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Application of Box-Behnken model to study biosorption of lead by *Saccharomyces cerevisiae* and *Candida tropicalis* isolated from electrical and electronic waste dumpsite

Abioye O.P.[1*], Usman A.U.[1], Oyewole O.A.[1], Aransiola S.A.[2]
[1]Department of Microbiology, Federal University of Technology, PMB 65, Minna, Nigeria
[2]Bioresources Development Centre, National Biotechnology Development Agency, KM 5 Ogbomoso/Iresapa Road, P.M.B. 3524, Onipaanu, Ogbomoso, Nigeria
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*to whom all correspondence should be addressed: e-mail: bisyem2603@yahoo.com
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Abstract

Samples of soil acquired from electrical and electronic waste (e-waste) dumpsite in Kansuwan gwari, Minna, Nigeria were utilized for the isolation of microorganisms utilized as a part of lead (Pb) biosorption studies. The soil specimen was cultured for *Saccharomyces cerevisiae* and *Candida tropicalis*. Box Behnken plan was utilized to advance the removal of lead by the organisms. The factors utilized as a part of the Box Behnken plan were temperature, pH and concentration of Pb. *Saccharomyces cerevisiae* was enhanced by the Box Behnken design, a temperature of 25 °C, pH of 6 and a lead of 2 ppm concentration prompted a 70.8% lead removal. The investigation of variance for *Candida tropicalis* demonstrated that the reaction had a probability estimation of 0.851 for the regression. This likewise showed the model was not a solid match for the plan since the p-value (0.851) was more prominent than 0.05. *S. cerevisiae* gave a probability estimation of 0.029 for the regression, which demonstrated that the model was a solid match for the outline since the p-value was less than 0.05. More models may be connected to different fields in microbiology as it diminishes the ideal opportunity for protracted examinations and tends to limit the test to how successfully the outcomes can be accomplished. This study can be connected to the master plan of the effective pH, temperature and different parameters for the clean up of contamination in the environment.

Keywords: Yeast, e-waste, Box Behnken, lead, pH, temperature.

1. Introduction

Electrical and electronic waste (e-waste) is a term used to cover almost all types of electrical and electronic equipment (EEE) that have or could enter the waste stream. Although e-waste is a general term, it can be considered to cover Televisions, computers, mobile phones, white goods (e.g., fridges, washing machines, dryers), home entertainment and stereo systems, toys, toasters, kettles – almost any household or business item with circuitry or electrical components with power or battery supply (Anand *et al*., 2015). In general, large household appliances represent the largest proportion (about 50 per cent) of e-waste, followed by information and communications technology equipment (about 30 per cent) as well as consumer electronics (about 10 per cent). It is estimated that globally, e-wastes generation is growing by about 40 million tonnes a year (Schluep *et al*., 2009).

Human exposure to soils contaminated with lead can lead to ingestion and inhalation of dust particles contaminated with lead, which in turn can lead to potentially serious health consequences due to lead intoxication (Jan *et al*., 2015; Chisti, 2018). The lead content in uncontaminated soils of remote areas is generally within the range of 10-30 mg Pb/kg (UNEP, 2010). Lead concentration in soil beside roadways and in towns is reported to be up to several thousand mg Pb/kg, whereas soils adjacent to smelters and lead battery factories are reported at up to 60,000 mg Pb/kg (UNEP, 2008). Lead compounds accounted for 10 percent of global consumption of lead between the years 1970 and 2003. Lead – target organs are the bones, brain, blood, kidneys and thyroid gland (Agency for Toxic Substances and Disease Registry (ATSDR) 2017; Chisti, 2018). It accounts for most of the cases of paediatric heavy metal poisoning. Lead poisoning is the leading environmentally induced illness in children. At greatest risk are children under the age of 6 years because they are undergoing rapid neurological and physical development (ATSDR, 2017). In the environment, lead bioaccumulates in most organisms and is toxic to plants, animals and micro-organisms. Young fishes are more susceptible to lead poisoning than mature fishes or their eggs. Symptoms of lead toxicity in fish include spinal deformity and blackening of the caudal region (rear part of the fish).

