
		pH		Time of contact (h)		Agitation (RPM)	
P. putida	Biosorption	4	5	2		0	40
	Bioaccumulation	4	5	4		0	40
S. cerevisiae	Biosorption	4	5	6		0	40
	Bioaccumulation	4	5	8		0	40
				24			
Microbial consortium *(P. putida + S. cerevisiae)*	Once the process whose parameters remove a higher amount of the corresponding metal was identified, it was applied to the microbial consortium						

Table 1. Parameters to optimize in bioreactors.

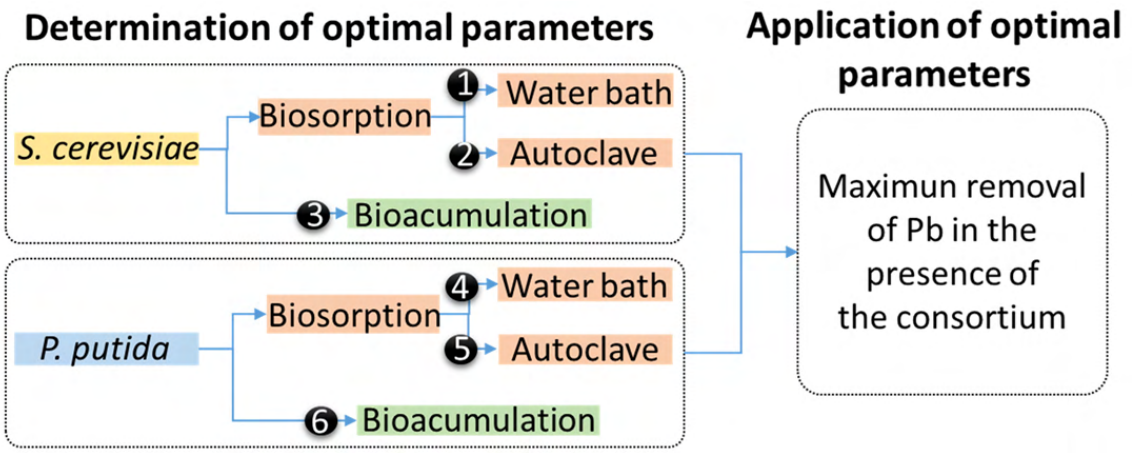

Figure 1. Illustrative diagram of the 6 experiments for optimization of parameters in biosorption and bioaccumulation processes, using pure cultures and the consortium.

Determination of the remaining Pb by atomic absorption spectrophotometry

In all the processes the remaining Pb was determined by means of EAA, for which it was necessary to carry out previously a digestion process to the samples to be analyzed, to avoid possible interfering in the readings. The determination of the concentration of Pb was carried out at a wavelength (λ) of 283.3 nm, using air-acetylene flame.

Digestion of samples

This was done in autoclave, placing 25 mL of each reactor in 50 mL test tubes with screw cap, together with 1 mL of HNO_3 and 1 mL of HCl, concentrated. The tubes were placed inside the autoclave for 1 hour at 121 °C and 15 psi, once the process was completed, the tubes were allowed to cool to room temperature; The solution was subsequently filtered with Whatman filter paper No. 1 and stored at 4 °C until read.

Calibration curve of Pb

The calibration curve was obtained using standard concentrations of: 1, 5, 10, 15, 20 and 25 mg/L and a Milli-Q water target. Each determination was made in duplicate.

Determination of Remaining Pb

Once the incubation time of the Pb removal processes was finished, 25 mL of each bioreactor were taken, which were centrifuged in 50 mL to 5 G Falcon tubes for 5 min to 4 °C, to separate the microorganisms. Subsequently, the supernatant underwent the digestion process and the remaining Pb was quantified by EAA. All the samples were analyzed in duplicate.

RESULTS AND DISCUSSION

INACTIVATION OF MICROORGANISMS

For Pseudomonas putida

It was determined that when applying the process of inactivation by water bath, the bacteria presented thermal resistance, since after being resected and incubated development was observed, so that process was discarded. With respect to autoclave inactivation, no development was observed, which confirmed that this process efficiently inactivated the bacteria.

For *Saccharomyces cerevisiae*

For yeast, both inactivation processes proved to be efficient.