Removal of toxic heavy metals from the environment is essential from the standpoint of environmental pollution control. The conventional processes used to remove heavy

metal ions from the contaminated environments include solvent extraction, chemical precipitation, ion exchange, activated carbon adsorption, chelation, reverse osmosis, coagulation–precipitation, electrochemical operation and filtration (Gunatilake, 2015; Kaljahi, 2016; Malik *et al*., 2017). However, Traditional techniques of heavy metal soil remediation are costly and may cause secondary pollution. Series of approaches is being practiced in order to reclaim land contaminated with lead (Abioye *et al*., 2013).

These disadvantages are especially apparent at low metal concentrations often encountered in wastewaters. Therefore, it is pertinent to develop efficient and environmentally compatible means able to remove or detoxify heavy metals in an economical way (Pawar and Bhosale, 2018). There are various *in situ* and *ex situ* remediation methods adopted to remove or reduce or extract the contaminant from the soils. Among those, bioremediation or biosorption is found to be more advantageous than any other methods because it is reliable, feasible, highly efficient, less chemical usage and sludge generation, regeneration of biosorbent as well as ease in process optimization, doesn't require any skilled labors, mechanical equipment and continuous monitoring (Pawar and Bhosale, 2018).

Biosorption using microbial biomass as the adsorbent has emerged as a potential alternative technique to the existing methods for metal removal (Gupta and Diwan, 2017).The use of biological material, including living and non-living microorganisms, in the removal and possibly recovery of toxic or precious metals from industrial wastes, has gained important credibility during recent years. This is because of the good performance, minimization of chemical/biological sludge and low cost of these materials (Kanamarlapudi *et al*., 2018). Microorganisms including bacteria, algae, fungi and yeasts, take up these metals either actively (bioaccumulation) and/or passively (biosorption) (Gupta and Diwan, 2017). The biosorption mechanism depends on whether the organisms are living or dead, the type of microorganisms, and the element species (Carolina *et al.*, 2017). The use of non-living microbial cells as biosorbents has been shown to be an effective means for removal or recovery of heavy metals from aqueous systems (Carolina *et al.*, 2017). The presence of lead has been observed in different types of industrial effluents. Lead is responsible for environmental pollution; with varying effects on human depending on the concentration. This is because of their toxic, persistent, and non biodegradable nature as they accumulate in the food chain causing several deleterious effects on human life (Kanamarlapudi *et al.*, 2018). In addition, lead, like other heavy metals is toxic even at low concentration. Biosorption of lead has attracted the attention in recent years as an alternative to conventional methods for its removal from water and wastewater. For example, the biosorption of Pb present in the storage battery industry wastewaters in polluted environment by *Rhizopus arrhizus* was investigated by Bahadir *et al*. (2007). Kanamarlapudi *et al*. (2018) also reported the ability of *Aspergillus niger* to biosorp Pb(II). Microorganisms have been used as biosorbent because of the presence of metal binding functional groups (Kanamarlapudi *et al.,* 2018).

The response surface methodology (RSM) is a collection of mathematical and statistical techniques for designing experiments, building models, evaluating the effects of factors and searching optimum condition of factors for desirable responses (Box *et al*., 1978). The optimisation process of this methodology involves studying the response of the statistically designed combinations, estimating the coefficients by fitting it in a mathematical model that fits best the experimental conditions, predicting the response of the fitted model and checking the adequacy of the mode. The most common designs include central composite design (CCD) and Box-Behnken design (BBD) (Box *et al*., 1978). Box-Behnken, a spherical and revolving design, has been applied in optimisation of chemical and physical processes (Muthukumar *et al*., 2003) because of its reasoning design and excellent outcomes. As a collection of statistical and mathematical techniques for developing, improving and optimizing processes, RSM is specifically applied in situations where several input variables potentially influence a performance measure or quality characteristic of the product or process (Suresh *et al*., 2011).

Box-Behnken design is having the maximum efficiency for an RSM problem involving three factors and three levels. The number of runs required is also less compared to a central composite design. This study is hence designed to remove lead contaminant from e-waste dumpsite site using yeast isolated from the dumpsite soil. This site was chosen because of the strong possibility of heavy metal contaminants that may be present in that site which (when present there) could be a good habitat for several microorganisms that could possibly include yeasts. Microbes around such an environment may have the ability to accumulate and remove metals. Yeasts were selected because of several literatures available on the use of bacteria and moulds for removal of metals in a contaminated environment and scanty information regarding the use of yeast isolates. The Box-Behnken is a good design for response surface methodology because it permits: (i) estimation of the parameters of the quadratic model; (ii) building of sequential designs; (iii) detection of lack of fit of the model; and (iv) use of blocks for the interpretations of lead biosorption. Therefore, the aim of this study is to apply Box-behnken model to study biosorption of lead by *Saccharomyces cerevisiae* and *Candida tropicalis* isolated from electrical and electronic waste dumpsite

2. Materials and methods

2.1. Study area and site

The study area was the popular market Kasuwan Gwari located at Minna, Niger State, Nigeria. The study site where soil samples were collected, contained discarded electronics of various kinds including refrigerators, discarded DVD players, air conditioners, radios and dismantled televisions.

2.2. Heavy metal used in the study

Lead nitrate was obtained from Panlac Scientific Equipment Store Minna, Niger State, Nigeria. The lead nitrate was of commercial grade, it had the following specifications: Formula: $Pb(NO_3)_2$, Molar mass: 331.2 g/mol, Density: 4.53 g/cm^3(20°C) and Melting point: 270°C.

2.3. Collection of soil samples

The 100 g of soil sample was collected into a glass jar using a sterile spatula from three different electronic waste dumpsite locations. The soil samples were transported to the Microbiology laboratory of the Federal University of Technology, Minna, Nigeria where the samples were analysed microbiologically.

2.4. Isolation and identification of yeast from soil sample

The standard pour plate method was used for the isolation of the yeast isolates (Fawole and Oso, 2007). The soil sample was serially diluted with sterile distilled water up to 10^{-5} dilution factor. Each colony that appeared on the plate was expressed as one colony forming unit (cfu/g) (Nazir *et al*., 2007). The yeast colonies were sub-cultured repeatedly on yeast extract agar to get a pure culture and was later stored in agar slant containing yeast extract agar. The organisms were characterized morphologically and viewed under a light microscope using (x100) objective lens, this was followed by various sugar fermentation test to identify the yeast isolates.

2.5. Lead solution preparation

Stock metal solution (500 ml) was prepared by dissolving 0.79 g of lead nitrate [$Pb(NO_3)_2$] in deionised water, shake for 15 minutes and then allowed to stand for 24 hours to obtain complete dissolution. Solution was adjusted to pH values 6 and 7 with 0.1 M of NaOH and HCl. The lead concentration was measured using Atomic Adsorption Spectrophotometer (AAS).

2.6. Yeast isolates suspension preparation for biosorption studies

Freshly grown single colonies of the isolates were picked up with an inoculation loop, stirred into 10 ml nutrient broth in a test tube incubated at 25 °C for 60 minutes and maintained as suspension stock for biosorption experiments.

2.7. Lead biosorption studies

Aliquots of 1 ml suspension of the bacterial were inoculated in 50 ml nutrient broth containing different concentrations of lead (0.5, 1.0, 1.5 and 2.0 ppm) (Homaidan *et al*., 2014). Before adding the isolates, the pH of the metal solution was adjusted to pH 6-7 with 0.1 M NaOH and HCl. The conical flasks were incubated at 37°C in an incubator with a constant shaking. A flask from the experimental setup was taken out every week (i.e. after 7, 14, 21 and 28 days) and were centrifuged at 4000 rpm for 25 minutes. After centrifugation, the supernatant was digested with HNO_3 and metal concentration was determined by Atomic Adsorption Spectrophotometer (AAS).