Verification of microbial morphology

Figure 2A-D shows the morphology of microorganisms observed by stained smears before and after the efficient inactivation processes. Figure 2A shows the bacillary bodies of *P. putida* prior to the inactivation process; in comparison with Figure 2B for the sample obtained after the autoclave inactivation process, it is noted that the morphology of short bacilli is preserved without any alteration. Figure 2C shows the ellipsoidal morphology of *S. cerevisiae* prior to the inactivation process; in comparison with Figures 2D and 2E, no alteration in morphology is observed after treatment by water bath and autoclave, respectively.

CALIBRATION CURVE OF PB

The mean absorbance values obtained by EAA for the calibration curve of Pb are shown in Table 2, corresponding to the standards of 1, 5, 10, 15, 20 and 25 mg/L of Pb.

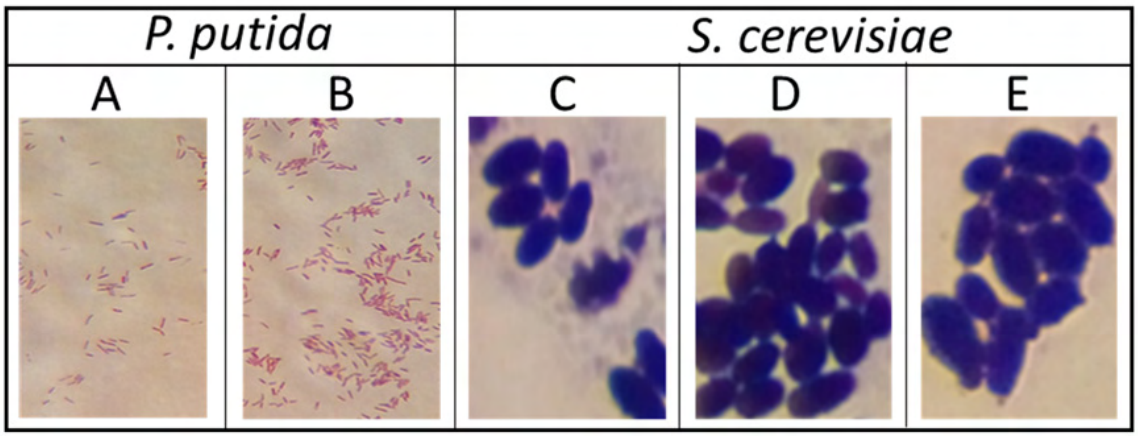

Figure 2. Comparison of the morphology of microorganisms before and after inactivation processes: *P. putida* before inactivation (A) and after inactivation in autoclave (B). *S. cerevisiae* before inactivation (C) and after inactivation in water bath (D) and autoclave (E), not in scale.

Standard	Concentration (mg/L)	Absorbance
1	1	0.004±0.002
2	5	0.021±0.003
3	10	0.041±0.002
4	15	0.062±0.000
5	20	0.083±0.001
6	25	0.102±0.003

Table 2. Absorbances obtained for the different standards of Pb.

Using the data in Table 2, the calibration curve was constructed, which can be seen in Figure 3, where it is observed that as the concentration of Pb increases, the absorbance increases proportionally, with a correlation coefficient of 0.9997.

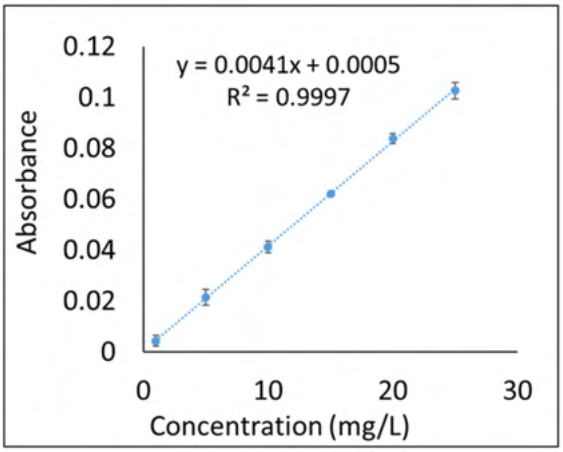

Figure 3. Calibration curve obtained for Pb by atomic absorption spectrophotometry.