2.8. Effect of incubation temperature on biosorption rate

Nutrient broth medium (50 ml) containing 0.5 ppm of lead with 1 ml aliquot of *Candida tropicalis* and *Saccharomyces cerevisiae* yeast suspension (24 hours old) in conical flasks were incubated at temperatures of 25 and 37 °C (Homaidan *et al*., 2014) with constant shaking respectively. All experiments were conducted in triplicates. The lead concentration in the digested supernatant was measured using Atomic Adsorption Spectrophotometer (AAS).

2.8.1. Effect of pH on biosorption rate

Nutrient broth medium (50 ml) containing varying amounts of lead (0.5, 1.0, 1.5 and 2.0 ppm) was adjusted to pH ranges 6 and 7 (Homaidan *et al.,* 2014) using 0.1 M HCl and 0.1 M NaOH at 37 °C and heavy metal concentration in the digested supernatant was measured using Atomic Adsorption Spectrophotometer (AAS).

2.8.2. Effect of heavy metal concentration biosorption rate

Candida tropicalis and *Sacchromyces cerevisiae* (yeast isolates) were inoculated into nutrient broth medium (50 ml) containing different concentrations of lead (0.5, 1.0, 1.5 and 2.0) respectively. The pH of the medium was adjusted to 7 with 37 °C incubation temperature and heavy metal concentration in the digested supernatant was analyzed using AAS. Percentage biosorption was calculated by using the Beer Lambert's law in the concentration range studied.

$$\%\text{biosorption} = \frac{\text{Initial metal concentration} - \text{final metal concentration}}{\text{Initial metal sorption}} \times 100$$

2.9. Box Behnken design

All results of this research work were tested with probability value of 5% (0.05). The probability value shows the level at which the hypothesis tested was either accepted or rejected. A model was constructed for each of the organism considered in this research work, which include; *Candida tropicalis* and *Saccharomyces cerevisiae***.** Minitab 17 version was used for analysing the data. The equation used for the coded value -1, 0 and 1 was,

$$Y = \beta_0 + \beta_1 A_1 + \beta_2 B_2 + \beta_3 A_3 + \beta_{12} A_1 B_2 + \beta_{13} A_1 C_3 + \beta_{23} B_2 C_3 + \beta_{11} A_1^2 + \beta_{22} B_2^2 + \beta_{33} C_3^2$$

Where, A = pH value, B = temperature, C = lead concentration, A, B and C are the factors considered.

The three factor Box Behnken design used in the study for the observed and predicted which successfully followed the model is shown in Table 1.

2.10. Statistical analysis

The analysis of variance (ANOVA) was used to check the adequacy of the developed models at 95% confidence level (Altin *et al*., 2007; Pawade *et al*., 2008). If the calculated value of the F-ratio of the regression model is more than the standard value specified (F-Table) for 95% confidence level, and then the model is considered adequate within the confidence limit (Liao and Shiue, 1996).

Table 1. Three factor Box Behnken design used in this study

Experimental runs			Values			
S/N	A (pH)	B (Temp)	C(lead con)	Observed	Predicted	Diff/error
1	-1	1	0	74.20	72.55	1.65
2	-1	1	0	70.20	66.77	3.43
3	-1	1	0	67.17	65.40	1.77
4	-1	1	0	66.06	66.77	-0.71
5	1	0	-1	66.13	67.57	-1.44
6	1	0	-1	64.17	62.72	1.45
7	1	0	-1	62.76	62.96	-0.2
8	1	0	-1	60.80	62.77	-1.97
9	0	-1	1	64.06	66.77	-2.71
10	0	-1	1	62.33	62.12	0.21
11	0	-1	1	61.10	64.51	-3.41
12	0	-1	1	59.50	56.08	3.42
13	0	0	0	60.73	58.76	1.97
14	0	0	0	51.20	52.96	-1.76
15	0	0	0	57.63	59.27	-1.64

3. Results

3.1. Yeast isolates

The yeasts isolates were identified as *Saccharomyces cerevisiae* and *Candida tropicalis*. *Saccharomyces cerevisiae* and *Candida tropicalis* were capable of utilizing glucose, galactose and lactose. *Saccharomyces cerevisiae* was in addition to these, capable of utilizing fructose and sucrose while *Candida tropicalis* was capable to utilizing maltose.

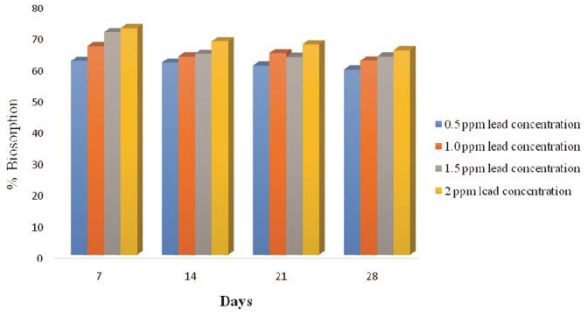

Figure 1. Biosorption of lead using *S. cerevisiae*

3.2. Lead biosorption by Saccharomyces cerevisiae and Candida tropicalis

The biosorption of lead by *S. cerevisiae* and *C. tropicalis* is shown in Figures 1 and 2. After 28 days of incubation, the highest % biosorption for *S. cerevisae* and *C. tropicalis* was obtained at lead concentration of 0.5 ppm, followed by lead concentration of 1 ppm and the least was obtained at lead concentration of 2 ppm (Figures 1 and 2).

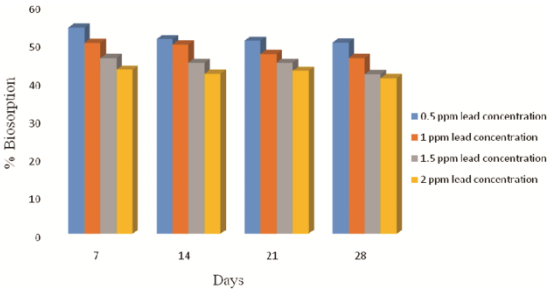

Figure 2. Biosorption of lead using *C. tropicalis*

3.3. The effect of incubation temperature and pH on biosorption of lead by S. cerevisiae and C. tropicalis

The effects of temperature values on the biosorption of lead by *S. cerevisiae* and *C. tropicalis* is shown in Figure 3. Of the 2 temperature values tested (25 and 37 °C), incubation at temperature value of 25 °C had a higher value of biosorption by the two organisms at days 7, 14, 21 and 28, with day 28 giving a higher values of biosoprtion (70.4%) for *S. cerevisiae* compared to incubation temperature of 37 °C with biosorption value of 64.6%, whereas for *C. albicans.* The higher % biosorption was recorded on day 7 (56.3%) compared to incubation at temperature of 37 °C with biosorption of 47.3%.

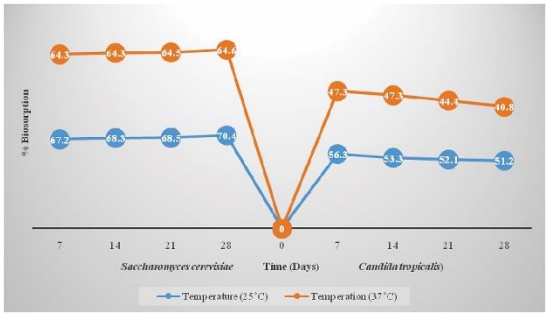

Figure 3. Effect of incubation temperature on percentage biosorption of lead by *Saccharomyces cerevisiae* and *Candida tropicalis*

3.4. Percentage of biosorption at different pH on biosorption of lead by S. cerevisiae and C. tropicalis

As shown in Figure 4, pH has effects on the rate of biosorption of lead by *S. cerevisiae* and *C. tropicalis.* When *S. cerevisiae* was incubated at pH 6, the highest biosorption value of 69.3% was obtained at day 28, whereas pH 7 had a biosorption value of 58.3%. For *C. tropicalis*, the highest biosorption value of 48.3% was recorded at day 7, but it had biosorption value of 46.2% at pH 7. Figures 5 and 6 show the 3d surface plot of lead removal versus pH and temperature and lead removal versus pH by *S. cerevisiae* and *C. tropicalis*. In both Figures, *S. cerevisiae* is shown to better uptake the lead when compared to the *C. albican.*