This curve served as the basis for determining the concentration of remaining Pb.

OPTIMAL PARAMETERS FOR MAXIMUM REMOVAL OF PB IN PURE CULTURES

The experimentally determined optimal parameters were: pH of 5, constant agitation of 40 RPM, incubation period of 24 h to 30 °C and a concentration of *P. putida* of 36.18 x 10^8 CFU/mL and 48 x 10^8 CFU/mL of *S. cerevisiae,* which were applied in the processes of removal of Pb in pure crops and in the consortium.

DETERMINATION OF THE REMAINING PB

Bioaccumulation by *Saccharomyces cerevisiae*

Figure 4 presents the results obtained during the Pb removal experiments, where it can be observed that at the beginning of the interaction between the yeast and the metal, there is immediately a removal of the same by biosorption. Later, at 24 hours, it is observed that the yeast also bioaccumulated Pb, decreasing the concentration of the metal to 5.942 ± 0.377 mg/L.

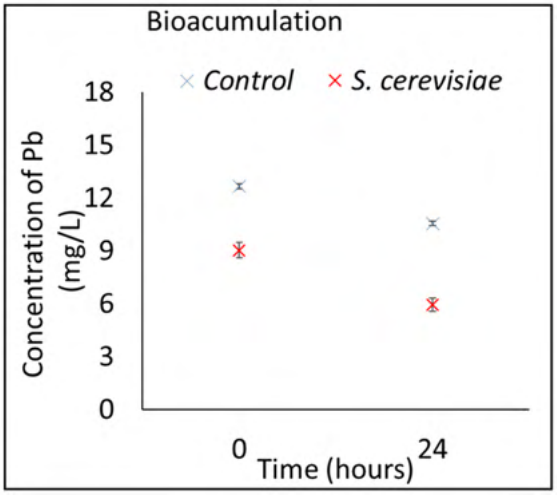

Figure 4. Results obtained by atomic absorption spectrophotometry for the bioaccumulation process of Pb by S. cerevisiae (red) and for the corresponding control (blue).

Biosorption by *Saccharomyces cerevisiae*

Figure 5A presents the results obtained for the evaluation of the removal process with *S. cerevisiae* inactivated in autoclave, observing an initial Pb concentration of 12.657 ± 0.153 in the bioreactor, which at 24 hours decreased to 3.22 7 ± *0.072 mg/L. On the other hand, in Figure* 5B, when the inactivation of the yeast was in the water bath, a final concentration of 1.029 ± 0.330 was observed in the bioreactor.

Table 3 shows the concentrations of Pb at the beginning and end for bioaccumulation and biosorption processes. In all the processes it was observed that when the yeast was added, there was immediately a decrease in the concentration of Pb at the beginning of the experiment, which increased at 24 hours. The removal percentages for each process are also shown; it can be observed that using *S. cerevisiae* inactivated by heating in a water