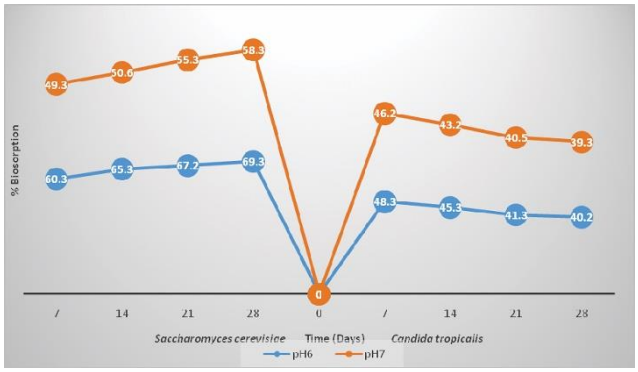

Figure 4. Effect of pH on biosorption of lead by *Candida tropicalis* and *Sacchromyces cerevisiae*

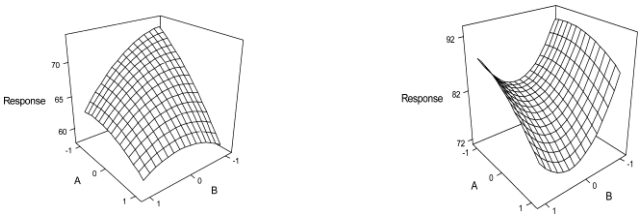

Figure 5. The 3d surface plot of lead removal versus pH and temperature by (a) *S. cerevisiae* and(b) *C. tropicalis*

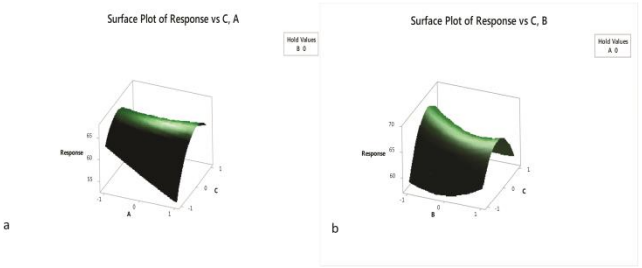

Figure 6. The 3d plot of lead removal versus pH and lead concentration by *S. cerevisiae*and *C. tropicalis*

Table 2 shows that the linear, quadratic and the interaction among the factors considered are statistically significant considering the low values of each of the probability values of the individual factors. (p-value < 0.05). From the analysis of variance table for response, probability value of 0.029 for the regression shows that the model is a good fit for the design since the p-value (0.029) is less than 0.05.

Table 2. Response surface regression for *Saccharomyces cerevisiae*

Term constant	Coef	StDev	T	P
	66.773	**2.107**	**31.689**	**0.000**
A	4.320	1.290	-3.349	0.020
B	-4.895	1.290	-3.795	0.033
C	-7.057	1.290	-5.471	0.001
A*A	-8.940	1.899	-4.708	0.011
B*B	-7.785	1.899	-4.100	0.021
C*C	-7.058	1.899	-3.717	0.047
A*B	6.225	1.825	3.411	0.017
A*C	-5.001	1.825	-2.634	0.063
B*C	-4.772	1.825	-2.615	0.065
S = 4.33779	R-Sq = 94.7%		R-Sq(adj) = 92.89%	

Table 3 shows the linear, quadratic and the interaction among the factors considered are not statistically significant considering the high value of each probability values (p-value > 0.05) of the individual factors (Table 4). From analysis of variance table for response, probability value of 0.851 for the regression shows that the model is not a good fit for the design since the p-value (0.851) is greater than 0.05.