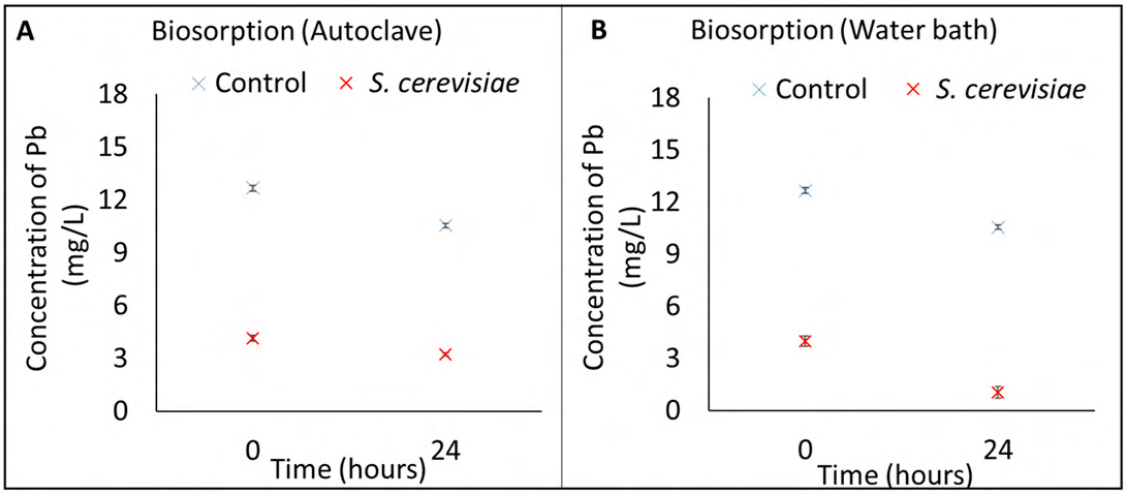

Figure 5. Results obtained by atomic absorption spectrophotometry for the biosorption process using S. cerevisiae (red) and its respective controls (blue), in which the yeast has been inactivated by autoclave (A) and by heating in a water bath (B).

Process			**Concentration** (mg/L)		**Percentage removals**
			Start	**Final**	
Bioaccumulation	Control		12.657 ± 0.153	10.541 ± 0.117	43.63 %
	Experiment		9.033 ± 0.438	5.942 ± 0.377	
Biosorption	Inactivation by autoclave	Control	12.657 ± 0.153	10.541 ± 0.117	69.38 %
		Experiment	4.144 ± 0.173	3.227 ± 0.072	
	Inactivation in water bath	Control	12.657 ± 0.153	10.541 ± 0.117	90.23 %
		Experiment	3.988 ± 0.308	1.029 ± 0.330	

Table 3. Concentration of Pb present in the bioreactor at the beginning and at the end of bioaccumulation and biosorption processes, and percentage of removal by *S. cerevisiae*.

bath, a greater removal of Pb was obtained, since from the beginning the concentration was reduced to 3.988 mg/L and at the end of the 24 hours it decreased even more, reaching 1.029 mg/L of Pb in the bioreactor, which resulted in a removal of Pb of 90.23%.

In the biosorption process in which the inactivation of *S. cerevisiae* was by autoclave, a lower percentage of Pb removal was obtained (69.38 %), and even more so in the bioaccumulation process (43.63 %).

Based on these results, to evaluate the removal of Pb by the bacteria-yeast consortium, the biosorption process was carried out, inactivating the yeast in water bath.

DETERMINATION OF THE REMAINING PB

Bioaccumulation by *Pseudomonas putida*

Figure 6 shows the concentrations of Pb obtained in bioreactors at baseline and after 24 hours. At the initial time of the bioaccumulation process, a decrease in the concentration of Pb is observed immediately that the bacteria (red) has been added with respect to the control (blue), but at 24 h the concentration increased (red), which is because the bacteria in the first few hours bioaccumulated Pb, but during the incubation time the bacteria did not tolerate the toxicity of this metal and released a part of what had already bioaccumulated at the beginning.

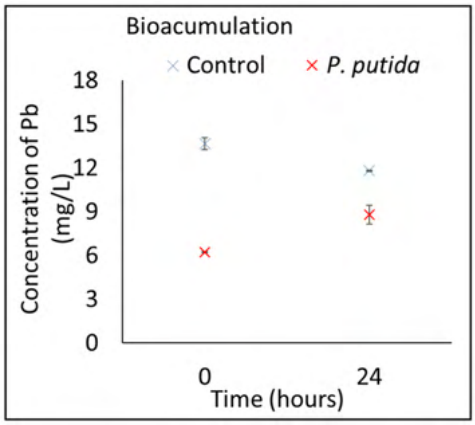

Figure 6. Results of atomic absorption spectrophotometry for the removal of Pb by bioaccumulation by P. putida (red) and corresponding control (blue).

Biosorption by *Pseudomonas putida*

Because the autoclave inactivation process was the only efficient process for the bacterium, it was applied for evaluation in the biosorption process. In Figure 7 it can be observed that *P. putida* has high efficiency in the removal of Pb, since at the beginning of the experiment the concentration of Pb (red) with respect to the control (blue) decreases considerably, at the end of 24 hours of incubation, the bacterium was able to completely remove Pb.

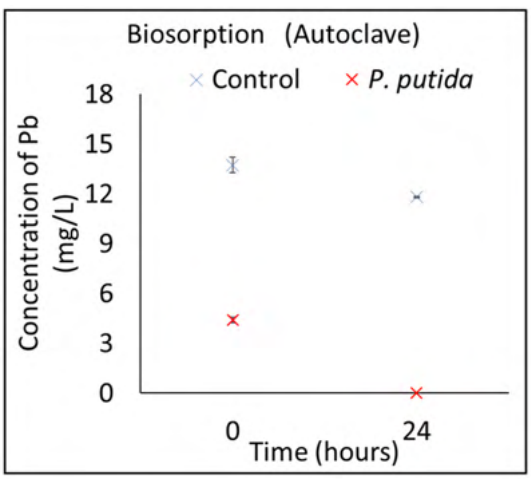

Figure 7. Results of atomic absorption spectrophotometry for the removal of Pb by P. putida by biosorption, inactivating this bacterium in an autoclave.

Table 4 shows the concentrations obtained in bioreactors at the beginning and at the end of the incubation time, as well as the percentage of Pb removed by *P. putida* for bioaccumulation and biosorption processes by inactivating the bacteria in autoclave. In both cases it can be observed that immediately when the bacteria are in contact with Pb, there is a decrease in the concentration of the metal. In the bioaccumulation process, the toxicity of the metal caused there to be no removal of the metal, as at 24 hours the bacteria released a large amount of the metal that had been biosorbed at the beginning of the process. With respect to the biosorption process, the bacterium was able to remove Pb in its entirety.

Based on these results it was determined that the efficiency in the removal of Pb using autoclaved inactivated *P. putida* is fully efficient, since it managed to remove 100% of the metal in 24 hours. This is because the bacteria are able to retain the metal in its cell wall, eliminating the availability of metal in water. Therefore, the process of biosorption with *P. putida* inactivated in autoclave is considered for use in the consortium.

Since the biosorption process inactivating *P. putida* by autoclave sterilization and *S. cerevisiae* by water bath heating were highly efficient for the removal of Pb, the performance of the consortium was evaluated using these conditions in the biosorption process.

IDENTIFICATION OF REMAINING PB IN A BIOSORPTION PROCESS BY THE CONSORTIUM

Figure 8 shows the comparison of the concentrations of Pb in the bioreactor at the beginning and at the end of the removal process by the consortium, observing that at the beginning of the process there is a decrease in the concentration of Pb (red) with respect to the control (blue), which further decreases after 24 hours of incubation.

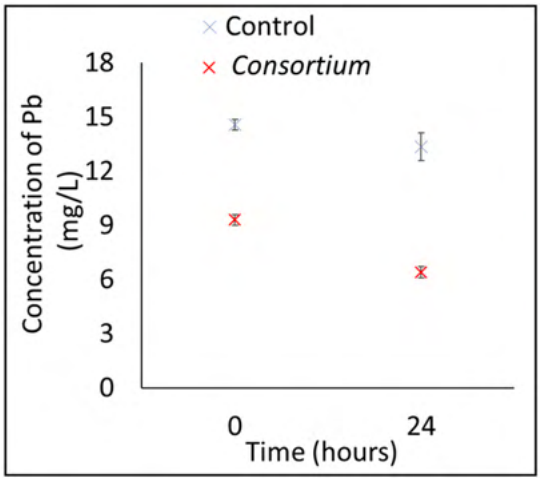

Figure 8. Results of atomic absorption spectrophotometry for the removal of Pb by the consortium through a biosorption process.

Process			Concentration (mg/L)		Percentage removals
			Home	Final	
Bioaccumulation		Control	13.646 ± 0.407	11.801 ± 0.042	There was no removal
		Experiment	6.223 ± 0.049	8.804 ± 0.639	
Biosorption	Inactivation by autoclave	Control	13.744 ± 0.477	11.818 ± 0.045	100 %
		Experiment	4.412 ± 0.186	0	

Table 4. Pb concentration in the bioreactor at the beginning and at the end of bioaccumulation and biosorption processes and percentage of *P. putida* removal.