4. Discussion

Physiochemical parameters such as pH, play an important role in increasing the rate of biosorption. There are three ways in which the pH can influence metal biosorption: (i) it affects the configuration of the active ion-exchange sites; **(**ii) it affects the ionic state of the sorbate in the solution and (iii) extreme pH values may damage the structure of the biosorbent material (Rearte *et al.*, 2013; Sen *et al*., 2015). The variation in biosorption of heavy metals by microbial biomass at different pH could be due to the differences in the sensitivity of cell wall molecules of the bacterial cells to pH. For instance, at a low pH, cell wall ligands tightly bind with the hydronium ions (H_3O^-) and hence restrict the approach of metal cations due to repulsive force. On the contrary, at higher pH values, more ligands like carboxyl, phosphate, imidazole and amino group would be exposed and carry negative charges with a subsequent attraction of metallic ions with positive charge and biosorption onto the cell surface (Pardo *et al*., 2003).

At initial low pH the cell surface becomes more positively charged thus reducing attraction between the biomass and lead metal ions. In contrast, higher pH values facilitate the uptake, since the cell surface is more negatively charged.

Table 3. Response surface regression for *Candida tropicalis*

Term	Coef	StDev	T	P
Constant	**79.787**	**7.311**	**10.914**	**0.000**
A	-3.992	4.477	0.892	0.413
B	-3.264	4.477	0.729	0.499
C	1.771	4.477	0.396	0.709
A*A	-2.098	6.590	-0.318	0.763
B*B	8.344	6.590	1.266	0.261
C*C	-2.309	6.590	0.350	0.740
A*B	-1.517	6.331	-0.240	0.820
A*C	-3.568	6.331	-0.563	0.597
B*C	-4.225	6.331	-0.667	0.534
S = 4.33779	R-Sq = 94.7%		R-Sq(adj) = 92.89%	

The coefficient of determination (R^2) was 45.4% which means that the independents variables were able to contribute up to 45.4% for *Candida tropicalis*. In the other sense, 45.4% of the R^2 indicates that the model developed explains 45.4% of the variance in the dependent variable (i.e. response) as seen in Table 3. From analysis of variance table for response, probability value of 0.851 for the regression shows that the model is not a good fit for the design since the p-value (0.851) is greater than 0.05. For *Saccharomyces cerevisiae* as seen in Table 2, the coefficient of determination (R^2) was 94.7%, which means that the independents variables was able to contribute up to 94.7%. In another sense, 94.7% of the R^2 indicates that the model developed explains 94.7% of the variance in the dependent variable (i.e.,response).From the analysis of variance table of the response, probability value of 0.029 for the regression shows that the model is a good fit for the design since the p-value(0.029) is lesser than 0.05.

From the above, it was observed that *Saccharomyces cerevisiae* model gave the most significant result with R^2 value of 92.8%, after comparing the coefficient of determination (R^2) of each of the biosorbents. This indicated that *Saccharomyces cerevisaie* was best optimized by the factors pH 6, temperature 25 °C and a lead concentration of 2.0 ppm. This result is similar to the work of Farhan and Khadom (2015) using *Saccharomyces cerevisiae*. Reported that the uptake capacityfor lead of *Sacchromyces cerevisiae* increased with increasing pH and the maximum capacity values have been observed at pH 6.0, 5.5, 2.5, 5.5, 6.0 and 5.5.

Thus, Box Behnken design was efficient in determining the optimum values of the factors (pH, temperature and lead concentration) that this is required by *Saccharomyces cerevisiae* for effective metal uptake (biosorption).

5. Conclusion

S. cerevisiae gave the most significant result with R^2 value when compared to *C. tropicalis*, after comparing the coefficient of determination (R^2) of each of the treatment models. Thus, *S. cerevisiae* was best optimized using Box Behnken Design by the factors pH, temperature and the lead concentration. Verification of the Fitness of each model using ANOVA technique shows that all the models can be used with confidence level of 0.95. The results also indicated that a pH of 6, a temperature of 25 °C and a lead concentration of 2.0 ppm lead to the lead removal by *S. cerevisiae.*

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7. Conflict of interest statement

There is no conflict of interest in the preparation of this article.

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