Biosorption process	Concentration (mg/L)		Percentage removal
	Home	Final	
Control	14.558 ± 0.304	13.345 ± 0.760	52.11 %
Consortium	9.288 ± 0.278	6.390 ± 0.325	

Table 5. Concentration of Pb present in the bioreactor at the beginning and end of the biosorption process with the consortium, as well as the percentage of removal.

Table 5 shows the concentrations of Pb present in the bioreactor at the beginning and at the end of the removal process by the consortium, as well as the percentage of Pb removed, corresponding to 52.11 %, which is a smaller percentage than that obtained during biosorption processes using each microorganism in pure culture.

CONCLUSIONS

In this work, the efficiency in the removal of Pb in a synthetic water was compared, using pure cultures of *P. putida* bacteria and *S. cerevisiae* yeast, as well as the yeast-bacteria consortium.

By applying and comparing Pb removal processes by bioaccumulation and biosorption using *P. putida (*36.18 x 10^8 CFU/mL), the bacterium was found to be more efficient through the biosorption process when inactivated in autoclave, as 100 % of the metal was removed in 24 hours at pH 5, with constant stirring of 40 RPM, incubating at 30 °C. As for the use of yeast *S. cerevisiae (*48 x 10^8 CFU/mL), through the process of biosorption inactivated by heating in the water bath, it was possible to remove 90.23 % of the Pb present in the solution, under the same incubation conditions as the bacterium, which favored that they could be applied in a consortium, which, far from expected, proved to be less efficient, since a removal percentage of 52.11% was obtained, which is significantly less than removed using microorganisms in pure culture.

With the above it is shown that the microbial bioremediation through a biosorption process using pure cultures of *P. putida* and *S. cerevisiae,* can be a great alternative for the removal of Pb present in water, in addition to the fact that the use of inactive microorganisms for the union of metal ions greatly reduces the costs of maintaining them, since they do not require nutrients, at the same time it is a non-invasive process which represents being a great alternative.

ACKNOWLEDGMENT

A.V.S.G.C. thanks to Consejo Nacional de Ciencia y Tecnología (CONACyT) for the support provided through the scholarship 634392.

REFERENCES

Abdel-Raouf, M. S. and Abdul-Raheim, A.R.M. (2017). Removal of Heavy Metals from Industrial Waste Water by Biomass-Based Materials. A Review: Journal of pollution effects and control 05(01), 1–13.

Ayangbenro, A.S., Babalola, O. (2017). A new strategy for heavy metal polluted environments. a review of microbial biosorbents. Int. J. Environ. Res. Public Health 14, 94.

Boskabady, M., Marefati, N., Farkhondeh, T., Shakeri, F., Farshbaf, A. y Mohammad Boskabady, M.H. (2018). The effect of environmental lead exposure on human health and the contribution of inflammatory mechanisms. A review: Environment International 120.

Beltrán-Pineda, M. E., and Gómez-Rodríguez, A. M. (2016). Biorremediación de metales pesados cadmio (Cd), cromo (Cr) y mercurio (Hg), mecanismos bioquímicos e ingeniería genética. Una revisión: Revista Facultad de Ciencias Básicas 12(2).

Caviedes, D. I., Muñoz, R. A., Gualtero, A. P., Rodríguez, D. y Sandoval, I. J. (2015). Tratamientos para la remoción de metales pesados comúnmente presentes en aguas residuales industriales. Revista Ingeniería y Región 13(1), 73–90.

Covarrubias, S. A., García, J. A. y Peña, J. J. (2015). El papel de los microorganismos en la biorremediación de suelos contaminados con metales pesados. Acta Universitaria 25, 40–45.

Deborah, S., y Raj, J. S. (2016). Bioremediation of heavy metals from distilleries effluent using microbes. Journal of Applied and Advanced Research 1(2), 23–28.

Gupta, A., Joia, J., Sood, A., Sood, R., Sidhu, C. y Kaur, G. (2016). Microbes as potential tool for remediation of heavy metals. A review: Journal of Microbial and Biochemical Technology 8, 364–372.

Hansda, A., Kumar, V. y Anshumali, (2016). A comparative review towards potential of microbial cells for heavy metal removal with emphasis on biosorption and bioaccumulation. World Journal of Microbiology and Biotechnology 32, 1–14.

Kanamarlapudi, S. L. R. K., Chintalpudi, V. K., y Muddada, S. (2018). application of biosorption for removal of heavy metals from wastewater. In: Biosorption, (J. Derco and B. Vrana, eds.), Pp. 69-116. IntechOpen, United Kingdom.

Labuto, G., Trama, B., Castro da Silveira, G., de Souza, B., Vitorino, F. y Collazo, R. (2014). Metals uptake by live yeast and heat-modified yeast residue. Ambiente y Agua 66,17–35.

Massoud, R., Rasoul, H.M., Hamzehlou, P. y Khosravi-Darani, K. (2019). Bioremediation of heavy metals in food industry: Application of *Saccharomyces cerevisiae*. Electronic Journal of Biotechnology. 37, 56–60.

Medfu Tarekegn, M., Zewdu Salilih, F., y Iniyehu Ishetu, A. (2020). Microbes used as a tool for bioremediation of heavy metal from the environment. Cogent Food and Agriculture, 6(01), 1–19.

Mendoza-Hernández, J. C., Perea, Y. S., Pretelin, C., Silveti, Á., Martínez, M. A., Pérez, G. Arriola, J. (2010). Biosorción de cromo, arsénico y plomo de soluciones acuosas por cultivos bacterianos en suspensión. Revista Latinoamericana El Ambiente y Las Ciencias, 1(2), 67–73.

Poblete-Castro, I., Becker, J., Dohnt, K., Martins, V. y Wittmann, C. (2012). Industrial biotechnology of *Pseudomonas putida* and related species. Applied Microbiology and Biotechnology 93, 2279–2290.

Rigoletto, M., Calza, P., Gaggero, E., Malandrino, M., & Fabbri, D. (2020). Bioremediation methods for the recovery of lead-contaminated soils. A review: Applied Sciences (Switzerland), 10(10).

Rodríguez, D. (2017). Intoxicación ocupacional por metales pesados. Revista Médica de Santiago de Cuba, 21(12), 3372–3385.

Shaban, N. S., Abdou, K. A., y Hassan, N. E.-H. Y. (2016). Impact of toxic heavy metals and pesticide residues in herbal products. Beni-Suef University Journal of Basic and Applied Sciences 5(1), 102–106.

Soares, E. V. y Soares, H. M. (2013). Cleanup of industrial effluents containing heavy metals: a new opportunity of valorising the biomass produced by brewing industry. Applied Microbiology and Biotechnology 97, 6667–6675.

Thippeswamy, B., Shivakumar, C., y Krishnappa, M. (2014). Study on heavy metals biosorption ability of *Saccharomyces cerevisiae*. International Journal of Biological Research 2, 106–115.

Timmis, K. (2002). *Pseudomonas putida*: a cosmopolitan opportunist par excellence. Environmental Microbiology 4, 779–781.

Uslu, G., y Tanyol, M. (2006). Equilibrium and thermodynamic parameters of single and binary mixture biosorption of lead (II) and copper (II) ions onto *Pseudomonas putida*: Effect of temperature. Journal of Hazardous Materials, 135, 87–93.

Vendruscolo, F., da Rocha, G., y Antoniosi, N. (2017). Biosorption of hexavalent chromium by microorganisms. International Biodeterioration and Biodegradation 119, 87–95.

Vimalnath, S. y Subramanian, S. (2018). Studies on the biosorption of Pb(II) ions using *Pseudomonas putida*. Separation Science and Technology 53, 2550-2562.

Wani, A.L., Ara, A., y Usmani, J. A. (2015). Lead toxicity. A review: Interdisciplinary toxicology, 8(2), 55–64.

Zango, U.U., Yadav, M., Sharma, V., Sharma, J.K., Panwar, S., Dan, S. y Sharma, A.K. (2020). Microbial Bioremediation of Heavy Metals: Emerging Trends and Recent Advances. Research Journal of Biotechnology 15, 164–178.

Zango, U.U., Ahluwalia, S.S. and Sharma, A.K. (2018). Microbial Consortium of *Aspergillus fumigatus, Aspergillus terreus* and *Paenibacillus dendritiformis* in the bioremoval of cadmium, International Journal of Pharmaceutical Research 10, 230-238.